PSYCHOPHARMACOGENETICS

PHILIP GORWOOD / MICHEL HAMON

EDITORS

Springer

Psychopharmacogenetics

Psychopharmacogenetics

Edited by

Philip Gorwood
Louis Mourier Hospital
Colombes, France

Michel Hamon
Faculty of Medicine Pitié-Salpêtriére
Paris, France

Springer

Philip Gorwood
Faculty Xavier Bichat16 rue
Henri Huchard
Dept. of Psychiatry, Louis Mourier
Hospital,
Colombes, France
Paris 95018

Michel D. Hamon
Hopital SalpetriereNeurobiol.
Cellulaire Fonction.
Neuropsychopharmacology,
Faculty of Medicine Pitie-Salpetriere,
Paris, France
91 boulevard de l'Hopital
Paris CX 13 75634

Library of Congress Control Number: 2005937076

ISBN-10: 0-387-30793-1
ISBN-13: 978-0387-30793-0

Printed on acid-free paper.

Printed in the United States of America. (SPI/SBA)

9 8 7 6 5 4 3 2 1

springer.com

FOREWORD

Michel HAMON, and Philip GORWOOD*

Considerable progress has been made for the last fifty years in the treatment of psychiatric disorders thanks to the empirical discovery of the psychotropic properties of a few drugs. Actually, antipsychotics first, then antidepressants, anxiolytics and mood stabilising agents have all been discovered at the beginning of the second half of the last century, causing a true revolution in the clinical practice of psychiatrists, and the definitive recognition of psychiatry as an actual clinical discipline, with the use of effective drugs in addition to other medical interventions, as for cardiology, internal medicine, etc.

However, serious limitations in this progress have been the relatively low proportions of patients responding to the drugs, the unpredictibility of the response, and the deleterious side effects of the first antipsychotics and antidepressants which sometimes considerably deteriorate the quality of life of patients, and explain the poor compliance to treatments. A second breakthrough in the clinical practice of psychiatrists has then been achieved from the seventies, i.e. 30 years ago, when novel drugs were developed on the basis of the extensive neuropharmacological investigations that followed the empirical discovery of the first psychotropic drugs. Indeed, because clear-cut data showed that tricyclic antidepressants act through the blockade of monoamine reuptake, chemists synthesized selective monoamine uptake inhibitors which then revealed to share with tricyclics potent antidepressive properties. Similarly, the demonstration that phenothiazine and butyrophenone antipsychotics actually act through the blockade of dopamine receptors led to the development of selective dopamine receptor antagonists, such as the benzamide compounds sulpiride and amisulpride, which are endowed with clear-cut antipsychotic properties. Such achievement was a clear breakthrough because these novel drugs, specifically designed to act selectively at clinically relevant molecular targets, are consequently endowed with much less secondary, deleterious, effects of the first psychotropes. Indeed, the quality of life of patients treated with this second generation drugs is markedly improved compared to that degraded by earlier drugs, which contributes to higher compliance, and, in turn, better efficacy. However, better does not mean optimum because a large proportion of

Michel Hamon, INSERM U677, Faculty Pitié-Salpétrière, 91 Bd de l'hôpital, 75634 Paris, France.
Philip Gorwood, INSERM U675, Faculty Bichat, 16 rue Henri Huchard, 75018 Paris, France.

depressed or psychotic patients still do not respond to these second generation drugs. Indeed, at least 30% of depressed patients are not responsive to potent antidepressants, but the reasons why they do not respond are not known.

A third step in the development of better treatments is therefore needed, and neuroscientists and clinicians are strongly determined to make it fully successful. This step actually involves two complementary approaches. The first one consists of improving the design and synthesis of pharmacologically active molecules in order to increase the effectiveness and safety of drugs aimed at alleviating psychiatric disorders. Chemists already produced multi-target drugs acting at several receptors, enzymes, transporters, relevant to these diseases, but still (mostly) devoid of undesirable side effects. These third generation drugs (such as atypical antipsychotics acting at both dopamine and serotonin receptors, or anti-depressants acting simultaneously at serotonin reuptake site and presynaptic auto- or hetero-receptors) are clearly a further progress toward better treatments. However, even much more can be expected from the second approach of this third step, which consists of considering the genotype as a possible reason for good, poor or no responding to drugs. This field of research is the domain of Psychopharmacogenetics.

The objective of this book is to present all aspects of this novel discipline which aims at identifying the possible genetic reasons causing a given patient to respond, or not respond, to a psychotropic drug, and to suffer, or not suffer, from side effects caused by this drug. For this purpose, we asked the best experts in the world to contribute to this enterprise, and all accepted with great enthusiasm. We are very grateful to all of them, for their remarkable and comprehensive contributions. The book is organized in three main parts. The first one, with the first 9 chapters, is devoted to the various major psychiatric disorders for which one can expect so much from Psychopharmacogenetics, as definition of patient's genotype should be of great help to design the best drug treatment specifically for this patient, with maximal chance of positive response and minimal risk of side effects. For instance, polymorphism in the promoter region of the gene coding the transporter responsible for serotonin reuptake seems to be critical in the response to second generation antidepressants. The second part aims at providing detailed knowledge on major molecular targets of psychotropic drugs, with particular focus on polymorphisms of relevant genes which play key roles in both the neurobiological mechanisms underlying the diseases and the mechanisms of actions of these drugs. In the last part of the book, possible genetic reasons accounting for side effects of psychotropic drugs are reviewed, concerning cardiac, motor and sexual functions, notably because of marked individual differences in the metabolism of drugs.

Clearly, drug treatment of psychiatric disease is a real challenge, and also a bet as it is extremely difficult to predict the quality of individual response. Psychopharmacogenetics is undoubtedly a potent approach toward better treatment by identifying responders based on genotype profile. We do hope this book will contribute to open this novel discipline in the field of psychiatry, and to promote a novel method of great potential for the design of more effective and surer treatment adapted to a given patient.

CONTENTS

1. INTRODUCTION ON PSYCHOPHARMACOGENETICS

Philip Gorwood, Elizabeth Foot*

'To wrest from nature the secrets which have perplexed philosophers in all ages,
To track to their sources, the causes of disease,
to correlate the vast stores of knowledge,
That they may be quickly available for the prevention and cure of disease
-These are our ambitions.'

Sir William Osler, 1849-1919

The ultimate goal of psychopharmacogenetics is improved patient health-care. The following chapters of this book will detail the different mechanisms potentially involved in psychiatric disorders giving clues for new pharmaco-genetic studies, and the description of various psychiatric disorders, their treatment and associated side-effects, that may shed light on the complexities of the different phenotypes.

The research findings presented will hopefully in the not too distant future, 5- 10 years, form critical knowledge on which future treatment guidelines will be made, to ensure psychiatry patients are benefiting from these advances in scientific knowledge and the new insights gained from genetics and pharmaco-genetics in ravelling the complexities of psychiatry disorders and their treatment.

* Philip Gorwood, INSERM Unit 675 (IFR02) Faculty Xavier Bichat. 16 rue Henri Huchard, 75018 Paris-France.
Elizabeth Foot, Glaxo Wellcome Research and Development Limited, Greenford, Middlesex, UK.

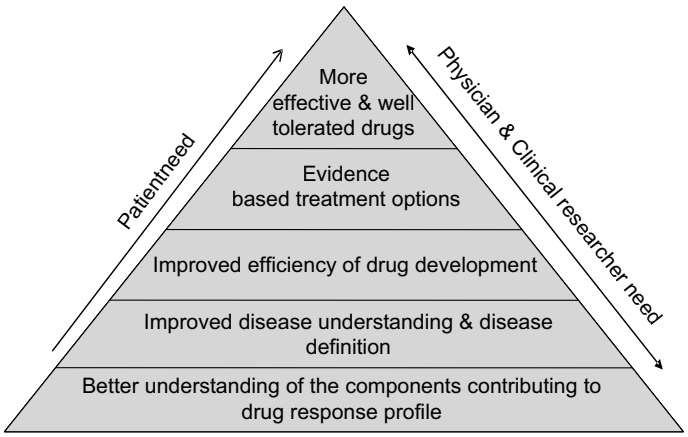
1. INTRODUCTION

The idea of the "science of the brain" and that the application of this science could be used to improve patient health is a belief well rooted in medical thinking. Indeed, since the time of the Ancient Greeks, the concept existed that psychiatric illness possessed an underlying neural structure. The last century saw an evolution of this medical thinking, with a growing body of research conducted within universities and institutions, to elucidate the chemistry and neurobiology of the brain and the changes that occur in psychiatric disorders. This pioneering research set the grounding for now viewing psychiatry as a science-driven medical field and the prospect of evidence-based treatment for these disorders.

Up until this time, drugs such as opiates had been used as the primary pharmacological treatment for psychiatry disorders such as depression and mania. These met with some success, although raised the problem of induced addiction. One can consider that it was the discovery of the first brain neurotransmitter, acetylcholine by Otto Loewi and Navrati E. (1926) back in the 1920s, that saw the beginnings of more science-based treatment strategies, with the end of the century seeing the wide spread use of the selective serotonin uptake inhibitors in the use of depression and mood disorders, the use of dopamine antagonists in schizophrenia and lithium for bipolar disorder.

However, despite the enormous advances made in our understanding of brain chemistry and the development of ground-breaking medicines, there remains considerable unmet patient need for more effective and better tolerated drugs (Figure 1). The sequencing of the human genome and the ability to investigate the genetic variation between individuals now offers a further window by which to untangle the complexities of psychiatry disorders and response to drug treatment. Pharmacogenetics, the science of the inherited component of variability in drug response, is now playing an increasingly important role to evolve the field of psychiatry medicine from a symptom-based medicine to a more mechanism and evidence-based field. Psychopharmacogenetics is the term used to describe pharmacogenetics as it specifically relates to the genetic understanding of the variability in response to psychiatry drugs.

As we ask the question as to how psychiatry medicine will look at the end of this century, or indeed in the nearer term, in next 5 to 10 years, a growing body of data is emerging to believe that new drugs for the future are hidden in our genes and to support a role for the use of genetic markers as part of a physician's decision-making criteria. Understanding the genetic contribution to the variability in response to drugs is predicted to reduce the reliance on trial and error prescribing and ultimately lead to more effective medicines and improved healthcare for patients. This genetic understanding of drug response, or pharmacogenetics, is also sometimes referred to as "giving the right drug at the right dose to the right patient at the right time". The evidence that this belief will soon become a reality, and the role for pharmacogenetics to support the clinical development and prescribing of novel psychiatry medicines is growing. Many of these key findings are described in the subsequent chapters of this book.



Legend: Patient need will be met by combined: (i) enhanced understanding of the disease phenotypes contributing to complex psychiatry diseases which in turn will provide new insights to help identify new drug targets and (ii) enhanced understanding of the factors contributing to drug response.

Figure 1. Patient Need Pyramid.

This introductory chapter aims to set the scene and provide a background to pharmacogenetics from the point of view of: (i) the patient and the potential benefits of the research findings for improving healthcare, (ii) the clinical research environment by providing an overview of some of the large on-going clinical studies and also Regulatory Authority initiatives in this field, (iii) the Science from the point of view of the methodology and issues that need to be considered in conducting pharmacogenetic studies, (iv) the Ethical consideration and finally (v) some thoughts on what is needed to move these scientific findings from research to the clinic.

2. PHARMACOGENETICS AND THE PATIENT

Current treatment strategies in psychiatry rely largely on symptom-based diagnosis, using a diagnostic score (DSM-IV and ICD-10), based on symptom clusters rather than the underlying neurobiology. Although current treatments can be highly effective in some individuals and offer significant improvements in patient quality of life, many psychiatry drugs unfortunately share a common absence of predictive factors for efficacy. Physicians have to rely on their own experience with a considerable element of trial and error prescribing. It has long been known by both physicians and patients that not all drugs work to the same extent or at the same dose in all patients diagnosed with the same disorder, based on symptomology. Nowhere is this more apparent than in psychiatry, where there is a high percentage of non-responders or incomplete response to initial

treatment, approximately 40%, countered by significant side-effects. The latter include rare instances of serious adverse reactions, such as the potentially life-threatening agranulocytosis observed in approximately 0.8% of patients receiving clozapine treatment, as well as non-life threatening, but still serious, metabolic side-effects such significant weight gain following anti-psychotic treatment.

A number of factors appear to contribute to this variable drug response profile, ranging from poor understanding of the etiology of the disease and symptom-based diagnosis leading to subjects with potentially heterogeneous disease etiologies being classified with the same disorder, to dose selection and environmental factors (Figure 2).

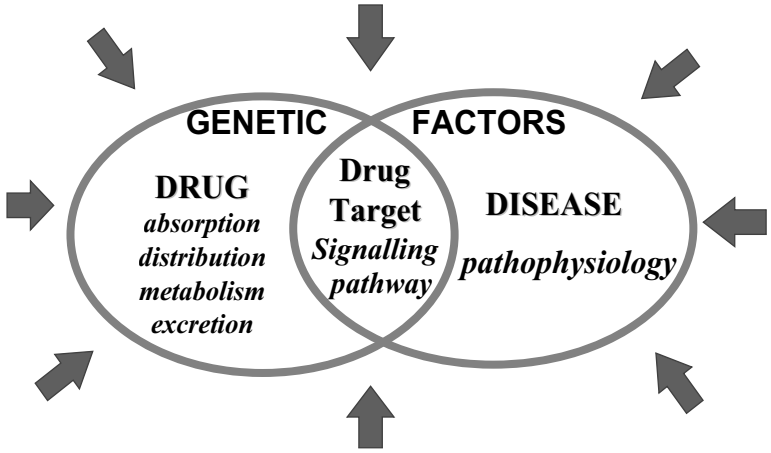


Figure 2. Variability in drug response: a genetics and environmental interplay.

All these factors interact to make prediction of individual response challenging. In many cases the mechanism of action of drugs and the signalling pathways involved in eliciting the therapeutic response are poorly understood. In addition, heterogeneous patient populations, composed of a number of different symptom clusters, are often treated as though they are one homogenous group. Currently there is no established test or "marker" that can assist the physician in guiding drug choice, although a large amount of research is currently being focused in this area. For example, a number of large clinical trials, sponsored by the NIMH (National Institute of Mental Health), are on-going, which aim to provide data derived from investigating response to drugs across mechanistic classes, and use this knowledge to develop treatment strategies to help guide treatment choice (Rush et al. 2003; CATIE, 2003).

The expectation, backed up by a growing body of scientific evidence, is that genetics and pharmacogenetics have the potential to dissect out the specific disease phenotypes that contribute to this currently symptom-based disease classification. This in turn would help identify more homogenous patient groups or sub-classifications to which drugs with specific mechanisms can be prescribed, thus offering the potential of greater therapeutic benefit (Goldstein et al.; Kirchheiner et al. 2004; Malhotra et al. 2004). There is often considerable variation in the frequency of these genetic variants between ethnic groups, and in addition the function of the variant may be modulated by its genetic background. The latter may help explain the apparently different functional effects reported for the long (*l*)/short (*s*) allele in the serotonin promoter gene observed between Caucasians and Asians; in Caucasians the *l* allele is associated with an increased probability of response to SSRIs *versus s* allele, whereas in Asians the association is reversed, with the *s* allele reported to be associated with improved response. The use, therefore, of a population with a mixture of ethnic backgrounds may confound the ability to determine an association and the functional significance of a given genetic variant (Ingelman-Sundberg M. 2004). Careful assessment of ethnic background, and incorporating this information in genetic analysis is therefore, critical in pharmacogenetic studies to avoid ethnic confounding effects (i.e., stratification bias). It is also important to remember that although pharmacogenetics will provide just one, and it is believed, a very important piece of the "uncertainty" in drug response, pharmacogenetics should be considered as one part of a bigger picture contributing to drug response. The findings from disease genetic studies, and clinical pharmacogenetic studies, together with information from other biomarkers and neuroimaging studies, will all serve to increase our understanding in this area and provide the knowledge base for moving its application into the clinic (Evans et al. 1999; Frank et al. 2003)

The madness of King George serves as perhaps an extreme, but interesting, example of how lack of understanding of the underlying disease mechanism can result in therapeutic failure, and in this particular case, lead to considerably more harm than good. For many years, the puzzle to understand the "madness" of King George III remained unexplained until, in the 1970's, a diagnosis of porphyria was made. However, still unexplained was the reason for the severity of the King's attacks and why they started relatively late in his life. Only recently was it shown that the attacks could have been triggered by a slow build up of arsenic in the blood, as a result of the treatments prescribed to him by the Royal physicians. As a result of their poor understanding of the disease and mechanism of action of their medications, the arsenic treatment prescribed was leading to an exacerbatiion of the king's illness rather than alleviating it.

Ensuring that the most appropriate medication is administered to treat the correct disease and underlying neurological mechanism is clearly central to achieve a successful treatment outcome. However, dose selection can also contribute to this picture of inconsistent response. The administration of a "standard" dose across a patient population may lead to variation in the resultant exposure as a result of genetic variants in the metabolic metabolising enzymes and transporters resulting in altered enzyme expression and function. Many of

the older anti-depressants and antipsychotics, for example, have a major metabolic clearance dependency on the polymorphic cytochrome P450 (CYP) isoenzyme CYP2D6 (Dahl et al. 2002) with other CYPs, such as CYP1A2 and CYP3A4 also contributing to this inter-variability in terms of both exposure and potential for drug interactions. All the major human drug-metabolising P450 enzymes have now been identified and cloned and the major gene variants identified. (Updated information on variant alleles in human CYPs, frequency and function can be found on the Human CYP allele Nomenclature Website, http://www.imm.ki.se/cypalleles). This information now provides the basis for using predictive pharmacogenetics markers to guide dose selection (Malhotra et al. 2004; Ingelman-Sundberg, 2004).

The consequences of variability in drug exposure will be drug dependent and determined by the benefit/risk ratio in terms of therapeutic benefit *vs* risk of tolerability issues for the patient. The need for genotyping patients for CYP genes prior to treatment is currently not required, although the label of many drugs refers to the potential functional impact of CYP genotype. This is most recently seen in the psychiatry field for the anti-depressant drug, Strattera (atomoxetine) for which the lable includes detailed information on the increased drug exposure observed in CYP2D6 poor metabolisers. Routine laboratory testing is, however, not mandated. Testing individuals once in their life for the major CYP genotypes involved in drug metabolism could be made in a similar way to testing blood groups. The question of testing the approximately 20 major P450 enzymes only or the whole group of P450 enzymes (more than 1.000 are known) is more a question of technology and cost.

Traditionally pharmacogenetics has been viewed in terms of pharmacodynamic and pharmacokinetic factors (Evans et al. 1999). However, it is perhaps more relevant to view pharmacogenetics from the patient perspective; on the one hand concerning the probability of achieving therapeutic benefit for their symptoms and, on the other hand assessing the risk that they will experience clinical significant adverse events. Variability in pharmacokinetics impacts both these components of drug response profile.

Factors such as poor compliance undoubtedly also contribute to the variation observed in drug response, but combinations of genetic variants in both the pharmacokinetic and signalling, pharmacodynamic pathways should be considered in assessing a patient's response to a given drug and dose, and included as part of prescribing decision making. Rather than single markers, it is likely, however, that a panel of markers will be required to predict the probability of efficacy and/or risk of side effects. The resultant response elicited by a drug in an individual subject is, therefore, the result of a complex interplay of many pathways consisting of intrinsic, mainly genetic, and extrinsic environmental, factors. Poor response to a given drug can arise due to defects at any point within this network of interactions (Figure 3).

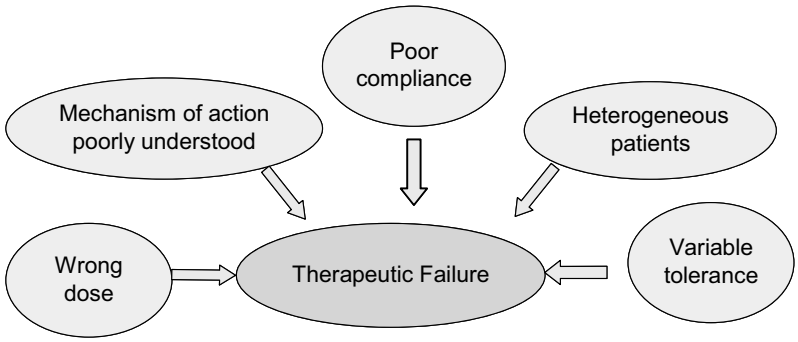


Figure 3. Reasons for therapeutic failure.

Such interplay presents a tough challenge for identifing predictive markers, and current psychiatry research has mainly focused on an approach using association studies and candidate genes based on the current understanding of the etiology of the disorders. Advances in technology, the lower cost and reduced time required to conduct such genetic analyses (for example with micro arrays) provide the prospect of unbiased, hypothesis-free approaches for genetic exploration and the identification of new markers, not predicted based on current disease understanding. Current understanding is constrained by our still relatively limited knowledge of the underlying neurobiology. The drugs developed based on this understanding are now in the clinic, but still leave considerable unmet need. Some new directions are needed. Genetics and pharmacogenetics can provide much needed new sign-posts to move in novel directions.

3. PHARMACOGENETICS IN CLINICAL RESEARCH AND DRUG DEVELOPMENT

The belief that pharmacogenetics will make a significant contribution to improving patient health care is widely held by both drug regulatory authorities and government bodies. Furthermore, there is a growing body of data to support the feasibility and reality of this view. This data is derived from research conducted both by academic groups and also from clinical studies conducted by pharmaceutical companies. Many of these findings are described in later chapters of this book, but some examples also are given below.

The need for more disease understanding and greater clarity and understanding of optimal patient drug treatment options is recognized by the NIMH and currently incorporated as part of the NIMH Treatment Research Initiative. This program includes 3 large “real-life” clinical trials studying the effectiveness of drugs for unipolar depression (STAR*D: Sequence Treatment Alternatives to Relieve Depression; http://www.edc.gsph.pitt.edu/stard), schizophrenia (CATIE: Clinical Antipsychotic Trials of Intervention effectiveness; www.catie.unc.edu) and bipolar disorder (STEP-BD: Systematic Treatment

Enhancement Program for Bipolar Disorder; www.stepbd.org) (Rush et al. 2003; CATIE, 2003). These clinical studies aim to determine the most appropriate treatment strategies for patients with mood and psychotic disorders. The findings from these studies have the potential to have a major impact on prescribing recommendations for their respective indication. The first data from the STAR*D program is expected in the next 2-3 years. All studies are collecting blood for DNA analysis and investigation of genetic markers. Within Europe, a number of additional large studies are being conducted that aim both to understand the aetiology of depression as well as identifying predictors of drug response; for example the GENDEP and NEWMOOD studies. The findings from these studies will serve to build our knowledge and provide data to better understand the role of pharmacogenetics and how to apply this as part of treatment strategies across the spectrum of psychiatry disorders, including mood disorders, psychosis, substance abuse and dementia.

From the Regulatory side, the FDA in North America (http://www.fda.-gov/cder/guidance/ 5900dft.doc) and other regulatory bodies, including the MHLW in Japan, have published draft guidelines (for example the FDA's VDGS: Voluntary Data Submission Guidelines) to provide guidance to pharmaceutical companies on how pharmacogenetic data will be used as part of regulatory decisions and to encourage the inclusion of pharmacogenetic data as part of drug development. It should be noted that such pharmacogenetic data may be used to support more efficient and successful clinical development strategies, without necessarily resulting in label restrictions based on genotype. Rather, data can be applied to ensure that novel, more effective medicines reach the patient as quickly as possible (Roses 2004). An example of this for psychiatry is the use of pharmacogenetics to dissect out placebo response, or perhaps more correctly, non-specific drug response, a major confounder in clinical studies not only for depression and anxiety, but also for other psychiatry disorders (Macedo et al. 2003). A number of studies now report an association between the common functional 44-base-pair insertion/deletion polymorphism in the promoter region of the serotonin gene transporter and both SSRI response and also placebo response (Smits et al. 2004; Smeraldi et al. 1998). The long (*l*) allele results in a gene with approximately twice the transcriptional efficiencies of the short (*s*) allele, and a consequent approximately 50% difference in serotonin uptake. Such data offers the potential to enrol subjects into clinical studies that will have a reduced placebo response and thus increase the opportunity to determine a placebo-drug treatment separation and to detect a more robust efficacy response signal for the investigational compound. Prospective studies and studies investigating the role of this variant across different mechanism classes is required, however, to more fully evaluate these initial findings.

4. PHARMACOGENETICS: TOOLS OF THE TRADE

If pharmacogenetics is a relevant science and tool for psychiatry, this implies that the psychotropic drug response has to be, at least in part, heritable (heritability being significantly above 0). Studies devoted to the heritability assessment in psychotropic response are, however, uncommon. This is probably

explained by the difficulty of such approach. For example, when using twin or family based studies to assess the heritability of a certain side-effect to a psychotropic drug, a large set of monozygotic and dizygotic twins or family members should both have the disease and also be treated by the same drug and for approximately the same period. Accordingly, the vast majority of these studies looking at heritability are based on aggregation studies, and conducted for the most frequent psychiatry disorder of major depressive disorder. In one study (Angst 1964) 38 out of 41 pairs (92,7%) of siblings were concordant for tricyclic response and the whole set of 12 depressed sibpairs were concordant (100%) for antidepressant response in another sample (Pare et al. 1962). In two independent samples of affected sibpairs, 10 out 12 were concordant (Pare et al. 1971), and 67% of the relative pairs were concordant for antidepressant response, a frequency only just above the one expected by chance, i.e. 50% (Franchini et al. 1998). Using a multiplex family, a further study showed that the four members of the same family were all resistant to classic antidepressants, and all sensitive to a monoamine oxidase inhibitor (O'Reilly et al. 1994). Studies devoted to antipsychotics are even less common. The few studies that have been performed are also concordant, in favour of a family and/or genetic component to treatment response. Two case reports of monozygotic twin pairs with schizophrenia were concordant for new antipsychotics after prior resistance to neuroleptics (Vojvoda et al. 1996; Mata et al. 2001). The only family study that did not find any evidence for familial aggregation of antipsychotic response, was a set of 28 schizophrenic sibling pairs (DeLisi et al. 1989). Family and twin studies, more specifically devoted to the heritability estimation are, however, required to more clearly dissect out the genetic component to drug response and the extent to which it contributes to the variance in response observed.

At a biological level, pharmacogenetics consists of associating a genetic variant or polymorphism with a specific drug response (either side effects or level of efficacy). If the drug response has a 100% of heritability, assuming no genetic heterogeneity, the genetic analysis will give a 100% confidence as to whether a patient will response or not respond to a specific treatment. However, this implies that the drug response is homogenous, without incomplete penetrance and cases of phenocopy (i.e., all patients having a specific drug response have the involved gene(s), and all subjects having the involved gene(s) have the specific drug response). This is rarely the case and in addition, the given treatment also needs to be identical, not only with respect to dose and patient compliance, but also concerning presence of co-prescribed treatments (which may competitively use the same enzymes for metabolism), food intake (for example, grapefruit and grapefruit juice which inhibit CYP3A4 enzyme activity), and have the same ethnic origins (anonymous SNPs variations are more likely to reflect ethnic specificities rather than genetic variations for a side-effect susceptibility). These different points serve to highlight the complexity of pharmacogenetics, and why the data provides probabilities and susceptibility rather than absolute certainties. Results are therefore usually reported as an Odds Ratio describing the probability that a patient carrying a certain allele will respond well to a given drug compared to a patient who does not have the allele, or if assessing safety risk, the likely increased risk or probability that the patient

with a certain allele will experience an adverse event as a result of taking a given medication.

Also contributing to this notion of pharmacogenetics not being an "exact science" is the absence of internal validity of the phenotypes on which pharmacogenetics is based in psychiatry. It is widely acknowledged that current psychiatry classification, mainly based on symptoms, results in heterogeneous patient populations with differing underlying disease mechanisms, but with the same disease classification. With the aim of identifying more homogenous phenotypes Ming Tsuang et al. (1993) proposed a "psychiatric genetic nosology" that classifies individuals to correspond to distinct genetic entities. This could be extended to include information gained from pharmacogenetics and a classification taking into account response to different drug mechanisms, most likely reflecting different underlying disease pathways. Difficulties in identifying homogeneous phenotypes can also be associated with the limitations of existing assessment methods that do not cover a variety of diagnostic classifications systems, ignore differential diagnosis and co morbidity, or omit an array of items suitable for providing broad descriptions of phenotypic subtypes. It is thus important to measure items such as recurrence or persistence of symptoms, impairment or incapacitation due to symptoms, and age of symptom onset and offset that may contribute to variability of the phenotype (Gorwood 2003). One way to cope with such uncontrolled phenotypic heterogeneity, is to conduct pharmacogenetic studies in as homogenous a population as possible, by ensuring subjects have an equivalent background for at least three different features. Firstly, they should have the same ethnic background, reducing the risk of stratification bias. Secondly, they should have the same type of disorder, not only according to diagnostic criteria but also regarding length, severity and core characteristics of the diagnosis. Thirdly, it is important to control, as much as possible, for pharmacological confounding factors, including dose, associated co-prescription, smoking and food intake. These latter, environmental factors, may be independent of genetic factors, although gene-environment interaction must be considered.

An example highlighting the impact of a more appropriate assessment of the involved phenotype, is the role of the dopamine receptor D3 gene in schizophrenia. The DRD3 gene was initially reported to be involved in the susceptibility to schizophrenia (Dubertret et al. 1998), but many inconsistent findings were reported. When focusing on one particular side-effect observed in treated schizophrenia patients, the association was in fact attributed to tardive dyskinesia (Spielman et al. 1998).

In both psychiatric disease genetic and pharmacogenetic studies, better designed protocols may help to reduce heterogeneity and increase the chance to detect genes with a significant effect. With reference to pharmacogenetic studies, some issues to consider include: the use of prospective rather than retrospective designs, more precise phenotype assessments and measurement of disease endophenotypes, avoiding admixture of samples with various ethnic background, and accounting for potential Gene x Environment interactions within the data analysis.

Two epidemiological genetic approaches are often used in human studies to identify genes associated with disease and drug response, the S-TDT (Sib Transmission Disequilibrium Test) (Spielman et al. 1998) and the QTL (Quantitative Trait Loci, Allison 1997) based analysis. The S-TDT involves recruiting an affected proband and an exposed, but unaffected sib. For pharmacogenetics, such an approach is easier, since the frequency of positive familial history for one psychiatric disorder is frequently high (see Table I) and the chance that the relative has also been treated by the same drug (or at least a drug from the same mechanistic class) is also relatively high. Statistical analysis then involves comparing two related subjects discrepant for the phenotype, and testing to see if this phenotypical discrepancy is in accordance with a genotypical discrepancy, i.e. sharing of 0, 1 or 2 alleles with a distribution that is not the one observed by chance only (which is, by definition, 25%, 50% and 25%). This technique has the advantage of being protected from the stratification bias (especially when parents are available to provide a DNA sample), and being closer to a pure genetic approach (as family-based). The QTL based analyses compares with the S-TDT by recruiting and taking DNA samples from a series of probands and their two parents. The phenotype is not classified as having or not having a certain diagnostic criteria, but rather determines at a dimensional level, a certain quantitative trait. For complex traits, a quantitative approach may be more relevant than the more classic categorical classifications. For example, reduction in the MADRS rating after 4 weeks of antidepressant therapy, blood-level of Clozapine or lithium for a certain dosage of treatment, specific weight gain following antipsychotic treatment, or the impact of psychotropic drugs on QT intervals in the ECG. With this approach, the patients who possess a given allele are compared with patients who do not. Regression analysis is then applied based on the assumption that if the allele is significantly involved in the studied trait, then the transmission of this allele should be associated with a difference from the average (lower or higher) of this trait.

At a more logistical level, methods for collecting DNA samples have also significantly improved the ability to conduct clinical genetic studies. Blood samples are still the gold standard for high quality DNA extraction, but collecting DNA from buccal swabs (Thomson et al. 1992), mouth wash (Lum et al. 1998) or even saliva (Irwin et al. 1996) are now often used, particularly for paediatric studies or large studies involving sites with limited blood collection facilities. These techniques give less DNA per sample, but samples are easier to handle and may be more appropriate for the given study population and study site or country. For example, a buccal-cell collection or buccal swab, does not require an immediate extraction, or other sample treatment such as freezing after collection, and may thus be stored until all samples have been collected and samples can then be shipped in one batch to the chosen extraction laboratory. A buccal swab may also be performed by the patient themselves or their relative and can be performed unsupervised at home without the need to attend the study site. The sample can then be posted to the investigator. Furthermore, the ease of collection contributes to lower sampling costs. Finally, large amounts of DNA can be obtained from these buccal-cell collections using the rapidly developing technique of non-specific amplification (Zhang et al. 1992). However, blood

samples remain the sample type of choice when study collection conditions, site and type of patient make this feasible.

The discovery of genes involved in other types of complex diseases, such as Crohn's disease (Hugot et al. 2001) or osteoporosis (Balemans et al. 2001) have shown that high complexity of the disorder, presence of moderate heritability, or the existence of phenotypical heterogeneity, should not be considered as factors incompatible with the use of genetics. Advances in both the technology for genotyping and bio analysis tools for analysis of the large amounts of data generated, are now contributing to the unravelling the complexities involved in the genetic analyses of psychiatry complex disorders and phenotypes. No psychiatric disorder has a 100% heritability, and no segregation analysis has shown that even a subgroup of families have a Mendelien inheritance. Psychiatric disorder do, however, show a high heritability (for example autism). Even so, many genes are likely to be involved, and even a larger number of interactions possible (Risch et al. 1999).

4.1. Coping with heterogeneity

As each individual genes involved may have only a moderate effect, large sample numbers are generally required to have the confidence to detect a significant effect. On the other hand, new analysis tools are now providing the ability to achieve an increase in statistical power and the ability to take into account disease heterogeneity. Such methods include the shift from parametric to non-parametric linkage studies, from linkage to sibpair analyses, and from siblings to case-control approaches. Although these approaches reduce the specificity of findings, on the more positive side, they also increase the chance of detecting genes with moderate impact (Risch et al. 1996). More sensitive methods are less specific, and thus more exposed to phenotypical heterogeneity. It is, therefore, important to compare the results of different studies, but ensuring that the between-sample heterogeneity is taken into account. Such meta-analyses are regularly used in psychiatric genetics, and could help to gain power in detecting the role of genes with relatively low, albeit important, contributions to the variance observed in response to psychotropic drugs. The Woolf method is an analysis frequently used (Woolf et al. 1955) and allows the calculation of total $\chi 2$ (representing the sum of the $\chi 2$ of each study), gives a $\chi 2$ estimating the heterogeneity between samples, and evaluates the specific $\chi 2$ (i.e., the $\chi 2$ considering all case-control studies, but excluding the part due to heterogeneity). Meta-analyses are also exposed to some methodological problems but are a particularly appropriate method of analysis for case-control studies, in addition to linkage studies (Gorwood 1999). For example, results from the meta-analysis of the different association studies performed on the role of the short allele of the 5-HTT gene (SLC6A4 gene) in suicidal attempts provided the most convincing arguments for the role of this gene in suicidal acts (Anguelova et al. 2003). A similar approach provided the evidence for the role of the DRD3 gene in tardive dyskinesia (Lerer et al. 2002).

It is also possible to face the difficulty of heterogeneity of the disease and population admixture by restricting the analysis to homogenous and recent

populations with founder effects. For example, the medical health care system in Iceland has provided a very large database on subject medical and genealogical information, which has provided researchers with not only medical history information, but also detailed family history. This latter information, not often available, dramatically increases the specificity of the findings and helps to reduce heterogeneity. The identification of a locus involved in asthma (Hakonarson et al. 2002) was, for example, based on such approach. Sometimes however, alleles have a clearly defined functional impact that over-shadows ethnic heterogeneity. A good example of this is seen for the drug metabolizing enzyme TPMT (thiopurine methyltransferase). Deficient patients carry one of three alleles (G238C, G460A and A719G). These have different frequencies according to their ethnic origins but with the same impact, namley to increase the risk of developing haematopoietic toxicity when subjects with these alleles are treated with conventional doses of thiopurines (Lennard et al. 1989).

4.2. Which genetic markers to use

With an average of one SNP every 1 kB (1,000 nucleotides), there are around 1.8 million publicly available SNPs (http://snp.cshl.org). Two individuals differ on average by 1.4 to 1.5 million base pairs (Sachidanandam et al. 2001) whatever their phenotype. This makes choosing the appropriate SNP to analyse for any given gene challenging. Many of these SNPS have no direct functional consequence on the transcribed protein, although they may be in linkage disequilibrium with other SNPs that do. SNPs for analysis are often chosen because they are highly polymorphic and of reasonably high frequency, usually above 5%. There are a number of approaches taken to select SNPs for genotyping and the approach taken will depend to a large extent on the experimental question being asked, number of genes of interest and number of samples, and hence study power. One may, for example, have a very specific hypothesis to test regarding the role of a given gene in predicting a given drug response. In this case, applying dense SNP mapping across a small number of genes, selected based on prior information as being important, may be most appropriate. In contrast, if one wishes to explore a larger gene list for hypothesis making and identification of a more restricted number of genes of interest to test in further studies, less dense mapping may be selected, but ensuring representative coverage across the gene. Haplotype information will also help to inform SNP selection, so that where two or more SNPs are in linkage disequilibrium, only one of these need be genotyped.

Because of our limited understanding of mechanism of action of most psychiatry drugs, taking a hypothesis-driven, candidate gene approach, based on current knowledge has the limitation of findings being limited to the currently known pathways and mechanisms. In contrast, a genome scan has the greater potential to identify hitherto unknown susceptibility genes for pharmacological response such as an adverse event, and broaden our understanding on drug mechanism of action. At a practical level, 70,000 putative anticancer agents have been tested for 8,000 genes in the National Cancer Institute panel of 60 human cancer cell lines (Shedden 2003). For psychiatry, focusing on a prioritised

set of SNP markers (whether because they are located in candidate genes or because they are informative makers of different regions of the genome) is preferable for the investigation of psychiatric treatments where few tissues are available (brain tissues not being easily obtained). An alternative approach, apart from sequencing large, more or less anonymous, parts of the genome, consists of focusing on panels of SNPs targeting genes with specific roles, for example genes implicated in drug absorption, distribution, metabolism and extraction (Ambrose 2002) or genes thought to be involved in the pathways of monoamine transmission. This strategy is frequently explained by economic reasons and/or availability of technical facilities.

The use of blocks of SNPs, i.e. haplotype, could offer an interesting alternative, which can more practically be applied at the present time and considerably reduce the number of SNPs required for a whole genome analysis, as well as for a candidate gene approach. Patil et al. (2001) reported that merely 4,563 of the original 24,047 SNPs were needed (19%) to capture 80% of haplotypic variation of human chromosome 21. This approach has been applied by Genaissance using the HAP^{TM}Array, to investigate susceptibility genes associated with agranulocytosis in patients treated by Clozapine (Oestreicher, 2002). Analysis of genetic data at the level of haplotypes provides both increased accuracy and power to infer genotype-phenotype correlations and evolutionary history of a locus. There are many leading methods of computational haplotype inference, such as PL-EM (Qin et al. 2002), Phase (Stephens et al. 2003), SNPHAP (Sham et al. 2004) and Haplotyper (Niu et al. 2002) that will be important to include as part of such pharmacogenetic sample analyses.

Another advantage of using haplotypes is the ability to capture rare SNPs. The hypothesis of "common-disease/common-variant" is often put forward for complex disorders, including psychiatry (Lander 1996; Collins 1997). SNP selection is frequently focused on the most common SNPs with less frequent and rare SNPs often not being analysed. However, the participation of rare alleles with major genetic effects (i.e. loss of protein function) may be important and provide valuable information and understanding. For example, BRCA1 and BRCA2 genes explain breast cancer in some families but are not associated with breast cancer in the global population (Domchek et al. 2003). In this regard, rare alleles should not be ignored since rare mutations (particular in the genes coding for drug targets) may provide unique insights into novel approaches to treat patients with common disorders (Pettipher et al. 2002). Analysing haplotypes should help increase the chance to detect blocks that contain a rare SNP involved in the disorder or drug response, whereas limiting the analysis to common polymorphic SNPs would have a higher probability of missing any association and therefore, not detecting an effect.

Haplotype analysis also provides the opportunity to detect yet unknown functional SNPs. For example, the Cys23Ser (C68G) was the main SNP tested in the 5-HT_{2C} gene in genetic studies of obesity and also pharmacogenetics studies of anti-psychotic-induced weight gain. This SNP was not found to be associated with obesity (Lentes et al. 1997), nor clozapine-induced weight gain (Basile et al. 2001). Several haplotypes for the promoter region of the 5-HT_{2C} receptor gene have now been described and further haplotype analysis has identified a specific

haplotype associated with the anti-psychotic-induced weight gain. This association has been attributed to a new T-759C SNP that has a protective effect against pharmacologically induced weight-gain (Reynolds et al. 2002).

4.3. What genotyping methods to choose

The introduction of arrays for the simultaneous assessment of multiple genes is the source of a major development for genetic analysis. The improvement in robotics and fluid physics is such that up to 64,000 gene clones can be evaluated on a single slide. Analysis of 1,000 to 5,000 genotypes per day is routine in many pharmacogenomic laboratories, with automated multiplex assays extending this to 100,000 genotypes per day. These developments generate large amounts of data in a single experiment, much more than can generally be evaluated using common statistics. Software has thus been developed that not only captures the experimental data, but also allows for the comparison of results with existing genome databases and for the generation of dendrograms for sequence homology. This method provides pattern recognition to enable genes to be collated into similar groups or patterns of expression (McLeod et al. 2001).

Recent technical developments thus give the ability to test, with a one-shot assay, hundreds of thousands of common SNPs providing a good coverage across the human genome. Indeed, the number of SNPs currently estimated to be required for successful whole-genome studies, not relying on haplotype blocks, range from between 60,000 and 600,000 (Zwick 2002). To support this scale of genotyping, the development of high-throughput genotyping assay approaches should have a major impact in psychopharmacogenetics and generating pharmacogenetic data from psychiatry clinical studies. Examples of such assays include allele-specific hybridisation, primer extension, oligonucleotide ligation and cleavage of a flap probe (Kwok 2000).

4.4. Facing the risk of false-positive and false-negative results

A problem shared by both psychiatric disease genetics and pharmacogenetics is the problem of false positive findings, and in many cases, non-replication of an initial association. This problem is difficult to avoid since the majority of genes involved in the susceptibility to major psychiatric disorders or contributing to variance in drug response, will only moderately increase the risk or modulate response; genotypical relative risk (GRR) is generally between 1.5 and 3 to 4. Taking into account the phenotypic heterogeneity of psychiatric disorders, and making the hypothesis that approximately half of the 30,000 genes of the genome could be considered as candidate genes in pharmacogenetics (since half are expressed in the brain), non-replications and a high false-positive rate, are not only possible but expected (when based on different independent sample sets). One possible way to deal with this problem is to increase the threshold of positive finding (for example, reducing the p-value, as when applying the Bonferonni correction). This, however, then results in

increasing the risk of false negative findings. In pharmacogenetics, many findings will be further contradicted in large samples with a better homogeneity.

Using larger sample sets, taking steps to reduce sample heterogeneity, coupled with the progress in both genotype and statistical methods may, it is hoped, progressively reduce these problems. Stratification bias, for example, which is frequently a confounding factor in case-control studies, can be assessed, and even controlled for, with the genomic control technique (Pritchard et al. 1999). The assumptions made with this method is that study groups (patients versus controls) are assessed for allele frequency of highly polymorphic markers that are unlinked to the analysed phenotype. Forty unlinked markers are reported to be necessary to achieve a 95% probability of detecting stratification in study group of over 200 subjects. When stratification is detected, subjects can be excluded until stratification is no longer present, or correction factors can be proposed to account for the level of between-groups stratification (Devlin et al. 1999).

4.5. From genomics to proteomics

An integrated pathway approach, i.e. investigating response at the level of both the genome and proteome, provide the opportunity to put genetic findings into biological context and give a more comprehensive picture of neuron function and pathology (Haab 2001; Gmuender 2002; Guzey et al. 2002). One obvious consideration for biomarker and proteomic research in psychiatry is access to relevant tissues, and whether information obtained from the most readily available tissues, namely blood or skin tissue samples, is relevant for what is happening in the brain. Further work may provide greater understanding to answer this latter question.

It has to be remembered, however, that there are more than 100,000 proteins in the human body and most, if not all, act on a variety of biological process. The proteomic approach is thus exposed to even greater complexity than genomics. Identifying proteins and their functions using genomic information, i.e. proteomics, presents the potential opportunity for more rapid identification of new biomarkers (Ledley 1999) that can in turn, act as a guide to find, evaluate, and support validation of genes and biomarker gene products for target diseases (Collburn 2003). This complimentary approach of genomics and proteomics in psychiatric genetics should also happen in pharmacogenetics.

The SSH (suppression subtractive hybridisation) technique, for example, has helped to identify new genes of interest, an important step before the identification of novel therapeutic targets. In this technique cDNA is generated from mRNA extracted from two types of tissues or cells, one tissue being collected from affected patients, and the other from healthy controls. For example, a group analysed genes induced by BRCA1 (an already known vulnerability gene in breast cancer) in order to find other potentially involved genes. The study thus compared control breast carcinoma cells (driver) with cells ectopically expressing BRCA1 (tester), and found that a new set of 30 genes might be involved in the risk of breast cancer (Atalay et al. 2002).

Other related techniques are also available that may have specific advantages for psychopharmacogenomics. These include differential hybridisation, subtraction cDNA libraries (Hedrick et al. 1984), mRNA differential display (Liang et al. 1992), serial analysis of gene expression (SAGE) (Velculescu et al. 1995) and microarrays for gene profiling (Schena et al. 1998).

Although these techniques are easier to apply for diseases with clear-cut lesions, such as ischemia following focal stroke (Wang et al. 2000), their potential interest and application in many psychiatric disorders is important now that large collections of brains from patients who have died are being made in different countries. These will provide samples for applying these techniques. Other developments in proteomics may be of considerable value in the development of new pharmaceutical targets through pharmacogenomics. Gene expression arrays are used to define the mechanism of action for new compounds, or to screen for direct influence of an agent on a specific pathway. Some new techniques may be specifically relevant for psychopharmacogenomics. For example, voxelation, using high-throughput analyses of spatially registered voxels harvested from the brain followed by three dimensions reconstruction, is performed before a Gene Expression Tomography (GET) which employs analyses of sets of parallel slices obtained from the brain by progressive rotation about multiple independent axes (Singh et al. 2003). Tomographic image reconstruction can then be employed for reconstructtion of gene expression patterns. This has been elegantly done for Alzheimer disease (Brown et al. 2002). Looking at the genes that are the most strongly differentially expressed between human brains from normal controls compared to patients with autism or schizophrenia, for example, could help to identify currently unknown genes. Such powerful techniques must, however, as for all scientific clinical research, be developed whilst paying close attention to the associated ethical, social and legal aspects.

5. PHARMACOGENETICS AND ETHICAL CONSIDERATIONS

Medical ethics supports the application of advances in science to progress medicine to the greater benefit of the patient and improving healthcare. However, as is the case with many scientific advances, for both pharmacogenetics and disease genetic research, there are two perceived sides of the coin; on the one-hand is the offer of great potential to enhance human health care, but on the other hand, there are fears for the misuse of information and the potential risk of discrimination, for example for employment and health insurance (Nuffield Council, 2003; Buchanan et al. 2002). For genetic research within clinical drug development, ethical considerations have largely focused on the possibility of using a genetic test to identify the most effective drug for a patient in terms risk:benefit i.e., risk of adverse events versus therapeutic benefit. A growing number of guidelines, advisory groups and white papers have been issued by groups across the Unitied States, Europe and Asia on this topic. The question as to if or how genetic information differs from other medical information is still debated. However, all clinical and medical patient information carries information of importance to a patient's health status and

often provides some prediction of future patient health and risk of disease e.g., cholesterol level and risk of heart disease. All such information, not just genetic information, must be handled with sensitive and steps taken to ensure confidentiality. A detailed review of these issues is provided in the recent report of the Nuffield Council of Bioethics (http://www.nuffieldbioethics.org/ pharmacogenetics/ latestnews.asp) (Melzer et al. 2003).

There are, however, some specific ethical considerations for psychiatry clinical studies, where there may be particular concerns from patients who are depressed or delusional. As a result fully informed consent may be more difficult to ensure and patients themselves may be more anxious and have heightened concerns and worries regarding their DNA being sampled and the generation of genetic information on themselves. They may also imagine exaggerated, often unrealistic, uses to which their sample may be used, for example used to clone another individual like themselves. In conducting psychiatry related pharmacogenetic research, as for all clinical research, it is therefore essential to have appropriate fully informed consent before any DNA is taken from an individual. All analyses conducted and the use to which the data is put must be governed by this consent under which the sample was collected. The challenge of ensuring informed consent must lie with the principal study investigator to answer any questions or concerns the patient may have and to ensure the patient clearly understands the nature of the research, future benefits for healthcare as well as potential risks.

Public opinion is likely to play an important role in the acceptability of genetic tests and also in directing both how and when pharmacogenetic knowledge becomes integrated into health care provision. Education as to what genetics and pharmacogenetics is, and importantly also what it is not, is essential to allay fears of the general population, and to enable individuals to understand both the benefits and risks. In this respect, pharmacogenetics is again no different to any other new scientific advance in medicine. Who is responsible for ensuring this occurs? There are many web-sites that provide educational material for individuals who are interested and proactive in searching for it, but for educating the broader patient population, the responsibility of education must be shared between those forming health-care policy and delivery. These include the physician and clinical researcher, and pharmaceutical companies.

Indeed, the prediction for the future is that a secure, patient-specific database will be established, once and only once, for each person. This would be available to authorized healthcare providers for the selection of optimal therapy for prevention of diseases and selection of the most appropriate medications. With more recent progress, previous technologies which were limited in their use due to high cost, are now much more affordable and hence more freely available to researchers. It is hoped that so called “patient gene identifications” or “genetic cards” will one day be affordable by the vast majority of patients, wherever they live, with the caveat that appropriate informed consent for use of this information is given. How long this might be is difficult to predict but potentially within the next 10 years.

6. PHARMACOGENETICS: MOVING FROM RESEARCH TO THE CLINIC

As this chapter has described, the field of pharmacogenetics is a rapidly evolving science, which heralds great promise for improving patient healthcare. However, to date, little of this new research is finding its way to the physician's office. So what is stopping these new scientific advances moving routinely into the clinic?

With a few exceptions, the current level of knowledge of efficacy pharmacogenetics in psychiatry is still in the exploratory stage, with as yet no robust results for genes predicting efficacy. However, some findings are replicating in independent sample sets and promising leads are developing, for example the serotonin gene promoter gene and SSRI response, dopamine D3 receptor gene and risk of anti-psychotic induced tardive dyskinesia. Many of these are presented within the following chapters of this book. Understanding of the impact of allelic variance on pharmacokinetic parameters can perhaps be considered to represent our current, most advanced state of pharmacogenetic knowledge. An example of this was recently seen in the approval of atomoxetine (Strattera; Eli Lilly), approved by the FDA in November 2002 for attention-defeceit/hyperreactive disorder. This drug is metabolised by CYP2D6 with the ratio (PM/EM) for an area under the curve (AUC) of ~10. Lilly conducted a post-facto stratification to determine the impact of genotype on adverse events risk. This analysis found the AUC to be increased in PM by 3% (EM, 6%; PM 9% for the main adverse events of insomnia and irritability). The FDA, however, stopped short of mandating a test for prescribing, since the benefit/risk for the drug was considered to be acceptable in the unselected patient group (Lesko & Woodstock 2002). However, genotype data was required to be available, along with other data, in the label to help guide physicians in their dose adjustments. In fact, information on CYP2D6 genotype is mentioned 7 times in label for atomoxetine. Tests for CYP2D6 alleles are widely available in CLIA laboratories. Furthermore, in the Unitied States, the FDA (Federal Drug Agency) as recently approved a genomic test to assess both CYP2D6 and CYP2C19, the AmplicChip CYP450 genomic test, developed by Roche Diagnostics). These two cytochrome P450 iso-enzymes play a role in the metabolism of ~25% of all prescription drugs and are of particular relevance for psychiatry where many of the drugs have a major contribution to their metabolism by CYP2D6, including risperdal, Haldo, Paxil, effexor and tricyclic antidepressants.

Also currently lacking is evidence from prospective clinical studies to more fully determine the impact of using genotype as part of prescribing algorithms on the benefit/risk outcome of drug response. Studies to date largely report on retrospective analyses. Such prospective studies are now underway, for example investigating the impact of CYP2C9 on warfarin treatment outcome. The outcome and reporting of these studies will provide critical data to help guide both regulatory authorities in their decisions on label requirements and physicians on how pharmacogenetic knowledge can best be used to more fully inform drug treatment and dosing decisions.

It is anticipated that regulatory authorities will increasingly expect to see this type of application of scientific advances, such as pharmacogenetics, being employed in the assessment of new medicines. The FDA Critical Path White paper, issued 14 March 2004 entitled "Innovation or stagnation? Challenge & opportunity on the critical path to new medical products" (http://fda.gov/oc/-iniatives/criticalpath/whitepaper.html), sets out this vision and expectation for clinical drug development. Regulatory authorities are under increasing public opinion scrutiny to understand safety risk, particularly in the wake of the recent voluntary withdrawal of the widely used pain-killer and anti-inflammatory inhibitor drug, Vioxx (rofecoxib) by Merck in September 2004 due to cardiovascular risk concerns. Pharmacogenetics is about assessing individuals and must be a core component of the strategy to explore safety signals of clinical concern observed in patients following drug administration. Regulatory authorities are likely to be a key driving force in bringing pharmacogenetic data to the clinic and its inclusion as part of treatment decisions. Pharmaceutical companies and clinical researchers alike need, therefore, to be applying genetic technologies to obtain robust data-sets, to build and share knowledge in this area and to apply this knowledge to enhance our understanding of the clinical safety and efficacy of psychiatry medicines.

In addition, patients themselves, their relatives and carers are predicted to play key roles in seeing pharmacogenetics, along with other advances in scientific knowledge, come to bear in guiding their drug treatment choices. Availability of information through the internet has resulted in patients and carers becoming increasingly more informed about the drug choices available to them and the latest research for their specific disorder. They seek to understand and gain more knowledge on the drugs they are given, particularly regarding potential safety risks and expect to be see these scientific advances being applied to their benefit and to improve their health care.

So in a time of increasing patient demand for information, increasing regulatory pressure for more detailed drug evaluation, coupled to economic constraints on health care budgets, pharmacogenetics holds great promise for playing a central role in guiding provision of health care and as standard information available to physicians. It is anticipated that the ever enquiring minds of physicians and clinical researchers, and their search to understand the complexities of psychiatric illness and patients' drug response will, over the next 3-5 years, begin to move the field of psychopharmacogenetics from the exploratory stage to one in which robust data-sets are available that can provide new knowledge to incorporate into prescribing algorithms. It will be this triad of physician, patient and regulator that are predicted to be key players in driving this new science into the clinic. Ultimately, it will be patients who will benefit, not only from the identification of new and novel psychiatry drugs, but also physicians will be equipped with greater ability to make more informed, evidence-based decisions to prescribe patients the most effective drug choices, providing therapeutic benefit, whilst minimising potential safety risks.

7. REFERENCES

Allison, D.B., 1997, Transmission-disequilibrium tests for quantitative traits. *Am J Hum Genet*. Mar; **60**(3): 676-90. Erratum in: *Am J Hum Genet*, Jun; **60**(6): 1571.

Ambrose, H., 2002, SNPs and pharmacogenomics, *Pharmacogenomics*. **3**: 583-586.

Angst, J., 1964, Antidepressiver effects und genetische faktoren, *Arzneimittelforschung*. **14**: 496-500.

Anguelova, M., Benkelfat, C., Turecki, G., 2003, A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: II. Suicidal behavior, *Mol Psychiatry*. Jul; **8**(7): 646-53.

Atalay, A., Crook, T., Ozturk M., Yulug, I.G., 2002, Identification of genes induced by BRCA1 in breast cancer cells. *Biochem Biophys Res Commun*. Dec 20; **299**(5): 839-46.

Balemans, W., Ebeling, M., Patel, N., Van Hul, E., Olson, P., Dioszegi, M., Lacza, C., Wuyts, W., Van Den Ende. J., Willems, P., Paes-Alves, AF., Hill, S., Bueno, M., Ramos, F.J, Tacconi, P., Dikkers, F.G., Stratakis, C., Lindpaintner, K., Vickery, B., Foernzler, D., Van Hul, W., 2001, Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST), *Hum Mol Genet*. Mar 1; **10**(5): 537-43.

Basile, V., Masellis, M., McIntyre, et al. 2001, Genetic dissection of atypical antipsychotic-induced weight gain; novel preliminary data on the pharmacogenetic puzzle, *J Clin Psychiatry*, **62**: 46-66.

Brown, V., Ossadtchi, A., Khan, A., Yee, S., Lacan, G., Melega, W., et al. 2002, Multiplex three-dimensional brain gene expression mapping in a mouse model of Parkinson's disease, *Genome Res*. **12**: 868-884.

Buchanan, A., Califano, A., Kahn, J., McPhaerson E., Robertson, J., Brody, B., 2002, Pharmacogenetics: ethical issues and policy options, *Kennedy Inst Ethics J*. **12**: 1-15.

Collburn, W., 2003 Biomarkers in drug discovery and development: from target identification through drug marketing, *J Clin Pharmacol*. **43**: 329-341.

Collins, F., 1997, Human genetics: where do we stand? *Origins*. Jan 2; **26**(28): 464-8.

Dahl, M-L., 2002, Cytochrome P450 phenotyping/genotyping in patients receiving antipsychotics, *Clin Pharmacokinet*. **41**: 453-470.

DeLisi, L., Dauphinais D., 1989, Neuroleptic responsivness in siblings concordant for schizophrenia, *Arch Gen Psychiatry*. **46**: 477.

Devlin, B., Roeder, K., 1999, Genomic control for association studies. *Biometrics*. **55**: 997-1004.

Domchek, S.M., Eisen, A., Calzone, K., Stopfer, J., Blackwood, A., Weber, B.L. Application of breast cancer risk prediction models in clinical practice, *J Clin Oncol*. Feb 15 ; **21**(4): 593-601.

Dubertret, C., Gorwood, P., Adès, J., Feingold, J., Schwartz, J.C., Sokoloff, P., 1998, A meta-analysis of DRD3 gene and schizophrenia: ethnic heterogeneity and significant association in caucasians, *American Journal of Medical Genetics (Neuropsychiatric Genetics*). **81**: 318-322.

Evans, W.E., Rellin, M.V., 1999, Pharmacogenomics: translating functional genomics into rational therapeutics, *Science*. **15**: 487- 491.

Franchini, L., Serretti, A., Gasperini, M., Smeraldi, E., 1998, Familial concordance of fluvoxamine response as a tool for differentiating mood disorder pedigrees, *J Psychiatry Research*. **32**: 255-259.

Frank, R., Hargreaves, R., 2003, Clinical biomarkers in drug discovery and development, *Nature*. **2**: 566-580.

Gmuender, H., 2002, Perspectives and challenges for DNA microarrays in drug discovery and development, *Biotechniques*. Jan; **32**(1):152-4, 156-158.

Goldstein, D.B., Tate, S.K., Sisodiya, S.M. Pharmacogenetics goes genomic, *Natrue Reviews*. **4**: 937-947.

Gorwood, P., 1999, Genetic association studies in behavioral neuroscience. In: *Handbook* of *Molecular Genetic Techniques for Brain and Behavior Research*, Cruzio W & Gerlai R., (Eds. Elsevier, Amsterdam) pp. 965.

Gorwood, P., 2003, Clinical assessment in psychiatric genetics. In *Psychiatric Genetics: Methods and Protocols*, edited by M Leboyer & F Bellivier. John M. Walker, (Humana Press Inc.), pp. 99-126.

Guzey, C., Spigset, O., 2002, Genotyping of drug targets: a method to predict adverse drug reactions? *Drug Saf.* **25**(8): 553-60.

Haab, B.B. 2001, Advances in protein microarray technology for protein expression and interaction profiling. *Curr Opin Drug Discov Devel*. Jan; **4**(1): 116-23.

Hakonarson, H., Bjorsdottir, U., Halapi, E., et al. 2002, A major susceptibility gene for asthma maps to chromosome 14q24, *Am J Hum Genet*. **71**: 183-491.

Hedrick, S.M., Nielsen, E.A., Kavaler, J., Cohen, D.I., Davis, M.M., 1984, Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins, *Nature*. Mar 8-14: **308**(5955): 153-8.

Hugot, J.P., Chamaillard, M., Zouali H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J.F., Sahbatou, M., Thomas, G., 2001, Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease, *Nature*. May 31: **411**(6837): 599-603.

Ingelman-Sundberg, M., 2004, Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future, *Trends in Pharmacological Sciences*: **25**: 193-200.

Irwin, M.H., Moffatt, R.J., Pinkert, C.A., 1996, Identification of transgenic mice by PCR analysis of saliva, *Nat Biotechnol*. Sep; **14**(9): 1146-8.

Kirchheiner, J., Nickchen, K., Bauer, M., Wong, M-L, Licino, J., Roots, I., Brockmoller, J. ,2004, Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response, *Mol Psy*. **9**: 442-473

Kwok, 2000.

Lander, E.S., 1996, The new genomics: global views of biology, *Science*. Oct 25; **274**(5287): 536-9.

Ledley, F., 1999, Can pharmacogenomics make a difference in drug development? *Nature Biotech*. **17**: 731.

Lennard, L., Van Loon, J.A., Weinshilboum RM. 1989, Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism, *Clin Pharmacol Ther*. Aug; **46**(2): 149-54.

Lentes, K., Hinney, A., Ziegler A., et al. 1997, Evaluation of a Cys23Ser mutation within the human 5-HT_{2C} receptor gene : no evidence for an association of the mutant allele with obesity or underweight in children, adolescents and young adults, *Life Sciences*. **61**: 9-16.

Lerer, B., Segman, R.H., Fangerau, H., Daly, A.K., Basile, V.S., Cavallaro, R., Aschauer, H.N., McCreadie, R.G., Ohlraun, S., Ferrier, N., Masellis, M., Verga, M., Scharfetter, J., Rietschel, M., Lovlie, R., Levy, U.H., Meltzer, H.Y., Kennedy, J.L., Steen, V.M., Macciardi, F., 2002, Pharmacogenetics of tardive dyskinesia: combined analysis of 780 patients supports association with dopamine D3 receptor gene Ser9Gly polymorphism, *Neuropsychopharmacology*. Jul; **27**(1): 105-19.

Lesko and Woodstock, 2002, Nature genetics review, **3**: 763-769.

Liang, P., Pardee., A.B., 1992, Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction, *Science*. Aug 14; **25**7(5072): 967-71.

Loewi, O., Navratil, E., 1926, Uber humorale Ubertragbarkeit der Herznervenwirkung. X Mitteilung. Uber das Schicksal des Vagusstoffs, *Pflugers Archiv fur die gesamte Physiologie*. **214**: 678-688.

Lum, A., Le Marchand, L., 1998, A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies, *Cancer Epidemiol Biomarkers Prev*. Aug; **7**(8): 719-24.

Macedo, A., Farre, M., Banos, J-E., 2003, Placebo effect and placebos: what are we talking about? Some conceptual and historical considerations, *Eur J Pharmacol.* **59**: 337-342.

Malhotra, A., Murphy, G.M., Kennedy, J.K.L., 2004, Pharmacogenetics of psychotropic drug response, *Am J Psychiatry*. **161**: 780-796.

Mata, I., Madoz, V., Arranz, M.J., Sham, P., Murray, R.M., 2001, Olanzapine : concordant response in monozygotic twins with schizophrenia, *Br J Psychiatry*. **178**: 86.

McLeod, H., Evans, W., 2001 Pharmacogenomics: Unlocking the human genome for better drug therapy, *Annu Rev Pharmacol Toxicol*. **41**: 101-121.

Melzer, D., Detmer, D., Zimmern, R., 2003, Pharmacogenetics and public policy: expert views in Europe and North American, *Pharmacogenomics*. **4**: 689-691.

Niu, T., Qin, Z.S., Xu, X., & Liu, J.S**.,** 2002, Bayesian haplotype inference for multiple linked single-nucleotide polymorphisms, *Am J Hum Genet.* **70**: 157-169.

Nuffield Council on Bioethics. Pharmacogenetics: Ethical Issues. 23 Sept. 2003. http://www.nuffieldbioethics.org/pharmacogenetics/latestnews.asp

Oestreicher, P., 2002, Company profile. Genaissance Pharmaceuticals, *Inc. Pharmacogenomics*. **3**: 273-276.

O'Reilly, R.L., Bogue, L., Singh, S.M., 1994 Pharmacogenetic response to antidepressants in a multi-case family with affective disorder, *Biol Psychiatry*. **36**: 467-471.

Pare, C.M.B., Mack, J.W., 1971, Differentiation of two genetically specific types of depression by the response to antidepressant drugs, *J Med Genet*. **8**: 306-309.

Pare, C.M.B., Rees, L., Sainsbury, M.J., 1962, Differentiation of two genetically specific types of depression by the response to antidepressants, *Lancet*. **2**: 1340-1343.

Patil, N., Berno, A., Hinds, D., et al. 2001, Blocks of limited haplotypes diversity revealed by high-resolution scanning of human chromosome 21, *Science*. **294**: 1719-1723.

Pettipher, R., Cardon, L., 2002, The application of genetics to the discovery of better medicine. *Pharmacogenomics*. **3**: 257-263.

Pritchard, J.K., Rosenberg, N.A., 1999, Use of unlinked genetic markers to detect population stratification in association studies, *Am J Hum Genet*. **65**: 220-228.

Qin, Z.S, Niu, T., & Liu, J.S., 2002, Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms, *Am J Hum Genet*. **71**: 1242-1247.

Reynolds, G., Zhang, Z., Zhang, X., 2002, Association of antipsychotic drug-induced weight gain with a 5-HT2C receptor gene polymorphism, *Lancet*. **359**: 2086-2087.

Risch, N., Merikangas, K., 1996, The future of genetic studies of complex human diseases, *Science*. Sep 13; **273**(5281): 1516-7.

Risch, N., Spiker, D., Lotspeich, L., Nouri, N., Hinds, D., Hallmayer, J., Kalaydjieva, L., McCague P., Dimiceli, S., Pitts, T., Nguyen, L., Yang, J., Harper, C., Thorpe, D., Vermeer, S., Young, H., Hebert, J., Lin, A., Ferguson, J., Chiotti, C., Wiese-Slater, S., Rogers, T., Salmon, B., Nicholas, P., Myers, R.M., et al. 1999, A genomic screen of autism: evidence for a multilocus etiology, *Am J Hum Genet*. Aug; **65**(2): 493-507.

Roses, A.D. 2004, Pharmacogenetics and drug development: the path to safer and more effective drugs, *Nature Reviews*. **5**: 643-654.

Rush, A.J., Trivedi. M., Fava, M., 2003, STAR*D treatment trial for depression, *Am J Psychiatry*. **2**: 160.

Sachidanandam, R., Weissman, D., Schmidt, S.C., Kakol, J.M., Stein, L.D., Marth, G., Sherry, S., Mullikin, J.C., Mortimore, B.J., Willey, D.L., Hunt, S.E., Cole, C.G., Coggill, P.C., Rice, C.M., Ning, Z., Rogers, J., Bentley, D.R., Kwok, P.Y., Mardis, E.R., Yeh, R.T., Schultz, B., Cook, L., Davenport, R., Dante, M., Fulton, L., Hillier, L., Waterston, R.H., McPherson, J.D., Gilman, B., Schaffner, S., Van Etten, W.J., Reich, D., Higgins, J., Daly, M.J., Blumenstiel, B., Baldwin, J., Stange-Thomann, N., Zody, M.C., Linton, L., Lander, E.S., Altshuler, D., 2001, International SNP Map Working Group. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms, *Nature*. Feb 15; **409**(6822): 928-33.

Schena, M., Heller, R.A., Theriault, T.P., Konrad, K., Lachenmeier, E., Davis, R.W., 1998, Microarrays: biotechnology's discovery platform for functional genomics, Trends *Biotechnol*. Jul; **16**(7): 301-6.

Sham, P.C., Rijsdijk, F.V., Knight, J., Makoff, A., North, B., Curtis, D., 2004, Haplotype association analysis of discrete and continuous traits using mixture of regression models, *Behav Genet*. Mar; **34**(2): 207-14.

Shedden, K., Townsend, L.B., Drach, J.C., Rosania, G.R., 2003, A rational approach to personalized anticancer therapy: hemoinformatic analysis reveals mechanistic gene-drug associations, *Pharm Res*. Jun; **20**(6): 843-7.

Singh, R.P., Smith, D.J., 2003, Genome scale mapping of brain gene expression, *Biol Psychiatry*. **53**: 1069-1074.

Smeraldi, E., Zanardi, R., Benedetti, F., Bella, D.D., Perez, J., Catalano, M., 1998, Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine, *Mol Psychiatry*. **3**: 508-511.

Smits, K.M., Smits, L.J.M., Schouten, J.S.A.G., Stelma, F.F., Nelemans, P., Prins, M.H., 2004, Influence of SERTPR and Stin in the serotonin transporter gene on the effect of selective serotonin reuptake inhibitors in depression: a systematic review, *Mol Psy*. 433-441

Spielman, R.S., Ewens, W.J. 1998, A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test, *Am J Hum Genet*. **62**: 450-458.

Stephens, M., Donnelly, P., 2003, A comparison of bayesian methods for haplotype reconstruction. *Am J Hum Genet.* Nov; **73**(5): 1162-9.

The National Institute of Mental Health Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) Project, 2003: schizophrenia trial design and protocol development, *Schizophrenia Bulletin*. **29**: 15-31.

Thomson, D.M., Brown, N.N., Clague, A.E., 1992, Routine use of hair root or buccal swab specimens for PCR analysis: advantages over using blood, *Clin Chim Acta*. May 15; **207**(3): 169-74.

Tsuang, M., Faraone, S., and Lyons, M., 1993, Identification of the phenotype in psychiatric genetics, *Eur Arch Psychiatry Clin NeuroSci*. **243**: 131-142.

Velculescu, V.E., Zhang, L., Vogelstein, B., Kinzler, K.W., 1995, Serial analysis of gene expression, *Science*. Oct 20; **270**(5235): 484-7.

Vojvoda, D., Grimmell, K., Sernyak, M., Mazure, C.M., 1996, Monozygotic twins concordant for response to clozapine, *Lancet*, **347**: 61.

Wang, X., Feuerstein, G., 2000, Suppression subtractive hybridisation: application in the discovery of novel pharmacological targets, *Pharmacogenomics*. **1**: 101-108.

Woolf, B., 1955, On estimating the relation between blood group and disease, *Ann Hum Genet*. **19**: 251-253.

Zhang, L., Cui, X., Schmitt, K., Hubert, R., Navidi, W., Arnheim, N., 1992, Whole genome amplification from a single cell: implications for genetic analysis, Proc Natl Acad Sci *U S A*. Jul 1; **89**(13): 5847-51.

Zwick, M., Cutler, D., Lin, S., et al. 2002, An empirical estimates of the number of SNPs required for a whole genome association study, *Am J Hum Genet*. **71**: 278.

2. GENETICS OF ANXIETY AND RELATED DISORDERS:

Implications for Pharmacogenetics

Klaus-Peter Lesch*

1. INTRODUCTION

Treatment response to antidepressant, anxiolytic, and antipsychotic drugs is influenced by genetic factors and depends on the structure or functional expression of gene products. While treatment response is believed to involve both genetic and environmental factors, the contribution of an individual gene to drug response is likely to be modest. However, interactions between different genes may result in a dramatic modification of drug response (additive, nonadditive or multiplicative gene effects). The challenge faced by research into the genetic basis of psychopharmacological drug responses is to identify genes of relative small effect against a background of substantial genetic and environmental variation.

Emotionality, cognition, and motor functions as well as circadian and neuroendocrine rhythms including food intake, sleep and reproductive activity are both modulated by the brainstem raphe serotonin (5HT) system, and distinctively altered in anxiety spectrum and mood disorders. While 5HT controls a highly complex system of neural communication mediated by multiple pre- and postsynaptic 5HT receptor subtypes including the serotonin 1A receptor ($5HT_{1A}$), high-affinity 5HT transport into the presynaptic neuron and thus maintenance of the 5HT pool available for subsequent release is

* K.P. Lesch, M.D., Molecular and Clinical Psychobiology, Department of Psychiatry and Psychotherapy, University of Wuerzburg, Fuechsleinstr. 15, 97080 Wuerzburg, Germany, E-mail: kplesch@mail.uni-wuerzburg.de

mediated by a unique protein (the 5HT transporter, 5HTT, SERT, SLC6A4), which is regarded as the initial sites of action of antidepressant/anxiolytics drugs and several potentially neurotoxic compounds, such as MDMA ("ecstasy"). Serotonergic antidepressants/anxiolytics, such as prototypical tricyclic clomipramine, and the selective 5HT uptake inhibitors (SSRIs), such as fluvoxamine, paroxetine, citalopram, and sertraline, occupy several pharmacologically distinct sites overlapping at least partially the substrate binding site. These agents are widely used in the treatment of depression, anxiety disorders, and impulse control disorders, as well as substance abuse including alcoholism.

Although several reviews have extensively discussed the general role of pharmacogenetics in individualising treatment of mood disorders with psychoactive compounds, pharmacogenetics implications of the treatment of anxiety spectrum disorders have not been addressed previously (Alda 2001; Catalano 1999; Lerer and Macciardi 2002; Mancama and Kerwin 2003; Serretti et al. 2002; Veenstra-VanderWeele et al. 2000). Therefore, the present chapter covers fundamental aspects of the genetics of anxiety-related traits, emotional responses, and anxiety disorders. The current status of conceptual issues in the search for candidate genes of human fearfulness and anxiety will also be considered. Finally, the evidence for a relationship between genetic variability of two serotonergic genes, 5HTT and $5HT_{1A}$, and response to antidepressant, anxiolytic, and antibipolar drugs as well as non-pharmacological treatments will be discussed.

2. GENETICS OF ANXIETY AND RELATED DISORDERS

2.1. Anxiety-related traits in mood disorders

A large body of evidence from family, twin, and adoptee studies has been accumulated that a complex genetic component is involved in anxiety-related traits and in the liability to anxiety spectrum disorders. While genetic research has typically focused either on normal personality characteristics or on psychiatric disorders, with few investigations evaluating the genetic and environmental relationship between the two, it is of critical importance to answer the questions whether a certain quantitative trait etiopathogenetically influences the disorder or whether the trait is a syndromal dimension of the disorder. Nevertheless, some studies have implicated anxiety-related personality traits, such as neuroticism or negative emotionality, in the comorbidity of mood disorders (Kendler et al. 1993a; Livesley et al. 1998). Separation of anxiety spectrum disorders from mood disorders including depression and bipolar disorder in current consensual diagnostic systems remarkably enhanced interest in the link between temperament, personality, and psychiatric disorders as well as the impact of this interrelationship on the heterogeneity within diagnostic entities, prediction of long-term course, and treatment response (Mulder et al. 1994). Based on multivariate genetic analyses of co-morbidity, generalized anxiety disorder and major depression have common genetic origins and the

phenotypic differences between anxiety and depression are dependent upon the environment (Kendler 1996; Kendler et al. 1992b). Moreover, indexed by the personality scale of neuroticism, general vulnerability overlaps genetically to a substantial extent with both anxiety and depression (Kendler et al. 1993a; b). These results predicted that when a quantitative trait locus (QTL), such as the 5HTT gene, is found for neuroticism, the same QTL should be associated with symptoms of anxiety and depression. Anxiety and mood disorders are therefore likely to represent the extreme end of variation in negative emotionality (Eley and Plomin 1997; Eley and Stevenson 1999). The genetic factor contributing to the extreme ends of dimensions of variation commonly recognized as an disorder may be quantitatively, not qualitatively, different from the rest of the distribution. This vista has important implications for identifying genes for complex traits related to a distinct disorders. Association of allelic variation of 5HTT function and mood disorders including unipolar depression and bipolar disorder has initially been reported by Collier and colleagues (1996) and subsequently by several other investigators, although some studies did not replicate these results (Lesch and Mössner 1998).

2.2. Generalized anxiety disorder

Generalized anxiety disorder (GAD) is defined by excessive and uncontrollable worry about a number of life events or activities for least 6 month, accompanied by at least 3 of 6 associated symptoms of negative affect or tension, such as restlessness, fatigability, concentration difficulties, irritability, muscle tension, and sleep disturbance. Relative to other anxiety and mood disorders, GAD is more likely to show a gradual onset and/or life-long history of symptoms. While early ages of onset are common, the syndrome itself may emerge only later in life and a considerable number of patients with GAD report an onset in adulthood that is usually in response to psychosocial and emotional stress. Research has consistently shown that GAD is associated with high comorbidity rates for other psychiatric disorders, including panic disorder, major depression, dysthymia, social phobia, and specific phobia (Kendler et al. 1992a; Kendler et al. 1995a; Roy et al. 1995; Skre et al. 1994; Weissman 1993). Based on inadequate diagnostic reliability and high comorbidity the discriminant validity of GAD has been controversial. Studies have begun to address the structural relationship of the dimensions comprising various anxiety disorders and there is evidence that GAD-associated negative affect and worry are dispositional traits common to both anxiety and mood disorders. GAD may therefore be conceptionalized as a trait dimension predisposing to other disorders and etiological models of GAD therefore integrate both psychosocial and biological factors. Twin and family-based studies indicate a clear genetic influence in GAD with a heritability of approximately 30%. GAD-associated genetic factors are completely shared with depression, while environmental determinants seem to be distinct (Kendler 1996; Kendler et al. 1992b). This notion is consistent with recent models of emotional disorders which view anxiety and mood disorders as sharing common vulnerabilities but differing on dimensions including, for instance, focus of attention or psychosocial liability.

2.3. Phobias

Phobias occur in several forms and specific phobias are linked to a particular object or situation. Social phobia as an example is an intense fear of becoming humiliated in a social setting or being painfully embarrassed in front of other people. Lifetime prevalence of social phobia was 9.5% in females and 4.9% in males, with about one-third being classified as individuals with generalized social phobia. Little is known about the psychobiology and heritability of specific phobias, although twin studies of common phobias and fears in unselected samples point toward a genetic influence (Stein 1998). Assessment of lifetime history of five unreasonable fears and phobias, including agoraphobia and social, situational, animal, and blood-injury phobia, in female twins resulted in heritabilities between 46% and 67% (Kendler et al. 1999). Correcting for unreliability of ascertainment, the liability to fears and their associated phobias is moderately heritable. Individual-specific environmental experiences play an important role in the development of phobias, while familial and other environmental factors appear to be of little etiological significance. Only few population-based association and linkage-disequilibrium studies have been conducted in phobias. Linkage-disequilibrium studies in a population capitalize on the likelihood that the susceptibility genes for a particular disorder probably came from one or a few founding members. Stein et al. (1998) excluded linkage between generalized social phobia and the genes of 5HTT and $5HT_{2A}$ receptor, although modifier effects could not be ruled out. Interestingly, Furmark and coworkers (2004) reported a relationship between allelic variation of 5HTT function, amygdala excitability, and symptom severity in patients with social phobia. Individuals with one or two copies of the low-activity, short allele of the 5HTT promoter polymorphism exhibited significantly increased scores of anxiety-related traits, state anxiety, and enhanced right amygdala responsivity to anxiety provocation, compared with subjects homozygous for the high-actitivity variant.

2.4. Posttraumatic stress disorder

Posttraumatic stress disorder posttraumatic stress disorder (PTSD) is a common, frequently chronic condition that occurs following life-threatening or horrific traumatic events. The lifetime incidence of PTSD in western societies is 10-15% and approximately 50% of individuals who have had an episode of PTSD develop chronic symptoms. Family and twin studies suggest a substantial genetic contribution to the pathogenesis of PTSD (Radant et al. 2001). However, PTSD is unique among psychiatric disorders since there is an explicit requirement for the presence of a precipitating environmental event. While some types of trauma exposure (e.g. natural disasters, assaults) are not influenced by individual characteristics, other types of trauma exposure may be associated with certain personality characteristics (e.g. engaging in high-risk activities) which are themselves under genetic influence.

Unfortunately, a quite broadly defined phenotype, specific requirement for an environmental exposure and high frequency of comorbid psychiatric illness

as well as genetic heterogeneity, incomplete penetrance, pleiotropy, and interaction multiple genes complicate genetic studies of PTSD and its treatment. One strategy to get around these problems is to perform genetic analysis of traits associated with PTSD, rather than PTSD itself, an approach that has yielded promising results for other diseases with complex genetics. Hypothalamic-pituitary-adrenal axis dysfunction, physiologic markers of increased arousal, and increased acoustic startle response are PTSD-associated traits accessible to genetic analysis. However, the power of these traits to distinguish PTSD from non-PTSD patients need to be determined before they can be employed in genetic studies. Only a few association studies have been reported. Comings et al. (1996) reported that the A1 allele of the dopamine D2 receptor gene (DRD2) was significantly more common among PTSD patients as compared to veteran controls. Differences in rates of substance abuse, which appear also be associated with the A1 allele may explain failure to replicate this finding (Gelernter et al. 1999). Similarily based on the assumption of an abnormal dopaminergic function in PTSD, Segman and associates (2002) examined the association of the dopamine transporter (DAT, SLC6A3) variable number tandem repeat (VNTR) polymorphism with PTSD. The study evaluated 102 chronic PTSD patients versus carefully-documented trauma survivors who did not develop PTSD. Significant excess of 9 repeat allele was observed among PTSD patients (43% *vs* 30.5% in TS controls) further supporting the notion that genetically influenced changes in dopaminergic reactivity may contribute to the occurrence of PTSD among trauma survivors. Finally, in a sample of male PTSD patients, dinucleotide repeat polymorphisms of the $GABA_A$ receptor β3 subunit gene (GABRB3) were associated with higher scores of somatic symptoms, anxiety, insomnia, social dysfunction, and depression (Feusner et al. 2001).

2.5. Panic disorder

Panic disorder (PD) is strikingly different from other types of anxiety in that panic attacks are sudden, appear to be unprovoked, and are often disabling. They may include intense fear, fear of dying or a sense that something unimaginably horrible is about to occur and one is powerless to prevent it or discomfort accompanied by several physiological symptoms, such as choking sensations, sweating, dizziness, fear of losing control or perceptual distortions. A panic attack typically lasts several minutes to hours and may be one of the most distressing experiences. Panic attacks are followed by persistent concerns about having additional attacks, worry about the implications of the attack or its consequences, and significant changes in behavior related to the attacks.

Following one or repeated panic attacks, patients may develop an irrational fear, or phobia, about these situations and begin to avoid them. At this stage, PD is complicated by agoraphobia. PD, agoraphobia, and depression display significant comorbidity. Familial patterns of aggregation also suggest that PD, GAD, depression, and agoraphobia may co-occur but it is still a matter of considerable debate whether they have related or different genetic etiologies (Maier et al. 1995; Weissman 1993; Woodman 1993). A higher correlation for

monozygotic twins (MZ) than for dizygotic twins (DZ) indicates genetic influences. Logistic regression analysis of PD family data yielded evidence of vertical transmission and the effect of sibship environment, whereas segregation analysis of family data resulted in moderate evidence of an incompletely penetrant dominant or recessive major gene (Bonney 1986; Hopper et al. 1990). Model fitting in a large twin sample found evidence that the familial transmission of panic-phobia was influenced by sex-dependent additive genetic effects, dominant genetic factors, individual-specific environmental factors, and a shared environmental effect for women only (16% vs 1%), while higher heritability in males (38%) compared to females (16%) was predicted (Kendler et al. 1995b).

Several genome-wide linkage scans for PD liability genes have been published. Although none of the findings based on lod scores or the proportion of allele sharing reached a level of statistical confidence according to stringent criteria, a region suggestive of a susceptibility locus for PD on chromosome 7p15 was independently identified in both studies. Crowe and associates (2001) detected the highest lod score of 2.23 at the D7S2846 locus, located at 57.8 cM on chromosome 7, in a region that lies within 15 cM from the D7S435 locus reported by Knowles et al. (1998). Linkage to numerous other markers over a substantial proportion of the human genome had previously been excluded under various parametric models in different sets of pedigrees (Crowe 1990; Crowe et al. 1990; Kato et al. 1996; Mutchler et al. 1990; Wang et al. 1992). Some of the conflicting results of linkage analyses in PD may be ascribed to methodological differences in family ascertainment, phenotype definition, diagnostic assessment, and approaches data analysis. Even more likely, they may represent true etiologic differences due to locus heterogeneity. Susceptibility to PD may thus be influenced either by an incompletely penetrant major gene in some families or by multiple genes of weak and varying effect in others.

Since evidence for a genetic liability in PD is persuasive, a small number of putative vulnerability genes have been assessed in association studies. A role of monoamine neurotransmitters in the etiology of PD has been suggested by the observations that increased serotonergic neurotransmission provokes anxiety even up to the level of panic attacks in PD patients. This corresponds well to the general observation that in rodent models increased serotonergic function is anxiogenic while a decrease is anxiolytic. Although it may be hypothesized that enhanced serotonergic neurotransmission in PD is due to decreased 5HT uptake, no association with allelic variation of 5HTT expression and PD was detected in different populations (Deckert et al. 1997; Hamilton et al. 1999; Ishiguro et al. 1997; Matsushita et al. 1997). These negative findings are compatible with the assumption that additional or alternative cellular pathways and neural circuits are involved in panic anxiety. Monoamine oxidase A (MAOA), an enzyme involved in the degradation of 5HT, dopamine, and norepinephrine and thus positioned at the crossroads of several monoaminergic system, is another plausible candidate gene. A 30-bp repeat polymorphism was identified in the promoter region of the MAOA gene that differentially modulates gene transcription (Deckert et al. 1999). Variation in the number of repeats of this

MAOA gene-linked polymorphic region (MAOALPR) displayed allele-dependent transcriptional efficiency. The effectiveness of the 3-repeat allele was 2-fold lower than those with longer repeats. Assessment of the MAOALPR for association with PD in two independent samples showed that the longer alleles were significantly more frequent in female patients than in females of the corresponding control populations (Deckert et al. 1999). Together with the observation that inhibition of MAOA is clinically effective in the treatment of panic disorder, particularly in women, these findings suggest that altered MAOA activity may be a gender-specific risk factor for PD. Recently, a functional single nucleotide polymorphism (SNP) in the transcriptional control region of $5HT_{1A}$ (HTR1A-1019) was reported which displays differential binding efficiency of a repressors/enhancer-type transcriptional regulator (Lemonde et al. 2003). The G variant of this polymorphism was shown to be associated with anxiety- and depression-related personality traits as well as with the agoraphobic subtype of panic disorder (Rothe et al. 2004; Strobel et al. 2003). Finally, no consistently significant associations between PD and alleles of the $GABA_A$, dopamine D2 and D4, cholecystokinin B as well as the adenosine A1 and A2a receptor genes have been detected (Crawford et al. 1995; Crowe et al. 1997; Deckert et al. 1998; Kato et al. 1996; Kennedy et al. 1999; Wang et al. 1998). Population-based studies also found no evidence for an association between PD and the gene for the DAT (Hamilton et al. 2000).

3. GENETIC VARIABILITY OF SEROTONIN TRANSPORTER FUNCTION

In humans, transcriptional activity of the 5HTT gene is modulated by a polymorphic repetitive element, 5HTT gene-linked polymorphic region (5HTTLPR) located upstream of the transcription start site. Comparison of different mammalian species confirmed the presence of the 5HTTLPR in simian primates but not in prosimian primates and other mammals (Lesch et al. 1997). The majority of alleles are composed of either 14 or 16-repeat units (short and long allele, respectively), while alleles with 15, 18-20, or 22 repeat copies, and most variants with single-base insertions/deletions or substitutions within individual repeats are rare. The distinctive structure of the 5HTTLPR gives rise to the formation of DNA secondary structure that has the potential to regulate the transcriptional activity of the associated 5HTT gene promoter. When fused to a luciferase reporter gene and transfected into human 5HTT expressing cell lines, the short (s) and long (l) 5HTTLPR variants differentially modulate transcriptional activity of the 5HTT gene and ultimately uptake function of the 5HTT protein (Lesch et al. 1996).

A growing body of evidence suggests a role of 5HTTLPR-dependent allelic variation in 5HTT expression and function in anxiety-, depression-, and aggression-related personality traits and syndromal dimensions of various psychiatric disorders (for review see Lesch 2003). The influence of genetically driven variability of 5HTT function on individual phenotypic differences in personality and behavior was explored in several independent population/family

genetic studies. The findings suggest that the 5HTTLPR influences traits of negative emotionality related to anxiety, depression, and stress responsiveness as well as aggressiveness. Nevertheless, several efforts to detect associations between the 5HTTLPR and personality traits have been complicated by the use of small sample sizes, heterogeneous subject populations, ethnic and sociocultural characteristics, and differing methods of personality assessment. In addition to the exploration of the impact of allelic variation in 5HTT expression on anxiety, depression, and aggression-related personality traits, a role of the low-activity s allele has been suggested in a variety of neuropsychiatric disorders (for review see Lesch 2003; Lesch and Mössner 1998).

Evidence for a modulatory effect of the 5HTTLPR on prefrontal cortex and amygdala activity suggests that genotype-phenotype correlations may be accessible to analysis of event-related potentials (ERP) or functional magnetic resonance imaging (fMRI) of the brain. In two subsequent studies, Fallgatter and associates (1999; 2004) reported an association between 5HTTLPR genotype and prefrontal cortex-limbic excitability detected with two different tasks of cognitive response control, Go-NoGo and error-processing task). Individuals with one or two s allele of the 5HTTPLR showed higher prefrontal brain activity as compared to subjects homozygous for the l variant, thus indicating that the 5HTTLPR s variant is linked to enhanced responsiveness of the prefrontal cortex, particularly the anterior cingulate cortex (ACC). These findings strongly suggest a relationship between cognitive brain function and allelic variation of 5HTT function. Hariri and coworkers (2002) reported that individuals with at least one copy of the 5HTTLPR s variant exhibit greater amygdala responsivity, as assessed by fMRI, in response to fearful stimuli compared with individuals homozygous for the high-activity l allele. This result confirms that genetically driven variation of serotonergic function contributes to the response of brain regions underlying human emotional behavior and indicate that differential excitability of the amygdala to emotional stimuli may contribute to increased fear and anxiety-related responses. The considerable effect sizes of both ERP and fMRI measures as well as their unique ability to assay information processing at the level of brain function during cognitive tasks in relatively small samples of individual and in the absence of noticeable behavioral differences, offers a powerful approach to functional genomics of the brain. The consistent results derived from these endophenotypic paradigms not only underscore the power of direct assessment of brain physiology in exploring the functional impact of genomic variation but also support the notion of a critical link between functional gene variation and differences in information processing within distinct neurocircuits that have been linked to the manifestation of distinct behavioral traits and behavioral disorders (Fallgatter et al. 2004).

4. SEROTONIN TRANSPORTER AND ANTIDEPRESSANT/ ANXIOLYTIC RESPONSE

Based on theoretical consideration a complex interaction between genotype, behavioral or syndromal dimensions, and drug response has been predicted

(Catalano 1999). A given genetic predisposition, such as allelic variation in 5HTT function, may lead to both increased susceptibility to anxious or depressive features and less favorable antidepressant responses in patients affected by mood disorders. Impaired 5HTT function confers, if any, only a very modest susceptibility to depressed states, because adaptive mechanisms are likely to compensate for the deficiency, while more robust alterations of 5HT turnover observed during antidepressant treatment revealed robust effects of allelic variation of 5HTT function, 7-20% of variance of treatment effect) that lead to variable SSRI efficacy. Pharmacogenetic and other treatment response studies of the serotonin transporter gene are summarized in the following sections and in Table 1.

Smeraldi and associates (1998) investigated whether the 5HTTLPR genotype is related to the antidepressant response to the SSRI fluvoxamine and/or augmentation with the $5HT_{1A}$ receptor antagonist pindolol in patients with major depression with psychotic features who had been randomly assigned to treatment with a fixed dose of fluvoxamine and either placebo or pindolol for 6 weeks. Both homozygotes for the l variant (l/l genotype) of the 5HTTLPR and heterozygotes (l/s) showed a better response to fluvoxamine than patients homozygous for the s variant (s/s). Interestingly, in the group treated with fluvoxamine plus pindolol all the genotypes acted like l/l treated with fluvoxamine alone and the genetic effect could not be detected. Thus, SSRI efficacy in delusional depression seems to be related, in part, to genetic variation of 5HTT function that in the subjects with s/s genotype pindolol may have compensated for the altered transcriptional activity of the 5HTT gene.

The effect of the 5HTTLPR genotype on antidepressant response was replicated in an independent sample of depressed patients treated with the SSRI paroxetine (Zanardi et al. 2000). In a study of elderly patients treated for depression, Pollock and associates (2000) found that patients with a l/l genotype displayed a faster response to paroxetine. The association appeared specific to paroxetine, as there was no genotypic difference with respect to the antidepressant response to nortriptyline, a predominantly noradrenergic drug. The same group also demonstrated an influence of the 5HTTLPR on platelet activation in geriatric depression (Whyte et al. 2001). More recently, Rausch and coworkers (2002) reported an association between the 5HTTLPR l/l genotype and improved response to fluoxetine and a placebo-controlled study confirmed a significant increase in response to the SSRI sertraline in elderly depressed patients homozygous for the l allele of 5HTTLPR compared with patients carrying one or two copies of the s variant. No significant difference was observed in the placebo group (Durham et al. 2003). Arias et al. (2003) confirmed the results of previous studies demonstrating a similar association between 5HTTLPR genotype and an therapeutic effect of the most selective SSRI citalopram. In patients with depression, the remission in the course of treatment was less likely in subjects with the s/s genotype.

Table 1. Pharmacogenetic and other treatment response studies of the serotonin transporter gene

Antidepressants/ Anxiolytics	Sample	Phenotype	Results
Smeraldi et al.1998	Major depression (n=56)	Response to fluvoxamine	Better response in l/l and l/s subjects compared to s/s
	Major depression (n=46)	Response to fluvoxamine + pindolol	No difference in response to combination
Zanardi et al. 2000	Major depression (n=58)	Response to paroxetine	Better response in l/l and l/s subjects compared to s/s
Pollock et al. 2000	Major depression (n=95), elderly patients	Response to paroxetine or nortriptyline	More rapid response to paroxetine in l/l subjects, no effect on nortriptyline response
Kim et al. 2000	Major depression (n=120), controls (n=252), Koreans	Response to paroxetine or fluoxetine	Better response in s/s subjects, intron 2 VNTR also associated with response)
Yu et al. 2002	Major depression (n=121), Chinese	Response to fluoxetine	Better response in l/l subjects, l/l genotype less common
Yoshida et al. 2002	Major depression (n=66), Japanese	Response to fluvoxamine	Better response in s/s subjects, l/l genotype rare
Durham et al. 2003	Major depression (n=206), elderly patients, placebo-controlled	Response time to sertraline	Shortened response delay in l/l subjects at week 1 and 2 compared to l/s and s/s; no difference in placebo group
Perlis et al. 2003	Major depression (n=36)	Adverse effects of fluoxetine	Higher rate of insomnia and agitation in s/s subjects compared to l/s and l/l
Joyce et al. 2003	Major depression (n=169)	Response to fluoxetine or nortriptyline	Better response to both fluoxetine and nortriptyline in l/l and l/s subjects older than 25 years compared to s/s
Arias et al. 2003	Major depression (n=131)	Remission during citalopram	Higher rate of s/s in non-remission group compared to remission group

These findings may, however, apply primarily to European populations. Treatment of Korean and Japanese patient samples with fluoxetine, paroxetine, and fluvoxamine showed contradictory results as a better response was found in patients with a s/s genotype (Kim et al. 2000; Yoshida et al. 2002), whereas Yu and coworkers (2002) reported a superior response to fluoxetine in l/l in depressed patients from China. Possible explanations for these discrepancies are manifold. For example, allele frequencies of the 5HTTLPR l variant between European populations and both the Korean and the Japanese population differ significantly (54% vs. 25% and 26%). A low frequency of the l allele in both of these studies resulted in a small number of homozygous l/l patients. Furthermore, ethnic as well as environmental differences could also influence the genotypic response to SSRIs.

Table 1 (continued): Pharmacogenetic and other treatment response studies of the serotonin transporter gene.

Sleep deprivation/ light therapy	Sample	Phenotype	Results
Benedetti et al. 1999	Bipolar disorder, depressed (n=68)	Antidepressant effect of sleep deprivation	Better effect in l/l subjects compared to l/s and s/s
Benedetti et al. 2003	Bipolar disorder, depressed (n=22)	Antidepressant effect of light therapy combined with sleep deprivation	Effect more marked in l/l subjects compared to l/s and s/s
Lithium			
Del Zompo et al. 1999	Bipolar disorder (n=67), controls (n=103)	Response to lithium, 49 responders, (18 nonresponders)	Higher frequency of l allele in nonresponders compared to controls
Serretti et al. 2001	Bipolar disorder (n=167), unipolar depression (n=34)	Effect of prophylactic lithium treatment (episode frequency)	Fewer episodes before lithium treatment, but less reduction of episodes in s/s patients
Switch, rapid cycling			
Mundo et al. 2001	Bipolar disorder, antidepressant-induced mania, IM+(n=27), bipolar disorder, IM-(n=29)	Presence/absence of mania (IM+/IM-) during antidepressant treatment	Increased frequency of s allele in IM+ compared to IM-; no effect of intron 2 VNTR
Rousseva et al. 2003	Bipolar disorder (n=305)	Lifetime history of antidepressant-induced mania/rapid cycling	Increased frequency of s allele in subjects with rapid cycling but not with antidepressant-induced mania
Antipsychotics			
Arranz et al. 2000	Schizophrenic disorder (n=200)	Response to clozapine	Higher rate of s/s in non-response group; five other primarily serotonergic genotypes contribute to the prediction of response
5HT Challenge			
Whale et al. 2000	Healthy females (n=7, l/l genotype), Healthy females, (n=7, s/s genotype)	Clomipramine-induced prolactin response	Higher response in l/l subjects
Reist et al. 2001	Male alcohol dependence (n=14), healthy (n=13)	Fenfluramine-induced prolactin response	Higher response in l/l subjects

Furthermore, an interaction between 5HTTLPR genotype and therapeutic efficacy of the antimanic/antibipolar agent lithium, which is assumed to act via serotonergic mechanisms, was demonstrated. Del Zompo and coworkers (1999)

reported a trend towards higher frequency of the l allele among lithium nonresponders compared to controls. Serretti et al. (2001) found an opposite result: the group homozygous for the s variant showed poorer response. However, this association appears to have been mainly due to a difference in pre-lithium frequency of episodes; there was no difference in episode frequency during lithium treatment.

Benedetti and coworkers (1998) reported that drug-free patients with bipolar depression who were homozygous for the l variant of 5HTTLPR show superior mood improvement after total sleep deprivation (TSD) than those with the s/l and s/s genotype. However, relapse following restoration of night sleep led to similar depression ratings in all genotype groups at the end of treatment. In a follow-up study the same group demonstrated that short-term relapse following acute response to TSD may be prevented by a combination of TSD with light therapy given during the TSD night and in the morning after recovery sleep and that the response is influenced by the 5HTTLPR with more marked effects in homozygotes for the l variant (Benedetti et al. 2003). These findings suggest that 5HTT function is critical for the antidepressant mechanism of action of sleep deprivation and light therapy, that presence of the l allele is associated with an increased reactivity of serotonergic system to a variety of stimuli, and support the notion that 5HTTLPR genotyping may represent a useful pharmacogenetic tool to individualize treatment of depression.

Finally, Mundo et al. (2001) found that 63% bipolar patients with a history of antidepressant-induced mania had the s allele compared to 29% in bipolar subjects who had been exposed to antidepressants, but did not develop mania. A role of the 5HTTLPR in rapid cycling, though not in antidepressant-induced mania, was supported in an independent cohort of patients with bipolar affective disorder, further supporting the notion that the low-activity s allele of the 5HTTLPR contributes to a pattern of affective instability (Rousseva et al. 2003). The role of allelic variation of 5HTT function in the development of adverse effects of SSRI treatment in patients with major depression was investigated by Perlis and coworkers (2003). A higher rate of fluoxetine-induced insomnia and agitation was found in s/s subjects compared to patients carrying the l/s and l/l genotypes. Although no antidepressant-induced mania was observed in this patient sample, these results seem plausible in the context of the findings that sleep disruption is a risk factor for the switch from depression to mania.

In addition to treatment response studies, 5HT system responsivity following pharmacologic challenge has been investigated with respect to genetic variability of the 5HTT function. Individuals with the l/l genotype exhibited greater prolactin response to either clomipramine or fenfluramine (Reist et al. 2001; Whale et al. 2000). Since the 5HTTLPR is likely to influence 5HT concentrations at all synapses, allelic variation in 5HTT function may affect the response to almost any agent affecting the 5HT system. This assumption would also explain an association between the s/s genotype and poor response to clozapine, an anxiolytically active antipsychotic displaying also a serotonergic mechanism of action (Arranz et al. 2000).

5. SEROTONIN 1A RECEPTOR AND ANTIDEPRESSANT/ ANXIOLYTIC RESPONSE

While multiple lines of evidence implicate the serotonin 1A receptor ($5HT_{1A}$) in the pathophysiology of anxiety and depression as well as in the mechanism of action of anxiolytics/antidepressants, its relevance to the therapeutic effectiveness of these drugs has been a matter of considerable debate (Griebel 1995; Hensler 2003; Hjorth et al. 2000; Lesch et al. 2003). The $5HT_{1A}$ receptor is encoded by an intronless gene (*HTR1A*) located on human chromosome 5q12.3. Several rare missense polymorphisms, including the Gly22Ser variant which results in altered agonist-elicited downregulation, have been found within the protein coding of *HTR1A*. Moreover, Lemonde and coworkers (Lemonde et al. 2003) reported a functional C-1019G single nucleotide polymorphism (SNP) in the transcriptional control region of *HTR1A* (HTR1A-1019) and demonstrated in *in vitro* experiments that the G variant displays differential binding efficiency of the repressors/enhancer-type transcriptional regulator NUDR/DEAF-1. NUDR/DEAF-1 is co-expressed with both pre- and post-synaptic $5HT_{1A}$ receptors, but its regulation of *HTR1A* transcription may differ in presynaptic raphe versus postsynaptic target cells (Lemonde et al. 2003).

Although initial association studies of the *HTR1A* variations produced ambiguous results in affective disorders (Arias et al. 2002; Nishiguchi et al. 2002), Lemonde and coworkers (2003) also showed that the G variant of the HTR1A-1019 polymorphism is associated with severe depression and suicidality. Taking the considerable comorbidity of depression and anxiety disorders into account it came as no surprise that associations of the G variant with anxiety- and depression-related personality traits, particularly with higher scores in Neuroticism and Harm Avoidance, as well as with the agoraphobic subtype of panic disorder were also reported (Rothe et al. 2004; Strobel et al. 2003). These findings have been further extended by Huang et al. (2004) who report an association of the HTR1A-1019 polymorphism with panic disorder as well as in schizophrenia and substance use disorder.

Preliminary evidence that allelic variation of $5HT_{1A}$ receptor expression influences the response to antidepressant treatment has recently been provided by two independent studies. Serretti and colleagues (2004) assessed the severity of depressive symptoms in 151 patients with major depression and 111 bipolar patients before and following six weeks of treatment with the SSRI fluvoxamine and demonstrate that in bipolar disorder but not in unipolar depression, patients homozygous for the C variant of the HTR1A-1019 polymorphism showed a better response as compared to carriers of the G allele. Interestingly, the results failed to reveal an interaction between the HTR1A-1019 polymorphism and reported effects of the 5HTTLPR. Lemonde et al. (2004) reported that antidepressant response to the SSRI fluoxetine, noradrenaline reuptake inhibitor nefadozone, and $5HT_{1A}$ agonist flibanserin, which desensitize the $5HT_{1A}$ autoreceptor as one their mechanisms of action, was associated HTR1A-1019 polymorphism 118 depressed patients. Patients homozygous for the G variant of the HTR1A-1019 polymorphism improved significantly less on flibanserin and

in pooled antidepressant treatment groups were twice as likely to be non-responders as those with the C/C genotype. These findings further corroborate the hypothesis that genetic variations in *HTR1A* may not only predispose to psychiatric disorders, but may also contribute to individual differences in responsiveness to antidepressant treatment.

Taken together, allelic variation in $5HT_{1A}$ receptor expression seems to play a critical role in the development and modulation of individual differences in anxiety- and depression-related personality traits as well as in the pathophysiology of anxiety disorders and syndromal dimensions of depression, psychosis, and substance abuse. Evidence that the HTR1A-1019 polymorphism also influences therapeutic responses to serotonergic agents may have implications for tailoring individual antidepressant/anxiolytic treatment. The availability of an increasing number of functional gene variants within the serotonergic pathway together with integration of emerging concepts of developmental genetics of complex traits will provide the groundwork for the molecular dissection of syndromal dimensions and treatment response.

6. CONCLUSIONS

Response to psychopharmacologic drugs is genetically complex, results from an interplay of multiple genomic variations with environmental influences, and depends on the structure or functional expression of gene products, which are direct drug targets or are indirectly modify the development and synaptic plasticity of neural networks critically involved in their effects. During brain development, the 5HT system, which is commonly targeted by anxiolytic and antidepressant drugs, controls neuronal specification, differentiation, and phenotype maintenance. While formation and integration of these neural networks is dependent on the action of multiple proteins, converging lines of evidence indicate that genetically controlled variability in the expression of serotonergic genes is critical to the development and plasticity of distinct neurocircuits. The most promising finding to date indicate associations between the response time as well as overall response to serotonin reuptake inhibitors (SSRIs) and a common polymorphism within the transcriptional control region of both the 5HTT and $5HT_{1A}$ genes. More functionally relevant polymorphisms in genes within a single neurotransmitter system, or in genes, which comprise a developmental and functional unit in their concerted actions, need to be identified and assessed in both large association studies to elucidate complex epistatic interactions of multiple loci. Finally, psychopharmacogenetic studies require to employ randomized, double-blind clinical trial methodology, and, in order to detect a small gene effects, a dimensional, quantitative approach to behavioral phenotypes and treatment effects arising from standardized psychometric trait and response assessment is needed. Given the limitation of the diagnostic and psychometric approach future studies will require extended, homogeneous, and ethnically matched samples.

7. ACKNOWLEDGEMENT

The author's work is supported by a grant from the European Commission (NEWMOOD LSHM-CT-2003-503474).

8. REFERENCES

Alda, M., 2001, Genetic factors and treatment of mood disorders. *Bipolar Disord*. **3**:318-324.

Arias, B., Arranz, M.J., Gasto, C., Catalan, R., Pintor, L., Gutierrez, B., Kerwin, R.W., Fananas, L., 2002, Analysis of structural polymorphisms and C-1018G promoter variant of the 5-HT,1A) receptor gene as putative risk factors in major depression. *Mol Psychiatry*. **7**:930-2.

Arias, B., Catalan, R., Gasto, C., Gutierrez, B., Fananas, L., 2003, 5-HTTLPR polymorphism of the serotonin transporter gene predicts non-remission in major depression patients treated with citalopram in a 12-weeks follow up study. *J Clin Psychopharmacol*. **23**:563-7.

Arranz, M., Nunro, J., Birkett, J., Bolonna, A., Manacama, D., Sodhi, M., Lesch, K., Meyer, J., Sham, P., Collier, D., Murray, R., Kerwin, R., 2000, Pharmacogenetic prediction of clozapine response. *Lancet.* **355**:1615-1616.

Benedetti, F., Colombo, C., Serretti, A., Lorenzi, C., Pontiggia, A., Barbini, B., Smeraldi, E., 2003, Antidepressant effects of light therapy combined with sleep deprivation are influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *Biol Psychiatry*. **54**:687-92.

Benedetti, F., Serretti, A., Colombo, C., Campori, E., Barbini, B., di Bella, D., Smeraldi, E., 1998, Influence of a functional polymorphism within the promoter of the serotonin transporter gene on the effects of total sleep deprivation in bipolar depression. *Am J Psychiatry*. **156**:1450-2.

Bonney, G.E., 1986, Regressive logistic models for familial disease and other binary traits. *Biometrics*. **42**:611-25.

Catalano, M., 1999, The challenges of psychopharmacogenetics. *Am J Hum Genet.* **65**:606-610.

Collier, D.A., Stöber, G., Li, T., Heils, A., Catalano, M., Di Bella, D., Arranz, M.J., Murray, R.M., Vallada, H.P., Bengel, D., Müller, C.R., Roberts, G.W., Smeraldi, E., Kirov, G., Pak, S., Lesch, K.P., 1996, A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. *Mol Psychiatry***1**:453-460.

Comings, D.E., Muhleman, D., Gysin, R., 1996, Dopamine D2 receptor, DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. *Biol Psychiatry.* **40**:368-72.

Crawford, F., Hoyne, J., Diaz, P., Osborne, A., Dorotheo, J., Sheehan, D., Mullan, M., 1995, Occurrence of the Cys311 DRD2 variant in a pedigree multiply affected with panic disorder. *Am J Med Genet.* **60**:332-4.

Crowe, R.R., 1990, Panic disorder: genetic considerations. *J Psychiatry Res.* **24**:129-34.

Crowe, R.R., Goedken, R., Steve Samuelson, S., Wilson, R., Nelson, J., R, N.J., 2001, Genomewide survey of panic disorder. *Am J Med Genet*. **105**:105-109.

Crowe, R.R., Noyes, R., Jr., Samuelson, S., Wesner, R., Wilson, R.,1990, Close linkage between panic disorder and alpha-haptoglobin excluded in 10 families. *Arch Gen Psychiatry.* **47**: 377-80.

Crowe, R.R., Wang, Z., Noyes, R., Jr., Albrecht, B.E., Darlison, M.G., Bailey, M.E., Johnson, K.J., Zoega, T., 1997, Candidate gene study of eight GABAA receptor subunits in panic disorder. *Am J Psychiatry.* **154**:1096-100.

Deckert, J., Catalano, M., Heils, A., Di Bella, D., Friess, F., Politi, E., Franke, P., Nothen, M.M., Maier, W., Bellodi, L., Lesch, K.P., 1997, Functional promoter polymorphism of the human serotonin transporter: lack of association with panic disorder. *Psychiatry Genet*. **7**:45-7.

Deckert, J., Catalano, M., Syagailo, Y.V., Bosi, M., Okladnova, O., Di Bella, D., Nothen, M.M., Maffei, P., Franke, P., Fritze, J., Maier, W., Propping, P., Beckmann, H., Bellodi, L., Lesch, K.P., 1999, Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. *Hum Mol Genet.* **8**:621-4.

Deckert, J., Nothen, M.M., Franke, P., Delmo, C., Fritze, J., Knapp, M., Maier, W., Beckmann, H., Propping, P.,1998, Systematic mutation screening and association study of the A1 and A2a

adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. *Mol Psychiatry.* **3**:81-5.

Del Zompo, M., Ardau, R., Palmas, M., Bocchetta, A., Reina, A., Piccardi, M., 1999, Lithium response: association study with two candidate genes. *Mol Psychiatr.* **4, Suppl**:66-67.

Durham, L.K., Webb, S.M., Milos, P.M., Clary, C.M., Seymour, A.B.,2003, The serotonin transporter polymorphism, 5HTTLPR, is associated with a faster response time to sertraline in an elderly population with major depressive disorder. *Psychopharmacology* (Berl).

Eley, T.C., Plomin, R.,1997, Genetic analyses of emotionality. *Curr Opin Neurobiol.* 7:279-84.

Eley, T.C., Stevenson, J., 1999, Exploring the covariation between anxiety and depression symptoms: a genetic analysis of the effects of age and sex. *J Child Psychol Psychiatry* **40**:1273-82.

Fallgatter, A., Herrmann, M.J., J,R., Ehlis, A.C., Wagener, A., Heidrich, A., Zeng, Y., Ortega, G., Lesch, K.P., 2004, Allelic variation of serotonin transporter function modulates the brain electrical response for error processing. *Neuropsychopharmacology* (in press).

Fallgatter, A., Jatzke, S., Bartsch, A., Hamelbeck, B., Lesch, K., 1999, Serotonin transporter promoter polymorphism influences topography of inhibitory motor control. *Int J Neuropsychopharm.* **2**:115-120.

Feusner, J., Ritchie, T., Lawford, B., Young, R.M., Kann, B., Noble, E.P., 2001, GABA(A) receptor beta 3 subunit gene and psychiatric morbidity in a post-traumatic stress disorder population. *Psychiatry Res*. **104**:109-17.

Furmark, T., Tillfors, M., Garpenstrand, H., Marteinsdottir, I., Langstrom, B., Oreland, L., Fredrikson, M., 2004, Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neurosci Lett*. **362**:189-92.

Gelernter, J., Southwick, S., Goodson, S., Morgan, A., Nagy, L., Charney, D.S., 1999, No association between D2 dopamine receptor (DRD2) "A" system alleles, or DRD2 haplotypes, and posttraumatic stress disorder. *Biol Psychiatry.* **45**:620-5.

Griebel, G., 1995, 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther.* **65**:319-95.

Hamilton, S.P., Haghighi, F., Heiman, G.A., Klein, D.F., Hodge, S.E., Fyer, A.J., Weissman, M.M., Knowles, J.A., 2000, Investigation of dopamine receptor (DRD4) and dopamine transporter (DAT) polymorphisms for genetic linkage or association to panic disorder. *Am J Med Genet.* **96**:324-30.

Hamilton, S.P., Heiman, G.A., Haghighi, F., Mick, S., Klein, D.F., Hodge, S.E., Weissman, M.M., Fyer, A.J., Knowles, J.A., 1999, Lack of genetic linkage or association between a functional serotonin transporter polymorphism and panic disorder. *Psychiatr Genet*. **9**:1-6.

Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002, Serotonin transporter genetic variation and the response of the human amygdala. *Science.* **297**:400-3.

Hensler, J.G., 2003, Regulation of 5-HT1A receptor function in brain following agonist or antidepressant administration. *Life Sci.* **72**:1665-1682.

Hjorth, S., Bengtsson, H.J., Kullberg, A., Carlzon, D., Peilot, H., Auerbach, S.B., 2000, Serotonin autoreceptor function and antidepressant drug action. *J Psychopharmacol*. **14**:177-185.

Hopper, J.L., Judd, F.K., Derrick, P.L., Macaskill, G.T., Burrows, G.D., 1990, A family study of panic disorder: reanalysis using a regressive logistic model that incorporates a sibship environment. *Genet Epidemiol.* **7**:151-61.

Huang, Y.Y., Battistuzzi, C., A, O.M., Harkavy-Friedman, J., Greenhill, L., Brodsky, B., Arango, V., Brent, D.A., Mann, J.J., 2004, Human 5-HT1A receptor C-1019G polymorphism and psychopathology. *Int J Neuropsychopharmacol.* (in press).

Ishiguro, H., Arinami, T., Yamada, K., Otsuka, Y., Toru, M., Shibuya, H., 1997, An association study between a transcriptional polymorphism in the serotonin transporter gene and panic disorder in a Japanese population. *Psychiatry Clin Neurosci.* **51**:333-5.

Kato, T., Wang, Z.W., Zoega, T., Crowe, R.R., 1996, Missense mutation of the cholecystokinin B receptor gene: lack of association with panic disorder. *Am J Med Genet.* **67**:401-5.

Kendler, K.S., 1996, Major depression and generalised anxiety disorder. Same genes, (partly) different environments–revisited. *Br J Psychiatry Suppl*. 68-75.

Kendler, K.S., Karkowski, L.M., Prescott, C.A., 1999, Fears and phobias: reliability and heritability. *Psychol Med.* **29**:539-53.

Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1992a, Generalized anxiety disorder in women. A population-based twin study [see comments]. *Arch Gen Psychiatry.* **49**:267-72.

Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1992b, Major depression and generalized anxiety disorder. Same genes, (partly) different environments? *Arch Gen Psychiatry.* **49**:716-22.

Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1993a, A longitudinal twin study of personality and major depression in women. *Arch Gen Psychiatry.* **50**:853-62.

Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1993b, Panic disorder in women: a population-based twin study [see comments]. *Psychol Med.* **23**:397-406.

Kendler, K.S., Walters, E.E., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1995a, The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. *Arch Gen Psychiatry.* **52**:374-83.

Kendler, K.S., Walters, E.E., Truett, K.R., Heath, A.C., Neale, M.C., Martin, N.G., Eaves, L.J., 1995b, A twin-family study of self-report symptoms of panic-phobia and somatization. *Behav Genet*. **25**:499-515.

Kennedy, J.L., Bradwejn, J., Koszycki, D., King, N., Crowe, R., Vincent, J., Fourie, O., 1999, Investigation of cholecystokinin system genes in panic disorder. *Mol Psychiatry.* **4**:284-5.

Kim, D.K., Lim, S.W., Lee, S., Sohn, S.E., Kim, S., Hahn, C.G., Carroll, B.J., 2000, Serotonin transporter gene polymorphism and antidepressant response. *Neuroreport*. **11**:215-9.

Knowles, J.A., Fyer, A.J., Vieland, V.J., Weissman, M.M., Hodge, S.E., Heiman, G.A., Haghighi, F., de Jesus, G.M., Rassnick, H., Preud'homme-Rivelli, X., Austin, T., Cunjak, J., Mick, S., Fine, L.D., Woodley, K.A., Das, K., Maier, W., Adams, P.B., Freimer, N.B., Klein, D.F., Gilliam, T.C., 1998, Results of a genome-wide genetic screen for panic disorder. *Am J Med Genet.* **81**:139-47.

Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P.R., 2004, Association of the C (-1019)G 5-HT1A functional promoter polymorphism with antidepressant response. *Int J Neuropsychopharmacol* (in press).

Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., Sequeira, A., Kushwaha, N., Morris, S.J., Basak, A., Ou, X.M., Albert, P.R., 2003, Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J Neurosci.* **23**:8788-99.

Lerer, B., Macciardi, F., 2002, Pharmacogenetics of antidepressant and mood-stabilizing drugs: a review of candidate-gene studies and future research directions. *Int J Neuropsychopharmacol*. **5**:255-75.

Lesch, K.P., 2003, Neuroticism and serotonin: a developmental genetic perspective. In: Plomin, R., DeFries, J., Craig, I., McGuffin, P. (eds) Behavioral Genetics in the Postgenomic Era. *American Psychiatric Press*, Washington, D.C., pp. 389-423.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Müller, C.R., Hamer, D.H., Murphy, D.L., 1996, Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science.* **274**:1527-1531.

Lesch, K.P., Meyer, J., Glatz, K., Flügge, G., Hinney, A., Hebebrand, J., Klauck, S.M., Poustka, A., Poustka, F., Bengel, D., Mössner, R., Riederer, P., Heils, A., 1997, The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: alternative biallelic variation in rhesus monkeys. *J Neural Transm.* **104**:1259-66.

Lesch, K.P., Mössner, R., 1998, Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? *Biol Psychiatry.* **44**:179-92.

Lesch, K.P., Zeng, Y., Reif, A., Gutknecht, L., 2003, Anxiety-related traits in mice with modified genes of the serotonergic pathway. *Eur J Pharmacol.* **480**:185-204.

Livesley, W.J., Jang, K.L., Vernon, P.A., 1998, Phenotypic and genetic structure of traits delineating personality disorder. *Arch Gen Psychiatry.* **55**:941-948.

Maier, W., Minges, J., Lichtermann, D., 1995, The familial relationship between panic disorder and unipolar depression. *J Psychiatr Res.* **29**:375-88.

Mancama, D., Kerwin, R.W., 2003, Role of pharmacogenomics in individualising treatment with SSRIs. *CNS Drugs.* **17**:143-151.

Matsushita, S., Muramatsu, T., Kimura, M., Shirakawa, O., Mita, T., Nakai, T., Higuchi, S., 1997, Serotonin transporter gene regulatory region polymorphism and panic disorder [letter]. *Mol Psychiatry.* **2**:390-2.

Mulder, R., Joyce, P., Cloninger, C., 1994, Temperament and early environment influence comorbidity and personality disorders in major depression. *Comprehensive Psychiatry.* **35**:225-233.

Mundo, E., Walker, M., Cate, T., Macciardi, F., Kennedy, J.L., 2001, The role of serotonin transporter protein gene in antidepressant-induced mania in bipolar disorder: preliminary findings. *Arch Gen Psychiatry.* **58**:539-44.

Mutchler, K., Crowe, R.R., Noyes, R., Jr., Wesner, R.W., 1990, Exclusion of the tyrosine hydroxylase gene in 14 panic disorder pedigrees. *Am J Psychiatry.* **147**:1367-9.

Nishiguchi, N., Shirakawa, O., Ono, H., Nishimura, A., Nushida, H., Ueno, Y., Maeda, K., 2002, Nishiguchi N, Shirakawa O, Ono H, Nishimura A, Nushida H, Ueno Y, Maeda K, 2002, Lack of an association between 5-HT1A receptor gene structural polymorphisms and suicide victims. *American Journal of Medical Genetics.* **114**:423-425.

Perlis, R.H., Mischoulon, D., Smoller, J.W., Wan, Y.J., Lamon-Fava, S., Lin, K.M., Rosenbaum, J.F., Fava, M., 2003, Serotonin transporter polymorphisms and adverse effects with fluoxetine treatment. *Biol Psychiatry*. **54**:879-83.

Pollock, B.G., Ferrell, R.E., Mulsant, B.H., Mazumdar, S., Miller, M., Sweet, R.A., Davis, S., Kirshner, M.A., Houck, P.R., Stack, J.A., Reynolds, C.F., Kupfer, D.J., 2000, Allelic variation in the serotonin transporter promoter affects onset of paroxetine treatment response in late-life depression. *Neuropsychopharmacology.* **23**:587-90.

Radant, A., Tsuang, D., Peskind, E.R., McFall, M., Raskind, W., 2001, Biological markers and diagnostic accuracy in the genetics of posttraumatic stress disorder. *Psychiatry Res.* **102**:203-15.

Rausch, J.L., Johnson, M.E., Fei, Y.J., Li, J.Q., Shendarkar, N., Hobby, H.M., Ganapathy, V., Leibach, F.H., 2002, Initial conditions of serotonin transporter kinetics and genotype: influence on SSRI treatment trial outcome. *Biol Psychiatry.* **51**:723-32.

Reist, C., Mazzanti, C., Vu, R., Tran, D., Goldman, D., 2001, Serotonin transporter promoter polymorphism is associated with attenuated prolactin response to fenfluramine. *American Journal of Medical Genetics.* **105**:363-8.

Rothe, C., Gutknecht, L., Freitag, C.M., Tauber, R., Franke, P., Fritze, J., Wagner, G., Peikert, G., Wenda, B., Sand, P., Jacob, C., Rietschel, M., Nöthen, M.M., Garritsen, H., Fimmers, R., Deckert, J., Lesch, K.P., 2004, Association of a functional -1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. *Int J Neuropsychopharmacol*. **7**:189-192.

Rousseva, A., Henry, C., van den Bulke, D., Fournier, G., Laplanche, J.L., Leboyer, M., Bellivier, F., Aubry, J.M., Baud, P., Boucherie, M., Buresi, C., Ferrero, F., Malafosse, A., 2003, Antidepressant-induced mania, rapid cycling and the serotonin transporter gene polymorphism. *Pharmacogenomics J.* **3**:101-4.

Roy, M.A., Neale, M.C., Pedersen, N.L., Mathe, A.A., Kendler, K.S., 1995, A twin study of generalized anxiety disorder and major depression. *Psychol Med.* **25**:1037-49.

Segman, R.H., Cooper-Kazaz, R., Macciardi, F., Goltser, T., Halfon, Y., Dobroborski, T., Shalev, A.Y., 2002, Association between the dopamine transporter gene and posttraumatic stress disorder. *Mol Psychiatry.* **7**:903-7.

Serretti, A., Artioli, P., Lorenzi, C., Pirovano, A., Tubazio, V., Zanardi, R., 2004, The C (-1019)G polymorphism of the 5-HT1A gene promoter and antidepressant response in mood disorders: preliminary findings. *Int J Neuropsychopharmacol* (in press).

Serretti, A., Lilli, R., Mandelli, L., Lorenzi, C., Smeraldi, E., 2001, Serotonin transporter gene associated with lithium prophylaxis in mood disorders. *Pharmacogenomics J*. **1**:71-7.

Serretti, A., Lilli, R., Smeraldi, E., 2002, Pharmacogenetics in affective disorders. *Eur J Pharmacol.* **438**:117-128.

Skre, I., Onstad, S., Edvardsen, J., Torgersen, S., Kringlen, E., 1994, A family study of anxiety disorders: familial transmission and relationship to mood disorder and psychoactive substance use disorder. *Acta Psychiatr Scand.* **90**:366-74.

Smeraldi, E., Zanardi, R., Benedetti, F., Di Bella, D., Perez, J., Catalano, M., 1998, Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine [see comments]. *Mol Psychiatry.* **3**:508-11.

Stein, M.B., 1998, Neurobiological perspectives on social phobia: from affiliation to zoology. *Biol Psychiatry.* **44**:1277-85.

Stein, M.B., Chartier, M.J., Kozak, M.V., King, N., Kennedy, J.L., 1998, Genetic linkage to the serotonin transporter protein and 5HT2A receptor genes excluded in generalized social phobia. *Psychiatry Res.* **81**:283-91.

Strobel, A., Gutknecht, L., Zheng, Y., Reif, A., Brocke, B., Lesch, K.P., 2003, Allelic variation of serotonin receptor 1A function is associated with anxiety- and depression-related traits. *J Neural Transm.* **110**:1445-1453.

Veenstra-VanderWeele, J., Anderson, G.M., Cook, E.H., Jr., 2000, Pharmacogenetics and the serotonin system: initial studies and future directions. *Eur J Pharmacol.* **410**:165-181.

Wang, Z., Valdes, J., Noyes, R., Zoega, T., Crowe, R.R., 1998, Possible association of a cholecystokinin promotor polymorphism (CCK- 36CT) with panic disorder. *Am J Med Genet.* **81**:228-34.

Wang, Z.W., Crowe, R.R., Noyes, R., Jr., 1992, Adrenergic receptor genes as candidate genes for panic disorder: a linkage study. *Am J Psychiatry.* **149**:470-4.

Weissman, M.M., 1993, Family genetic studies of panic disorder. *J Psychiatr Res.* **27**, 69-78.

Whale, R., Quested, D.J., Laver, D., Harrison, P.J., Cowen, P.J., 2000, Serotonin transporter (5-HTT) promoter genotype may influence the prolactin response to clomipramine. *Psychopharmacology.* (Berl) **150**:120-2.

Whyte, E.M., Pollock, B.G., Wagner, W.R., Mulsant, B.H., Ferrell, R.E., Mazumdar, S., Reynolds, C.F., 3rd ,2001, Influence of serotonin-transporter-linked promoter region polymorphism on platelet activation in geriatric depression. *Am J Psychiatry.* **158**:2074-6.

Woodman, C.L., 1993, The genetics of panic disorder and generalized anxiety disorder. *Ann Clin Psychiatry.* **5**: 231-9.

Yoshida, K., Ito, K., Sato, K., Takahashi, H., Kamata, M., Higuchi, H., Shimizu, T., Itoh, K., Inoue, K., Tezuka, T., Suzuki, T., Ohkubo, T., Sugawara, K., Otani, K., 2002, Influence of the serotonin transporter gene-linked polymorphic region on the antidepressant response to fluvoxamine in Japanese depressed patients. *Prog Neuropsychopharmacol Biol Psychiatry.* **26**:383-6.

Yu, Y.W., Tsai, S.J., Chen, T.J., Lin, C.H., Hong, C.J., 2002, Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. *Mol Psychiatry.* **7**:1115-9.

Zanardi, R., Benedetti, F., Di Bella, D., Catalano, M., Smeraldi, E., 2000, Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *J Clin Psychopharmacol.* **20**:105-107.

3. MAJOR DEPRESSIVE DISORDERS:

Depressive Disorders

Alessandro Serretti and Paola Artioli*

1. INTRODUCTION

Depressive disorders represent a significant problem in public health; the associated high morbidity represent an important cause of psychosocial functional impairment and increased mortality, both for natural causes and for suicide, make the treatment of these diseases one of the most demanding task in mental health. According to a World Health Organization (WHO) report, depression is going to be the condition with the second greatest disease burden world-wide by 2020 (Murray 1996). Mood disorders have a large impact on social health. They contribute for 11% of all the inabilities registered in the International Classification of Disease manual, (ICD-9), and only in the USA they cause a loss of 147 billions dollars for year considering both direct and indirect costs (Pincus 2001; Bauwens 1991).

Major Depression (MD) is twice as common in adolescent and adult female as in adolescent and adult male. Its point prevalence in adults in community samples varies from 5% to 9% for women and from 2% to 3% for men, while its international lifetime prevalence ranges from 4.6 to 17.1% of the general population, with Recurrent Major Depression being about 7-10% (Kaplan 1995; Ustun 2001). These prevalence rates appear to be unrelated to ethnicity, education, income, or marital status, but culture may influence the experience and the communication of depressive symptoms. The mean age of onset is in the third decade of life, although onset in adolescence is rising common. The reported prevalence for mood disorders may depend on diagnostic criteria. In fact, considering less severe forms such as Minor Depression or Brief Recurrent Depression, the lifetime rate may reach up to one third of the population.

* Alessandro Serretti, Vita Salute University – San Raffaele Hospital, Milan, Italy.
E-mail: serretti.alessandro@hsr. Paola Artioli, Vita Salute University – San Raffaele Hospital, Milan, Italy.

Psychiatric and substance-abuse co-morbidities are the most common complications, and mortality rates are increased in the population of depressed subjects, as a result of high suicidal risks, accidents, complications of substance abuse and increased fatality of stress-sensitive medical illnesses. Up to 17% of individuals with severe MD die of suicide (Kaplan 1995), and a population based study performed in Sweden showed increased mortality ratios in patients with depressive disorders, both for all natural causes of death and for suicide only, natural causes representing about half the excess of deaths (Osby 2001), while a recent follow up study found that inpatient affected by affective disorders have elevated mortality rates for suicide but also for circulatory disorders (Angst 2002).

In particular, depressive symptomatology increases mortality in the elderly. Minor depression in older men and major depression in both older men and women was shown to increase the risk of dying, even after adjustment for socio-demographic variables, health status and health behaviours (Penninx 1999). In older patients, depression could also act by increasing cognitive decline, with a significant worsening of their quality of life (Yaffe 1999; Bassuk 1998).

On the other hand, somatic diseases are often complicated by depressive symptomatology. In fact, depression showed to be an independent risk factor for increased post-myocardial infarction morbidity and mortality (Carney 2001; Ziegelstein 2001; Malhotra 2000; Burvill 1995), and at least 25% of hospitalised cancer patients meet criteria for major depression or adjustment disorder with depressed mood (Massie 1990). Also, depression represent a frequent complication of diabetes, and other metabolic illnesses (Lynch 1994).

The above mentioned reasons confirm the importance of research in the field of pathophisiology and treatment of depressive disorders. There are still a lot of unknown issues about mechanisms and biochemical pathways which underlie the appearance of mood disorder and the efficacy – effectiveness of pharmacological treatments.

The research in mood disorders concerns to different topics: pathogenetic mechanisms (genetic studies, PET-SPECT or neuro-cognitive studies, in vitro or animal models experiments), diagnostic biological characterization (because a biological marker or a specific measurable “sign” to identify diagnostic categories is not available yet), and treatment features (trials to test efficacy and mechanisms of action of drugs, appearance of side effects, differences in effectiveness for different patients).

Within this large field of application, pharmacogenetic research is receiving more and more attention, for the promising results and its attractive perspectives. In fact, the identification of precise criteria for drug administration is as much desirable as difficult to reach. Moreover, pharmacogenetic criteria could represent a sort of biological definition of nosologic categories, overwhelming the difficulties in categorizing psychiatric disorders on the basis of symptomathologic diagnostic criteria. In recent years, pharmacogenetic research on mood disorders had pointed out some findings (for a review see (Serretti 2002) but researchers are far to reach a complete knowledge of the complex heritability mechanisms of antidepressant response.

2. THE HISTORY

The idea that mood disorders are heritable disturbances dates back to Hippocrates theory of constitutional types: the hypothesis that psycho-pathological traits could be inherited (known as Atavism) was present since the origins of psychiatry, as suggested by the observation that affected subjects clustered in families. However, a more precise hypothesis did not appear until the 19th century (Cooper 1986). Wilhelm Griesinger (1817-1868) distinguished in fact between traumatic external events and internal states (including hereditary predisposition), which could combine, in varying rates, to give rise to insanity. This causal model had a great success between contemporary scientist and was adopted at this time by many Authors (Ackerknecht 1959).

With the beginning of the 20th century and the spring of biological psychiatry, the first twin studies were performed to investigate heritability of mental diseases and psychopathological traits. Ernst Rudin and its group at the Munich Research Institute improved the fist statistical research techniques to quantify the familial concentration of some psychiatric diseases: they tended to neglect completely the role of environmental influences, so their results were easily criticized by the so called “environmentalists”. This widespread interest was particularly flowering in Nazi Germany and research findings about heritability were at the bases of the eugenic movement. The term “eugenic” was created by F. Galton in 1889. The followers of this doctrine had, as their main wish, to reduce illnesses and abnormality in humankind, through aimed elimination and mating selection (Weber 1997). They sustained that mental illness, feeble-mindedness, criminality, alcoholism and sexual promiscuity were all expression of racial degeneracy, whose remedy lay in a policy of selective birth control, extending to sterilization of those unfit to bear children. But first hopes were downsized when they understood that the necessary social laws contrasted with individual rights, and were therefore difficult to apply. Ideas of this nature lead to mass extermination of the mentally ill and handicapped under Hitler’s regime (Meyer-Lindenberg 1991). However, recent knowledge demonstrated that, from a purely genetic point of view, this strategy is both unfruitful and, most probably, deleterious (Cavallisforza L. Genes 1997). In fact, the selection of individuals according to phenotype appearance would never eliminate recessive factors carried by individuals without any “negative” phenotype trait completely. Moreover, the reduction of genetic variability induced by selection has been shown to be disadvantageous for the species.

The following decades were strongly influenced by the events of the II World War and psychiatric genetics, together with biological psychiatry as a whole, suffered from a long and widespread stigma in favour of phenomenology, psychoanalysis or, more recently, social approaches. Finally, in the late sixties and seventies the need to bridge the gap between psychiatry and the other rapidly advancing fields of medicine, prompted many worldwide general and mental health authorities (World Health Organization, American Psychiatric Association) to turn the psychiatric research to biological and genetic studies.

3. CLASSIFICATION

The aim of nosology has been to distinguish homogeneous subtypes of mood disorders corresponding to different clustering of these signs and symptoms, clearly delimited from other disorders. Modern psychiatry follows Leonhard's (Leonhard 1959) suggestion to subdivide mood disorders according to the type of mood disturturbance experienced by the patient. The most worldwide followed nosologic manual is currently the Diagnostic and Statistical Manual of Mental Diseases, (DSM) which was compiled for the fist time in 1951 by a special Committee (Committee on Nomenclature and Statistics) instituted by the American Psychiatric Association. It is periodically revised, and the fifth revision is going to be published in the short term.

According to DSM criteria the symptoms of a Major Depressive Episode consist of: depressed mood, diminished interest or pleasure in all activities, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue and loss of energy, feelings of worthlessness or excessive guilt, inability to concentrate or act decisively, decreased appetite with weight loss and recurrent thoughts of death or suicide. Usually, Major Depressive Episodes arises slowly, with an onset period varying from a week to some months. Each episode typically lasts for several months and ends gradually. The different combinations of major depressive episodes, manic episodes, mixed episodes and hypomanic episodes define the diagnosis of mood disorders. Single episodes do not have their own diagnostic codes and cannot be diagnosed as separate entities, they serve as the building blocks for diagnoses. Finally, psychotic features such as delusions or hallucinations may occur during both manic or depressive episodes.

Do these definitions fit for genetic studies? In other words, are mood disorders subtypes genetically distinct? And are genes that cause a specific disorder completely different from those involved in other disorders, or do they overlap each other? Even thought it is possible a clear diagnosis between typical schizophrenia and Bipolar Disorder (BP), genetic distinction between other disorders is not so clear (Winokur 1993 and 1995; Tsuang 1990; Maziade 1995).

Molecular genetic studies tried to overcome this bias by using a stratified phenotype definition: from strict to broad. From family studies, in fact, emerges a reasonable definition of a BP spectrum of mood disorders that would include, from narrow to broad, BP Type I, BP Type II, Schizoaffective Disorder, Recurrent Major Depressive Disorder (MD) and Single Episode Major Depression, this last being considered the disorder with the lowest genetic loading (Winokur 1995; Tsuang 1990; Gershon 1982; Weissman 1984; Maier 1993). It is unclear, however, if this spectrum represents a pleiotropic expression of a single genetic susceptibility.

The overlap between different diagnostic phenotypes is even more extensive when considering less severe disorders. The presence at the same time of several psychic disorders in a single subject is, for example, common. This phenomenon is called comorbidity. Do they really represent different nosologic entities or do they evidence deficits in the present diagnostic systems criteria? People suffering of mood disorders often experience anxiety symptoms. Are genes implicated in anxiety disorders the same involved in depression? Does the

overlap between different diagnostic phenotypes reflect an overlap between involved genes? Family and twin studies show a large overlap between them (Torgersen 1990; Kendler 1992). A more careful twin study about comorbidity analysing different disorders, found evidences of two clusters of genetic factors: one is related to MD and Generalized Anxiety Disorder, the other is involved in Panic Disorder, Bulimia and Phobic Disorder (Kendler 1995). Even if comorbidity could represent a bias, molecular and genetic researches would provide some evidence for disentangling the overlap of these two pathologies.

From the abovementioned description it clearly appears that the definition of an individual as "affected subject" is not based on biologically valid measurements, but mainly on clinical features. This fact has been probably the cause for the lack of definite results in molecular genetic studies. Therefore during recent years researchers, prompted by inconclusive results, developed alternative phenotype definitions, based on symptomatology, neuropsychologic testing, neuroimaging, time course of the disease or drug response. This is a quite difficult issue because any phenotype for the search of liability genes must demonstrate a number of properties. Firstly, it should be a reliable measurement, then the pathway linking it to the underlying biological mechanism should be at least plausible, finally it should demonstrate a significant heritability in formal genetic studies.

4. PHARMACOGENETICS

Pharmacogenetics is the use of genetic information to guide pharmacotheraphy and improve outcome by providing individualized and science-based treatment decisions. It gained increasing attention and holds great promise for clinical medicine in the latest years (Roses 2000; Dettling 2001; Segman 1999; Pickar 2001). Pharmacogenetic strategy studies how genetic variation could affect the response of patients to psychotropic drugs and their susceptibility to adverse drug reactions.

The emerging field of pharmacogenetics holds great potential, particularly in psychiatry, for refining psychopharmacology, given the lack of biologically based treatment guidelines (American Psychiatric Association 1994, 1997 and 2000; Catalano 1999). The main goal would be the possibility for clinicians in the future to optimise the use of medications by choosing the drug most likely to work in patients according to their particular genetic profile. Pharmacogenetics could also be considered as a solution to bypass the problem of the biological heterogeneity of psychiatric diseases. In fact heritability of response patterns to psychotropic drugs showed to be more homogeneous and not influenced by diagnostic biases.

Pharmacogenetic studies in mood disorder were performed only during recent years (Serretto 2002). Drug response has the property to be reliably measured (Hamilton 1967), the involved pathway is, at least partly, known (Post 1995; Maes 1995) and finally it showed to be heritable.

The pharmacotherapy of affective disorders has reduced morbidity and improved outcomes for millions of individuals worldwide. In fact, since 1950 specific antidepressant drug treatments, able to improve symptomatology and

increase the chance of a good long term outcome, have been used. Unfortunately, not all subjects benefit from treatment, and efficient clinical predictors have not been yet identified, but there is some evidence suggesting that genetic factors play a substantial role (Orsini 1987; Sederer 1986; Berrettini 1998; Pare 1971). In 1994, it was reported that, in a family with eight relatives suffering from major depression, individuals tended to respond to the monoamine oxidase inhibitor, tranylcypromine (O'Reilly 1994).

Our group analysed data from forty-five pairs of relatives treated with fluvoxamine (Franchini 1998); the sample included individuals with unipolar and individuals with bipolar depression. Among their first-degree relatives, thirty (67%) also responded to fluvoxamine and the families they belonged to showed the higher genetic loading for affective disorders. Subsequently, in a related study, we applied complex segregation analysis to 171 families of bipolar and unipolar probands responsive to fluvoxamine: the results favoured a single major locus transmission of mood disorders in a subset of 68 families of bipolar probands (Serretti 1998).

5. CANDIDATE GENES

Polymorphisms at some genes have been studied to date, to test their association with antidepressant response. The choice of candidate genes has taken into account the possible involvement of each gene in the pathophysiology of the disease and in the mechanism of action of the analysed drugs. The most commonly worldwide used antidepressant drugs are Selective Serotonin Reuptake Inhibitors

5.1. Tryptophan Hydroxylase

The Tryptophan Hydroxylase (TPH) gene, which codes for the rate-limiting enzyme of serotonin biosynthesis, has been cloned (Boularand 1990) and mapped on 11p15.3-p14 (Craig 1991).

Studies on administration of N-ethyl-3,4-methylenedioxyamphetamine (MDE) to rats showed a significant concentration decline of 5-HT and of TPH activity in the hippocampus (Johnson 1989). In addition, long-term treatment of rats with sertraline has shown to up-regulate mRNA and protein levels of the TPH, as determined by in situ hybridisation and immunocytochemistry, respectively (Kim 2002). These findings suggest a crucial role of TPH in pharmacological action of AD.

The best known TPH variants are the two biallelic polymorphisms in strong disequilibrium on position 218 (A218C) and 779 (A779C) of intron 7 (Nielsen 1997). The polymorphism A218C is located in a potential GATA transcription factor-binding site, so that it may influence gene expression, and consequently the antidepressant response. The rarer TPH*a of A218C allele showed in fact to be associated to a decreased serotonin synthesis (Jonsson 1997).

Our group published two pharmacogenetic studies on A218C polymorphism. The first study involved a sample of major and bipolar depressives, with or without psychotic features. 217 inpatients were treated with

fluvoxamine 300 mg and either placebo or pindolol in a double blind design for 6 weeks, assessing the severity of depressive symptoms weekly with the Hamilton Rating Scale for Depression. TPH allelic variants were determined in each subject by using a PCR-based technique. No significant finding was observed in the overall sample as well as in the pindolol group, while TPH*A/A was associated with a slower response to fluvoxamine treatment in subjects not taking pindolol (P = 0.001) (Serretti 2001). The other study was performed on a sample of 121 inpatients with major depressive episode treated with paroxetine 20-40 mg with either placebo or pindolol in a double blind design for 4 weeks. TPH*A/A and TPH*A/C variants were associated with a poorer response to paroxetine treatment when compared to TPH*C/C (P=0.005); this difference was not present in the pindolol augmented group. Other variables, such as sex, diagnosis, presence of psychotic features, severity of depressive symptomatology at baseline and paroxetine plasma level, were not associated with the outcome (Serretti 2001).

Recently the same polymorphism was investigated with MAOA-VNTR in 66 Japanese patients with major depressive disorder during a 6-week controlled study. Only 54 patients completed the study, and they failed to demonstrate the association of the two polymorphisms with the antidepressant effect of fluvoxamine (Yoshida 2002); however, it must be observed that the sample was smaller than those of the previous studies, and belonging to a different ethnicity. The same Japanese group also published a paper reporting lack of association between this and other polymorphisms with the development of the development of fluvoxamine-induced nausea (Takahashi 2002).

5.2. Mono Amino Oxidase

The catabolism of catecholamines is catalysed by catechol-o-methyltransferase (COMT) and monoamine oxidase A (MAOA), which catalyses also 5HT catabolism (Berry 1994). These enzymes, being involved in the elimination of biogenic amines from the synaptic cleft, could also be involved in the different individual response to ADs, as potentially impaired variants could be more constitutionally active in amine catabolism, contrasting the action of ADs. In addition, MAOA is the specific target of the first synthesised ADs, MAO inhibitors (MAOI).

MAO-A gene is located on Xp11.23 (Sabol 1998). Animal models suggested that deletion of MAO-A gene causes behavioural alterations (Cases 1995). MAO-A inhibitors were the first effective AD drugs to be discovered. They were used as anti tubercular agents, and showed to have AD properties, by increasing levels of catecholamines and serotonin (Berry 1994). The first were not reversible and not selective inhibitors of both MAO-A and MAO-B, and caused severe side effects when associated to foods or beverages containing tyramine; this inconvenient was overwhelmed with reversible and selective MAO-A inhibitors (RIMA) such as moclobemide. MAO-A is also supposed to influence the mechanism of action of SSRIs through interaction with the 5-HT transporter (Maes 1995).

A polymorphism located 1.2 kb upstream of the MAO-A coding sequences has been shown to affect the transcription of the MAO-A gene promoter. This mutation consists of a 30 bp repeat in 3, 3.5, 4, or 5 copies, The polymorphism was shown to affect transcriptional activity of the MAOA gene promoter by gene fusion and transfection experiments involving 3 different cell types. Alleles with 3.5 or 4 copies of the repeat sequence are transcribed 2 to 10 times more efficiently than those with 3 or 5 copies of the repeat, suggesting an optimal length for the regulatory region (Sabol 1998; Cases 1995; Denney 1999).

A pharmacogenetic study on moclobemide response (Muller 2000), found no association between AD response and the variants at the polymorphism in the promoter of MAO-A gene. A recent study found no association between the same polymorphism and AD response to fluvoxamine in a Japanese sample (Yoshida 2002). Our group tested the association between fluvoxamine and paroxetine efficacy in a sample of 248 unipolar and 195 bipolar depressed patients, with or without psychotic features. 307 in-patients were treated with 300 mg fluvoxamine and 136 with 20-40 mg paroxetine for 6 weeks. The severity of depressive symptoms was assessed weekly with the HAMD, and allele variants were determined by a PCR-based technique. We observed no association between MAOA genotypes and antidepressant response (Cusin 2002).

5.3. Alpha 1 adrenergic receptor

Alpha 1 adrenergic receptors are members of the G protein-coupled receptor superfamily and activate mitogenic responses regulating growth and proliferation of many cells.

There are 3 alpha-1-AR subtypes: alpha-1A, (ADRA1A)-1B (ADRA1B) and -1D (ADRA1D) (Bylund 1994), all of which signal through the Gq/11 family of G-proteins.

ADRA1B subtype mapped on 5q23-q32; this normal cellular gene is identified as a protooncogene, and comprises 2 exons and a single large intron of at least 20 kb that interrupts the coding region. The distribution of the adrenergic receptor subtypes in the various organs and tissues and the functional response mediated by each one were not easy to be established. In some areas of rat's brain, mRNA for both ADRA1A and ADRA1B are equally expressed, while in others one subtype predominates (Lomasney 1991).

particular attention has been paid to the expression regulation of this receptor[75]: nuclear factor 1 (NF1) has been reported to be a transcriptional activator of ADRA1B promoter gene (Gao 1998).

At nucleotide 1165 a guanine (wild type) or cytosine was found leading to the amino acid variation Gly389Arg which is located in the intracellular cytoplasmatic tail near the seventh transmembrane-spanning segment. The C allele of this polymorphisms is associated with an enhanced coupling to the stimulatory Gs-protein and increased adenylyl cyclase activation, disturbances, often observed in affective disorders.

This polymorphism, located in a putative G-protein binding domain might be the basis of inter-individual differences in the pathophysiologic characteristics or in the therapeutic response in major depression (Mason 1999).

It was investigated for association with major depression or with the response to antidepressant treatment in a sample of 259 patients compared to 206 healthy controls. A tendency for a relation between CC homozygosity and a better and even faster response to various antidepressant treatments was found, determined by the HAMD and CGI score ($P = 0.05$). The patients were treated with tricyclic antidepressants, noradrenergic and serotonergic specific agents, selective norepinephrine reuptake inhibitors. Even if after correction for multiple testing (Bonferroni) these results did not remain significant, these findings suggest that the presence of the C allele might be an indicator for antidepressant treatment response.

5.4. Dopaminergic receptors

The potentiation of dopamine transmission by antidepressant treatments, as revealed by the increased motor stimulant response to dopamine agonists in experiment animals, takes place after 2-3 weeks of treatment, and it is still present 3 days, but not 10 days after treatment discontinuation. Antidepressant treatments showed to enhance dopaminergic neurotransmission by increasing the behavioural sensitivity to the stimulation of dopamine receptors in the mesolimbic dopamine system, and such super sensitivity might underlie the antidepressant therapeutic effect. The only AD that ignores the serotonin system and acts exclusively on dopaminergic and adrenergic systems is bupropion (Civelli 1995).

At least five different dopamine receptors were identified: D1, D2, D3, D4, D5/D1b. Dopamine Receptor D2 (DRD2) was mapped on 11q23 (Grandy 1989). This G-protein coupled receptor inhibits adenylyl cyclase activity. Alternative splicing of this gene results in two transcript variants encoding different isoforms: D2S and D2L. A third variant has been described, but it has not been determined whether this form is normal or due to aberrant splicing. these receptors have distinct functions in vivo; D2L acts mainly at postsynaptic sites and D2S serves presynaptic autoreceptor functions, inhibiting D1 receptor-mediated functions. The coding sequence is interrupted by 6 introns. The additional amino acids present in the human pituitary receptor are encoded by a single exon of 87 base pairs. DRD2 gene extends over 270 kb and includes an intron of approximately 250 kb separating the putative first exon from the exons encoding the receptor protein. D2-receptor-deficient mice are akinetic and bradykinetic in behavioural tests and showed significantly reduced spontaneous movements (Balk 1995).

DRD2 seems to exert a central role in the neuromodulation of appetitive behaviours, as DRD2 knockout mice show a total suppression of rewarding behaviour with morphine, with normal responses when food was used as a reward. Knockout studies suggested a relevant behavioural activity of DRD2 (Balk 1995). D2-receptor-deficient animals were akinetic and bradykinetic in behavioural tests and showed significantly reduced spontaneous movements. The human D2 receptor (DRD2) gene has been cloned and mapped to 11q22-23 (Grandy 1989).

Itokawa et al. (1993) reported a putatively functional polymorphism causing a structural change from Serine to Cysteine at codon 311 of DRD2 (S311C). The signal transducing action of the DRD2 receptor following ligand binding is to inhibit cAMP synthesis and the Cys311 variant is less effective in inhibiting it.

DRD4 showed considerable homology to DRD2 and to DRD3. Mutant mice show to be less active than wild type controls in open field tests in both novel and familiar environments. However, mutant mice outperformed wild type mice on the rotarod and displayed locomotor super sensitivity to ethanol, cocaine, and methamphetamine. Biochemical analyses indicated that dopamine synthesis and its conversion to DOPAC were elevated in the dorsal striatum of mutant mice. Rubinstein et al. (1997) proposed that DRD4 modulates normal, coordinated, and drug-stimulated motor behaviours as well as the activity of nigrostriatal dopamine neurons. Dopamine D4 receptor activation was shown to inhibit presynaptically glutamatergic neurotransmission in the rat supraoptic nucleus (Price 2001).

DRD4 was mapped on chromosome 11p15.5; it is one of the most variable human genes known. Most of this diversity is the result of length and single-nucleotide polymorphism (SNP) variation in a 48-bp VNTR in exon 3 (Van Tol 1992), which encodes the third intracellular loop of this dopamine receptor. Variant alleles containing 2 (2R) to 11 (11R) repeats are found, with the resulting proteins having 32 to 176 amino acids at this position. The frequency of these alleles varies widely, the 4-fold repeat (D4.4) being most frequent.

Concerning Dopaminergic receptors, our group investigated the possible association of the DRD2 (Ser 311Cys) and DRD4 exon 3 VNTR gene variants with the antidepressant activity of selective serotonin reuptake inhibitors (SSRIs). The sample consisted of 266 depressed inpatients treated with fluvoxamine 300 mg/day, and 98 treated with paroxetine, 20-40 mg/day. The severity of depressive symptoms was assessed weekly with the Hamilton Rating Scale for Depression. DRD2 and DRD4 allelic resulted not associated with response to treatments. Possible stratification factors, such as sex, diagnosis, presence of psychotic features, depressive symptoms at baseline, paroxetine and fluvoxamine plasma levels, and pindolol augmentation did not significantly influence the observed results (Serretti 2001).

5.5. Serotonergic receptors

Serotonin (5HT) is found in neuronal cell bodies clustered specifically in the brainstem. The axons of these cells, however, innervate almost every area of the central nervous system. Beside its fundamental role in mood tone, 5HT is also involved in eating, sleeping, sexual behaviour, the circadian cycle, and other neuroendocrine functions. In addition, the serotonergic pathway is the main target of SSRIs, the most widely used antidepressant compounds.

There are multiple subtypes of 5HT receptors, with varying affinity. They are categorised into seven main classes (5HT1-7).

5HT2A gene was mapped on chromosome 13q14-q21 (Sparkes 1991); it consists of 3 exons separated by 2 introns and spans over 20 kb. When the 5HT2A receptor is stimulated by 5HT, it causes the production of second

messengers, modulating phosphatidylinositol production and intracellular Ca2+ flux. The activation of 5HT2A receptors of medial prefrontal cortex and anterior cingulate cortex is thought to mediate the hallucinogenic properties of LSD, whereas in amygdala 5HT2A receptor activation is a component of the response. The 5HT2A receptors may mediate some of the antidepressant effects seen in experimental animal models of depression (Skrebuhhova 1999). Nefazodone exerted its antidepressant effects partially through 5HT2A receptor antagonism (Hemrick-Luecke 1994).

A polymorphism in the promoter region was identified (A-1438G) which creates an HpaII restriction site (Spurlock 1998) and associated to clozapine response (Arranz 1998). A silent polymorphism T102C was identified by Warren (1993) and was associated to individual responses to risperidone (Lane 2002) and clozapine (Arranz 1995).

A study was performed to investigate whether -1438G/A polymorphism in the promoter region of the 5-HT2A receptor gene is associated with therapeutic response to fluvoxamine in 66 Japanese patients with major depressive disorder. Fifty-four patients completed the study, and the genotype distribution and the allele frequencies showed no significant difference between responders and non-responders. The time-course of the Montgomery-Asberg Depression Rating Scale scores showed no significant difference among -1438G/G, -1438G/A, and -1438A/A genotype groups (Sato 2002).

Our group tested the effect of 5HT2A gene T102C variants on the antidepressant activity of fluvoxamine and paroxetine in the same sample of MAOA study (248 major and 195 bipolar depressed patients, with or without psychotic features, see above), and we observed a marginal association between 5HT2A variants and antidepressant response (Cusin 2002). Another paper, besides investigating association between T102C polymorphism and major depression, tested the association with antidepressant treatment response in a sub-sample of patients; the treatment were non standardized and the sub-sample size was exiguous but they find an association between good response (calculated as a decrease in HAMD 17 and CGI item 1) and the presence of C allele (Minov 2001).

5HT6 receptor is G protein coupled and stimulates adenylyl cyclase. In the rat it shows high affinity for several therapeutically important antidepressant such as mianserin and clomipramine and antipsychotic drugs, particularly the atypical antipsychotics such as clozapine. A mutant receptor in which serine 267 in the c-terminal region of the third intracellular loop was changed to lysine shows 10-fold higher affinity for serotonin than the native receptor and demonstrates agonist-independent activity; clozapine decreased the basal activity of this mutated receptor to vector control levels.

Kohen et al. (1996) cloned and characterized the human serotonin 5HT6 receptor gene, which encodes a 440-amino acid polypeptide and mapped to human chromosome 1p36-p35, not far from the location of the 5HT1D alpha receptor. They showed that the receptor is expressed in several human brain regions, most prominently in the caudate nucleus. Study of genomic clones revealed that the open reading frame is interrupted by 2 introns in positions corresponding to the third cytoplasmic loop and the third extracellular loop,

respectively. 5HT6 receptor antagonists seem to improve retention performance and behavioural studies on animals implicated a role for 5HT6 in cognition enhancement: this has been supported by in vivo microdialysis studies that showed how a selective 5HT6 antagonist produce an increase in extracellular glutamate levels in the frontal cortex.

A silent thymidine to cytosine polymorphism at position 267 (TC 267), within the first exon of HTR6, has been identified[96]. This genetic polymorphism was investigated for association with antidepressant response in 34 Major Depressive Disorder patients completing a 4-week treatment with various antidepressants, administered at not fixed doses, assessed with the HAMD before antidepressant treatment and at the end of the trial. The results provide a lack of association between C267T genetic variants and antidepressant response for these patients (Wu 2001).

5.6. Serotonin transporter

The brain 5HT transporter (SERT) appears to be a principal site of action of many antidepressants and may mediate behavioural and toxic effects of cocaine and amphetamines. Its role is to take up 5HT into the presynaptic neuron, which thus terminates the synaptic actions and recycles it into the neurotransmitter pool.

Ramamoorthy et al. (1993) identified and cloned a single gene encoding the human SERT, localised to chromosome 17q11.1-q12. The gene spans 31 kb and consists of 14 exons (Lesch 1994). Ogilvie et al. (1996) identified a variable number tandem repeat (VNTR) polymorphisms. Heils et al. (1996) reported a polymorphism in the transcriptional control region upstream of the 5HTT coding sequence (SERTPR). Initial experiments demonstrated that the long and short variants of this 5HTT gene-linked polymorphic region have different transcriptional efficiencies. The polymorphism is located approximately 1 kb upstream of the transcription initiation site and is composed of 16 repeat elements. It consists of a 44-bp insertion or deletion involving repeat elements 6 to 8. Lesch et al. (1996) called it 5-HTTLPR and studying lymphoblastoid cell lines found that the basal activity of the long variant was more than twice that of the short form of the 5HTT gene promoter. They next studied the expression of the native 5HTT gene in lymphoblast cell lines cultured from subjects with different 5HTT promoter genotypes. Cells homozygous for the l form produced steady-state concentrations of 5HTT transporter mRNA that were 1.4 to 1.7 times those in cells containing 1 or 2 copies of the s variant. At the protein level, membrane preparations from l/l lymphoblasts bound 30 to 40% more of a labelled marker than did membranes from l/s or s/s cells. Moreover, uptake of labelled serotonin in cells homozygous for the 1 form of the promoter polymorphism was 1.9 to 2.2 times that in cells carrying 1 or 2 endogenous copies of the s variant. In all of their studies, the data associated with the s/s and l/s genotypes were similar, whereas both differed from the l/l genotype, suggesting that the polymorphism has a dominant-recessive effect.

Our group performed several studies on SERPR (the new official name of 5HTTLPR polymorphism), the firs paper being published in 1998. We tested the

hypothesis that allelic variation of the 5-HTT promoter could be related to the antidepressant response to fluvoxamine and/or augmentation with pindolol. 102 inpatients with major depression with psychotic features were randomly assigned to treatment with fluvoxamine and either placebo or pindolol for 6 weeks. Depression severity was assessed weekly with HAMD, and allelic variations were detected by a PCR-based method. Data were analysed with a three-way repeated measures analysis of variance. Both homozygotes for the long variant (l/l) and heterozygotes (l/s) showed a better response to fluvoxamine than homozygotes for the short variant (s/s). In the group treated with fluvoxamine plus pindolol all the genotypes acted like l/l treated with fluvoxamine alone (Smeraldi 1998). A following study analysed a wider sample of major and bipolar depressives, with or without psychotic features, composed by 155 inpatients treated in the same standardized method of the previous study, and doing the same assessment of the antidepressant outcome. Also in this study SERTPR s allele was associated with a poor response to fluvoxamine treatment, and the diagnosis, the presence of psychotic features, and the severity of depressive symptomatology did not influence the association (Zanardi 2001). In a third study we investigated the persistence of the finding for another antidepressant treatment, analysing the association of alleles at SERTPR polymorphism with paroxetine treatment and evidencing an analogue finding (Zanardi 2000). The s allele was in fact associated with a less favourable and slower response, and the finding was independently replicated by Pollock et al. (2000) who examined 95 elderly patients receiving paroxetine or nortriptyline in a standardized treatment. Patients were treated for up to 12 weeks and assessed weekly with clinical ratings and measurements of plasma drug concentrations; they found a significantly more rapid mean reductions from baseline in HAMD for patients with the l/l genotype, despite equivalent paroxetine concentrations. Similar findings were obtained with citalopram, in a Spanish sample (Arias 2001).

Studies performed in Asian population showed contrasting results: Kim et al. (Kim 2000), found an association in the opposite direction, as in their sample homozygotes subjects for the s allele showed a better response both to fluvoxamine and to paroxetine. Two subsequent studies confirmed this result in Asian population: one analyzed 66 Japanese patients with major depressive disorder in a 6-week study with fluvoxamine; the short allele frequency was significantly higher in responsive individuals than in non responsive ones (Yoshida 2002). Conversely, the second study on 121 Chinese patients diagnosed with major depression revealed that patients with the l/l genotype had a significantly better response to fluoxetine, as evaluated on the basis of total, core, psychic-anxiety and somatic-anxiety HAMD score percentage change, according to the results in Caucasians populations (Yu 2002). Finally, Ito et al. studied the association between SERTPR and response to fluvoxamine prescribed up to 200 mg/day for 6 weeks in 66 patients with major depressive disorder, and found no significant association either for s or for l variant (Ito 2002). In Asian population the results are thus very conflicting, most probably the small sample sizes do not allow to draw a definite conclusion on the role of the SERTPR polymorphism.

In a recent study the presence of s allele was also associated to antidepressant-induced mania (Mundo 2001), but this finding was not confirmed in our data (Artioli 2002).

5.7. Nitric Oxide Syntase

Nitric Oxide (NO) is probably involved in the processes of learning and memory, as it influences synaptic plasticity in the striatum and elsewhere. NO is produced from its precursor L-arginine by the enzyme NO synthase (NOS), which includes at least three distinct isoforms - neuronal (NOS1), endothelial, and inducible NOS. The neuronal but so far not the endothelial NO syntase isoform (NOS3) was detected in some striatal interneurons with a large axonal arborisation. On the other hand, in the hippocampus, NOS1 is localized to GABAergic interneurons, whereas endothelial NOS is found in pyramidal neurons.

A microdialysis study in endothelial NOS- and NOS1-deficient mice revealed differences in NMDA-stimulated amino acid release in the striatal probes: GABA release was reduced in eNOS-/- and not in NOS1-/- mice, while glutamate release showed an opposite pattern (Doreulee 2003). Initial observations indicated that male NOS1-deficient mice engaged in chronic aggressive behaviour, not apparent among NOS1-deficient female mice or wild type male or female mice housed together.

Relevance of the observations to human behaviour was suggested. Voltage-dependent calcium channel antagonists have been reported to produce antidepressant-like effects in rodents. A major target of increases in subcellular calcium concentration is NOS which liberates NO in response to stimulation. In addition, NOS antagonists were shown to produce antidepressant-like response (Paul 2001). Recent studies have implicated NOS in the mechanism that underlies the therapeutic efficacy of antidepressant medication. In addition, MD patients were found to have significantly higher plasma nitrate concentrations than normal subjects, an index of NO production, in comparison to normal subjects.

The NOS1 gene was mapped to chromosome 12q24.2-q24.31. In a population-based association study, the NOS1 C276T polymorphism was investigated for its involvement in conferring susceptibility to MD, and its association to fluoxetine response in 114 MD patients who underwent a 4-week fluoxetine treatment. The results demonstrate the NOS1 variants to be found at similar frequencies in MD patients and healthy control subjects. Further, the variants did not influence the fluoxetine response in MD patients (Yu 2003).

5.8. G-protein beta 3 subunit

G proteins are key components of intracellular signal transduction in neurons and in all cells of the body (Neer 1995). They are trimers, whose function depends on the ability to dissociate into an alpha monomer bound to GTP dissociated from a beta-gamma dimmer. In the trimeric state the G protein is inactive, and it is constitutively associated with the receptor; Chronic

treatment with fluoxetine showed to attenuate GTP binding to gamma subunit in the dorsal raphe nucleus of rats, thus inducing desensitisation of 5HT1A receptors (Elena Castro 203).

Beta subunit could be subdivided into three subtypes: 1, 2 and 3. G beta3 subunit (GNB3) gene was mapped on locus 12p13, and spans 7.5 kb and is composed of 11 exons and 10 introns. Its promoter lacks a TATA box but harbours GC-rich regions. A polymorphism of a G-protein beta3 subunit (C825T) has been shown to be associated with increased signal transduction and ion transport activity (Siffert 1995); GNB3 825T variant is associated with the occurrence of the splice variant Gbeta3s, which, despite a deletion of 41 amino acids, is functionally active in reconstituted systems. Although the polymorphism did not affect the amino acid sequence of the beta-3 subunit, the T allele was associated with deletion of nucleotides 498-620 of exon 9; this was found to be an example of alternative splicing caused by a nucleotide change outside the splice donor and acceptor sites.

Gbeta3 polymorphism was associated with response to AD treatment in 88 depressive patients (10 bipolar disorder, 78 major depression) compared with 68 schizophrenic patients and 111 healthy controls. A statistical significant association was found between TT homozygosity and response to various AD treatments after four weeks (Zill 2000). In our centre we could replicate this finding in a sample of 490 subjects, bipolars and major depressives, treated with SSRIs fluvoxamine and paroxetine. Subjects with Gbeta3 T/T variants showed better response to treatment ($p=0.009$) and this effect was independent from analysed demographic and clinical variables (Serretti 2003). These are to our knowledge the first studies investigating association between a polymorphism in a molecule involved in intracellular signal transduction pathway and AD action.

5.9. Angiotensin Converting Enzyme

Angiotensin I-converting enzyme, or kininase II, is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation and electrolyte balance. Although angiotensin-converting enzyme has been studied primarily in these contexts, this widely distributed enzyme has many other physiologic functions. The ACE gene encodes 2 isozymes (Ramaraj 1998).

Ehlers et al. (Ehlers 1989) determined the cDNA sequence for human testicular ACE, identical, from residue 37 to its C terminus, to the second half or C-terminal domain of the endothelial ACE sequence. The inferred protein sequence consists of a 732-residue preprotein including a 31-residue signal peptide. Using a DNA marker at the growth hormone gene locus, which they characterized as ‘extremely polymorphic’ and which showed no recombination with ACE, Jeunemaitre et al. (1992) mapped ACE to 17q22-q24.

After the ACE gene was cloned, it was shown that 50% of the interindividual variability of plasma ACE concentration is determined by an insertion (I)/deletion (D) polymorphism situation in intron 16 of the ACE gene and known as the ACE I/D polymorphism (Rigat 1990), with the D allele showing dominance rather than codominance relative to the I allele (Jeffery 1999).

Angiotensin-converting enzyme (ACE) inhibitor has mood-elevating effects (Vuckovic 1991), and central ACE activity is increased for suicidal patients (Arregui 1979). In addition, substance P (SP), which is degraded by ACE, has been implicated in the pathogenesis of depressive symptoms (Arinami 1996) and evaluated in the treatment for MD (Kramer 1998).

A study on 99 Caucasians unrelated patients with MD and 99 age- and sex-matched healthy controls from the general population, rated with the Hamilton rating scale for depression (HAM-D17) and the clinical global impression scale (CGI, Item 1 - severity of disease) and receiving different treatments (tricyclic anti-depressants, mirtazapine, selective serotonin reuptake inhibitors, venlafaxine, electroconvulsive therapy, repetitive transcranial magnetic stimulation and combinations, found that after 4 weeks of treatment, D-allele carriers showed significantly lower HAM-D17 scores, remitted more often and had a significantly shorter duration of hospitalisation. Also, the number of treatment alterations during hospitalisation was significantly higher in I/I-genotypes. Thus, the D-allele might positively influence the onset of therapeutic efficacy, while homozygosity for the I-allele seems to be associated with delayed response (Baghai 2001). Another recent study has tested the hypothesis that an ACE-gene I/D polymorphism could be associated with antidepressant response in 58 MD patients treated with venlafaxine slow-release 75mg or fluoxetine 20 mg, not replicating the previous finding (Hong 2002).

5.10. Interleukin 1-beta

Interleukin-1, produced mainly by blood monocytes, mediates the host reactions of acute phase response. It is identical to endogenous pyrogen. The interleukin-1 (IL-1) complex consists of 3 linked genes mapping to chromosome 2q13-14 that encoding the secreted glycoproteins IL-1, IL-1, and IL-1 receptor antagonist (IL-1Ra). All three molecules bind to IL-1 receptors. Auron et al. (1984) isolated human IL1 cDNA, while Webb et al. (Auron 1985) assigned the IL1 gene to chromosome 2q13-q21. IL1B was assigned to the end of 18q (Le Beau 1986). Patterson et al. (1993) assigned the IL1RN gene to 2q14.2, mapping the human genomic region containing the 3 related genes (Patterson 1993).

Owen et al. (2000) report elevated plasma concentrations of interleukin-1 (IL-1) in major depression and post-viral depression. Previously it has been reported that the production rate of IL-1 is increased in dysthymia and major depression (Maes 1993; Anisman 1999 and 2002); thus, increased production of IL-1 and other proinflammatory cytokines may provide a mechanism for the psychological and organic etiologies of the disease. The acute stage of unipolar and bipolar depression is accompanied by activation of the inflammatory response system (IRS), with an increase in the number and percentage of peripheral blood leukocytes, neutrophils and activated T cells, increased neopterin secretion in serum and urine, an increased production of prostaglandins in plasma or cerebrospinal fluid (CSF), and the presence of an acute phase response (Maes 1999).

IL1 has behavioural effects, and may induce a behavioural complex in female rats, called sickness behaviour, characterized by loco-motor retardation,

sleep disorders, soporific effects, anorexia, weight loss, hyperalgesia, decreased social exploration, and inhibition of sexual behaviour (Maier 1998). The mechanisms whereby IRS activation may induce depression is still not known, but it is hypothesised that modulation of the HPA-axis activity, with increased corticotropin-releasing hormone (CRH) secretion, and modulation of the serotonergic turnover may be involved.

Prolonged desipramine administration (seven and 28 days) significantly increased the bioactivity of IL-1 (Kubera 2000), and several lines of evidence indicate that brain cytokines, principally IL-1beta and IL-1 receptor antagonist may have a role in the biology of major depression, and that they might additionally be involved in the pathophysiology and somatic consequences of depression as well as in the effects of antidepressant treatment (Licinio 1999).

Four SNPs have been reported in the IL-1 gene: -31C/T (promoter), -511C/T (promoter), +3954C/T (exon 5) and A/G (intron 4) at position 5810 (Di Giovine 1992; Pociot 1992; Guasch 1996). The -31 SNP is in strong linkage disequilibrium with the -511 SNP [148]. The association of the biallelic polymorphism -511C/T, located in the promoter region of the IL-1beta gene, to fluoxetine response was studied in 119 MD patients who received a 4-week fluoxetine treatment. MD patients who were homozygous for the -511T allele of the IL-1beta gene had a trend of less severity of depressive symptoms and more favourable fluoxetine response than -511C carriers (Yu 2003).

6. PERSPECTIVES

6.1. New approaches in molecular studies

Molecular genetic techniques are changing very rapidly. Until recently, few hundreds evenly spaced markers on the whole genome were used for genome scans, but it has been shown that disequilibrium may be not detectable for distances larger than 60 kb, or even less in hot regions (Ardlie 2002). This leaded to the use of the single nucleotide polymorphism (SNP), the most abundant type of polymorphism in the genome. SNPs occur about once every 1000 base pairs, there are thus more than 3 million of them in the genome.

Performing simultaneously large numbers of SNP genotypes using “gene chips” (Lipshutz 1999; Service R.F. 1998; Shoemaker 2001), which are small slides where thousands of genes may be analysed at time, a lot of information could be collected. Despite their potential usefulness, their use is still limited by the high costs (about 1000 USD each) and the lack of adequate statistical analysis for the large amount of data they provide. In fact a simultaneous analysis of thousands of genes, many of which are non functional or which are not related with the trait under analysis, leads to an unacceptable risk of false positive findings.

6.2. Research strategies

The emergence of pharmacogenetics will require advances in the selection of appropriate candidate genes. Such genes are to be sought for among those related to the mechanism of drug action and illness pathophysiology.

Drug response is just as complex as disease genetics, resulting not only from underlying genotypic variation at several mostly unknown loci, but also from variation in gene expression, post-translational modification of proteins, drug dose, drug interactions, diet, and other non-genetic factors. Therefore, we expect to see relatively slight effects of individual genes regarding drug response as well. In fact, pharmacogenomic markers reported on to date confer only about a twofold increased likelihood of response (Poirier 1995; Drazen 1999).

The pharmaceutical industry has the potential to apply pharmacogenomics strategies; pre-marketing studies could include genetic analyses in order to identify both whether a compound is clinically effective and for whom it is likely to be most effective (Pickar 2001).

6.3. Genetic counseling

Genetic counseling (GC) evolved with the aim to collect information about families burdened by some disease, to estimate and to communicate the risk of contracting it. In last years, the applications of GC have become increasingly wider, due to the possibility of DNA-level genetic test and to the knowledge deriving from the Human Genome Project.

The purposes of contemporary GC are formulated as strongly non-directive. The objectives are to provide a possibly neutral information, to protect and promote the personal autonomy, to help the individuals directing their lives in accord to their own liking. For long time, GC has been a rather uncommon practice for clinicians, concerning only rare conditions in especially burdened "risk-families". However, due to the fast development of technologies, genetic test and GC will soon be utilised for a much larger population and for a wider spectrum of conditions.

With the completion of the SNP map of the whole genome, it could be possible to enhance the power of genetic studies. Several genotyping platforms have been developed, including nucleic acid hybridisation on filter (Orr 2002), gene chips (Anthony 2001), single strand conformational polymorphism (Nataraj 1999) and primer-extension methods (Kwok 2000), but at present the expensiveness of these technologies makes their large scale use impossible. In the future they may potentially allow rapid and cost effective screens for all the possible mutation and sequence variation in genomic DNA.

Maybe it will not address on traditional genetic counseling targets, because heritability of the disease is complex and influenced by many variables. It could be rather addressed on the direction to make prediction about drug response and adverse effects, to choose the most appropriate and effective treatment for each schizophrenic patient.

In a future, a kit for genetically determined characterisation of MD would be developed, involving multiple genes relevant to the disease characteristics

and to the therapeutic outcome. This fact could lead to the selection of the medication and dosages, to minimise adverse effects and to reduce the overall direct treatment costs, with a considerable improvement of the quality of life and other functioning aspect (Kawanishi 2000).

6.4. Ethical issues

A great deal of works and books have been written about the ethics of genetic studies, including issues surrounding informed consent, DNA storage and communication of information regarding genetic features of individuals. The study of psychiatric disorders represents a particular challenge for the uncertain and complex nature of its pathogenesis and the stigma surrounding mental health. The particular nature these disorders, which give an emotional but also a cognitive impairment rises problems concerning the freedom of choice and the accessibility of information, the basic features for informed consent. Currently, from an ethical and legal perspective an individual with a psychiatric diagnosis is nor necessarily deemed to be incompetent, to lack the capacity to give informed consent or to oppose a refusal to participation in a genetic study.

The general greater paternalism in the research environment is justified by a lot of reasons. Nevertheless, it should leave some place to a general favourable opinion for a research made to follow the wellbeing of the human community, instead of worrying excessively about the privacy of the single person. Basically, the human community is made of single individuals, who should take benefit of the science conquests, even if the price to pay is to give up a little bit of their privacy. In fact, recent views about ethical principles of research makes wish of this new trend, by underlying the importance of research for the future of psychiatric treatments (Forster 2001; Human 2001; Ramsay 1999).

7. TABLE

Table. Pharmacogenetic studies on major depressive disorder treatments

Ref.	Gene	Drug	Results
103	SERTPR	Fluvoxamine	l allele subjects were more likely to respond (p=0.017)
104	SERTPR	Fluvoxamine	l allele subjects were more likely to respond (all sample p=0.029 - without pindolol p=0.002)
105	SERTPR	Paroxetine	s allele associated with less favourable and slower response (p<0.001)
106	SERTPR	Paroxetine	s allele associated with slower response (p=0.028)
108	SERTPR SERT-VNTR (intron 2)	Fluoxetine, Paroxetine	s/s genotype showed better response (p=0.007)
109	SERTPR	Fluvoxamine	s variant more frequent in responsive individuals than in non responsive
107	SERTPR	Citalopram	s/s genotype was significantly more frequent in no remission group (p=0.006)
111	SERTPR	Fluvoxamine	No association
110	SERTPR	Fluoxetine	l/l genotype shows a better response (p=0.013)
112	SERTPR	SSRI, TCA	Patients with manic or hypomanic episodes induced by AD treatment had an excess of s alleles (p<0001)
63	TPH A218C	Fluvoxamine	A/A genotype was associated with slower response (no pindolol p=0.001)
64	TPH A218C	Paroxetine	A/A and A/C genotypes were associated with slower response (no pindolol p=0.005)
65	MAOA VNTR, TPH A218C	Fluvoxamine	No association in a Japanese sample
71	MAOA VNTR	Moclobemide	No association
72	MAOA VNTR, 5HT2A T102C	Fluvoxamine, paroxetine	Marginal association between 5HT2A C variants and AD response; No association with MAOA genotypes
95	5HT2A T102C	Various ADs	Association between C variants and AD response
94	5HT2A -1438G/A	Fluvoxamine	No association
97	5HT6 C267T	Venlafaxine, fluoxetine	No association
85	DRD2 S311C, DRD4 VNTR	Fluvoxamine, paroxetine	No association
132	ACE I/D polymorphism	Venlafaxine, Fluoxetine	No association
131	ACE I/D polymorphism	Various ADs	D allele associated with better outcome
120	G-protein beta3 C825T	SSRI, TCA, ECT, combinations	TT homozygous associated with response (p=0.01)

Table (continued): Pharmacogenetic studies on major depressive disorder treatments

Ref	Gene	Drug	Results
121	G-protein beta3 C825T	Fluvoxamine, paroxetine	TT homozygous associated with response (p=0.009)
164	ADRB1 G1165C	TCA SSRI, NARI, NSRI	tendency for association between CC homozygosity and better and faster antidepressant response
116	NOS C276T polymorphism	Fluoxetine	No association
149	IL-1beta C-511T polymorphism	Fluoxetine	homozygous for the -511T allele had a trend of more favorable fluoxetine response

8. REFERENCES

Ackerknecht, E. H., 1959, A short history of psychiatry. New York: Harper and Row.

American Psychiatric Association, 1994, Practice guideline for the treatment of patients with bipolar disorder. American Psychiatric Association, *Am J Psychiatry* **151**, 1-36.

American Psychiatric Association, 1997, Practice guideline for the treatment of patients with schizophrenia. American Psychiatric Association, *Am J Psychiatry* **154**, 1-63.

American Psychiatric Association, 2000, Practice guideline for the treatment of patients with major depressive disorder (revision). American Psychiatric Association, *Am J Psychiatry* **157**, 1-45.

Angst, F., Stassen, H. H., Clayton, P. J., Angst, J., 2002, Mortality of patients with mood disorders: follow-up over 34-38 years, *J Affect Disord* **68**, 167-81.

Angst, J., 1995, The epidemiology of depressive disorders, *Eur Neuropsychopharmacol* **5** Suppl, 95-8.

Anisman, H., Kokkinidis, L., Merali, Z., 2002, Further evidence for the depressive effects of cytokines: anhedonia and neurochemical changes, *Brain Behav Immun* **16**, 544-56.

Anisman, H., Ravindran, A. V., Griffiths, J., Merali, Z., 1999, Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features, *Mol Psychiatry* **4**, 182-8.

Anthony, R. M., Brown, T. J., French, G. L., 2001, DNA array technology and diagnostic microbiology, *Expert Rev Mol Diagn* **1**, 30-8.

Ardlie, K. G., Kruglyak, L., Seielstad, M., 2002, Patterns of linkage disequilibrium in the human genome, *Nat Rev Genet* **2**, 299-309.

Arias, B., Catalan, R., Gasto, C., Imaz, M. L., Gutierrez, B., Pintor, L., Fananas, L., 2001, Genetic variability in the promoter region of the serotonin transporter gene is associated with clinical remission of major depression after long term treatment with citalopram. *World Federation of societies of Biological Psychiatry,* Berlin, Germany: 9S.

Arinami, T., Li, L., Mitsushio, H., Itokawa, M., Hamaguchi, H., Toru, M., 1996, An insertion/deletion polymorphism in the angiotensin converting enzyme gene is associated with both brain substance P contents and affective disorders, *Biol Psychiatry* **40**, 1122-7.

Arranz, M. J., Munro, J., Owen, M. J., Spurlock, G., Sham, P. C., Zhao, J., Kirov, G., Collier, D. A., Kerwin, R. W., 1998, Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine, *Mol Psychiatry* **3**, 61-6.

Arranz, M., Collier, D., Sodhi, M., Ball, D., Roberts, G., Price, J., Sham, P., Kerwin, R., 1995, Association between clozapine response and allelic variation in 5-HT2A receptor gene, *Lancet* **346**, 281-2.

Arregui, A., MacKay, A. V., Iversen, L. L., Spokes, E. G., 1979, Reduction of angiotensin-converting enzyme in substantia nigra in early-onset schizophrenia, *N Engl J Med* **300**, 502-3.

Artioli, P., Serretti, A., Lattuada, E., Lorenzi, C., Lilli, R., Smeraldi, E., Clinical and genetic features of antidepressant induced mania in bipolar disorder. Xth World Congress of Psychiatric Genetics 2002, Brussels: 755.

Auron, P. E., Rosenwasser, L. J., Matsushima, K., Copeland, T., Dinarello, C. A., Oppenheim, J. J., Webb, A. C., 1985, Human and murine interleukin 1 possess sequence and structural similarities, *J Mol Cell Immunol* **2**, 169-77.

Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M., Dinarello, C. A., 1984, Nucleotide sequence of human monocyte interleukin 1 precursor cDNA, *Proc Natl Acad Sci U S A* **81**, 7907-11.

Baghai, T. C., Schule, C., Zwanzger, P., Minov, C., Schwarz, M. J., de Jonge, S., Rupprecht, R., Bondy, B., 2001, Possible influence of the insertion/deletion polymorphism in the angiotensin I-converting enzyme gene on therapeutic outcome in affective disorders, *Mol Psychiatry* **6**, 258-9.

Balk, J., Picetti, R., Salardi, A., Thirlet, G., Dierich, A., Depaulls, A., Le Meur, M., Borrelli, E., 1995, Parkinsonian-like locomotorimpairment in mice lacking dopamine D2 receptors, *Nature* **377**, 424-428.

Bassuk, S. S., Berkman, L. F., Wypij, D., 1998, Depressive symptomatology and incident cognitive decline in an elderly community sample, *Arch Gen Psychiatry* **55**, 1073-81.

Bauwens, F., Tracj, A., Pardoen, D., Vander Elst, M., Mendlewicz, J., 1991, Social adjustment of remitted Bipolar and Unipolar out-patients. A comparison with age-and sex-matched controls, *Brit J Psychiatry* **159**, 239-244.

Berrettini, W., 1998, Progress and pitfalls: bipolar molecular linkage studies, *J Affect Disord* 50, 287-97.

Berry, M. D., Juorio, A. V., Paterson, I. A., 1994, The functional role of monoamine oxidases A and B in the mammalian central nervous system, *Progress in Neurobiology* **42**, 375-391.

Boularand, S., Darmon, M. C., Ganem, Y., Launay, J. M., Mallet, J., 1990, Complete coding sequence of human tryptophan hydroxylase, *Nucleic Acid Res* **18**, 42-57.

Burvill, P. W., Johnson, G. A., Jamrozik, K. D., Anderson, C. S., Stewart-Wynne, E. G., Chakera, T. M., 1995, Prevalence of depression after stroke: the Perth Community Stroke Study, *Br J Psychiatry* **166**, 320-7.

Bylund, D. B., Eikenberg, D. C., Hieble, J. P., Langer, S. Z., Lefkowitz, R. J., Minneman, K. P., Molinoff, P. B., Ruffolo, R. R., Jr., 1994, Trendelenburg U., International Union of Pharmacology nomenclature of adrenoceptors, *Pharmacol Rev* **46**, 121-36.

Carney, R. M., Blumenthal, J. A., Stein, P. K., Watkins, L., Catellier, D., Berkman, L. F., Czajkowski, S. M., O'Connor, C., 2001, Stone P. H., Freedland K. E., Depression, heart rate variability, and acute myocardial infarction, *Circulation* **104**, 2024-8.

Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J. C., De Maeyer, E., 1995, Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA, *Science* **268**, 1763-6.

Catalano, M., 1999, The challenges of psychopharmacogenetics, *Am J Hum Genet* **65**, 606-10.

CavalliSforza, L., 1997, Genes, Peoples, and Languages: University of California Press.

Civantos Calzada, B., Aleixandre de Artinano, A., 2001, Alpha-adrenoceptor subtypes, *Pharmacol Res* **44**, 195-208.

Civelli, O., 1995, Molecular biology of the dopamine receptor subtypes. In: Bloom FE, Kupfer DJ, eds: Psychopharmacology: the fourth generation of progress. New York: Raven press, pp. 155-161.

Cooper, B., 1986, Mental disorder as reaction: the history of a psychiatric concept. In: HK, ed: Life events and Psychiatric disorders: Controversial Issues. *Cambridge: Cambridge University Press.* pp.1-32.

Craig, S. P., Boularand, S., Darmon, M. C., Mallet, J., Craig, I. W., 1991, Localization of human tryptophan hydroxylase (TPH) to chromosome 11p15.3—p14 by in situ hybridization, *Cytogenet Cell Genet* **56**, 157-9.

Cusin, C., Serretti, A., Zanardi, R., Lattuada, E., Rossini, D., Lilli, R., Lorenzi, C., Smeraldi, E., 2002, Influence of monoamine oxydase A and serotonin receptor 2A polymorphisms in SSRIs antidepressant activity, *Int J Neuropsychopharmacol* **5**, 27-35.

Denney, R. M., Koch, H., Craig, I. W., 1999, Association between monoamine oxidase A activity in human male skin fibroblasts and genotype of the MAOA promoter-associated variable number tandem repeat, *Hum Genet* **105**, 542-51.

Dettling, M., Cascorbi, I., Roots, I., Mueller-Oerlinghausen, B., 2001, Genetic determinants of clozapine-induced agranulocytosis: recent results of HLA subtyping in a non-jewish caucasian sample, *Arch Gen Psychiatry* **58**, 93-4.

Di Giovine, F., Takhsh, E., Blakemore, A., 1992, Single base polymorphism at -511 in the human interleukin-1 gene (IL1)., *Hum Mol Genet* **1**, 450.

Doreulee, N., Sergeeva, O. A., Yanovsky, Y., Chepkova, A. N., Selbach, O., Godecke, A., Schrader, J., Haas, H. L., 2003, Cortico-striatal synaptic plasticity in endothelial nitric oxide synthase deficient mice, *Brain Res* **964**, 159-63.

Drazen, J. M., Yandava, C. N., Dube, L., Szczerback N., Hippensteel, R., Pillari, A., Israel, E., Schork, N., Silverman, E. S., Katz, D. A., Drajesk, J., 1999, Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment, *Nat Genet* **22**, 168-70.

Ehlers, M. R., Fox, E. A., Strydom, D. J., Riordan, J. F., 1989, Molecular cloning of human testicular angiotensin-converting enzyme: the testis isozyme is identical to the C-terminal half of endothelial angiotensin-converting enzyme, *Proc Natl Acad Sci U S A* **86**, 7741-5.

Elena Castro, M., Diaz, A., del Olmo, E., Pazos, A., 2003, Chronic fluoxetine induces opposite changes in G protein coupling at pre and postsynaptic 5-HT(1A) receptors in rat brain, *Neuropharmacology* **44**, 93-101.

El-Omar, E. M., Carrington, M., Chow, W. H., McColl, K. E., Bream, J. H., Young, H. A., Herrera, J., Lissowska, J., Yuan, C. C., Rothman, N., Lanyon, G., Martin, M., Fraumeni, J. F., Jr., Rabkin, C. S., 2000, Interleukin-1 polymorphisms associated with increased risk of gastric cancer, *Nature* **404**, 398-402.

Forster, H. P., Emanuel, E., Grady, C., 2001, The 2000 revision of the Declaration of Helsinki: a step forward or more confusion?, *Lancet* **358**, 1449-53.

Franchini, L., Serretti, A., Gasperini, M., Smeraldi, E., 1998, Familial concordance of fluvoxamine response as a tool for differentiating mood disorder pedigrees, *J Psychiatr Res* **32**, 255-259.

Gao, B., Kunos, G., 1998, Cell type-specific transcriptional activation and suppression of the alpha1B adrenergic receptor gene middle promoter by nuclear factor 1, *J Biol Chem* **273**, 31784-7.

Gershon, E. S., Hamovit, J., Guroff, J. J., Dibble, E., Leckman, J. F., Sceery, W., Targum, S. D., Nurnberger, J. I. J., Goldin, L. R., Bunney, W. E. J., 1982, A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands., *Arch Gen Psychiatry* **39**, 1157-1167.

Grandy, D., Litt, M., Allen, L., Bunzow, J., Marchionni, M., Makam, H., Reed, L., Magenis, R., Civelli, O., 1989, The human D2 dopamine receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP, *Am J Hum Genet* **45**, 778-785.

Guasch, J. F., Bertina, R. M., Reitsma, P. H., 1996, Five novel intragenic dimorphisms in the human interleukin-1 genes combine to high informativity, *Cytokine* **8**, 598-602.

Hamilton, M., 1967, Development of a rating scale for primary depressive illness, *Br J Soc Clin Psychol* **6**, 278-296.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., Lesch, P., 1996, Allelic variation of human serotonin trasporter gene expression., *J Neurochem* **66**, 2621-2624.

Hemrick-Luecke, S. K., Snoddy, H. D., Fuller, R. W., 1994, Evaluation of nefazodone as a serotonin uptake inhibitor and a serotonin antagonist in vivo, *Life Sci* **55**, 479-83.

Hong, C. J., Wang, Y. C., Tsai, S. J., 2002, Association study of angiotensin I-converting enzyme polymorphism and symptomatology and antidepressant response in major depressive disorders, *J Neural Transm* **109**, 1209-14.

Human, D., 2001, Declaration of Helsinki, *Lancet* **357**, 236.

Ito, K., Yoshida, K., Sato, K., Takahashi, H., Kamata, M., Higuchi, H., Shimizu, T., Itoh, K., Inoue, K., Tezuka, T., Suzuki, T., Ohkubo, T., Sugawara, K., Otani, K., 2002, A variable number of tandem repeats in the serotonin transporter gene does not affect the antidepressant response to fluvoxamine, *Psychiatry Res* **111**, 235-9.

Itokawa, M., Arinami, T., Futamura, N., Hamaguchi, H., Toru, M., 1993, A structural polymorphism of human dopamine D2 receptor, D2(Ser311–>Cys), *Biochem Biophys Res Commun* **196**, 1369-75.

Jeffery, S., Saggar Malik, A. K., Crosby, A., Bland, M., Eastwood, J. B., Amoah-Danquah, J., Acheampong, J. W., Plange-Rhule, J., 1999, A dominant relationship between the ACE Dallele and serum ACE levels in a Ghanaian population, *J Med Genet* **36**, 869-70.

Jeunemaitre, X., Rigat, B., Charru, A., Houot, A. M., Soubrier, F., Corvol, P., 1992, Sib pair linkage analysis of renin gene haplotypes in human essential hypertension, *Hum Genet* **88**, 301-6.

Johnson, M., Hanson, G. R., Gibb, J. W., 1989, Characterization of acute N-ethyl-3, 4-methylenedioxyamphetamine (MDE) action on the central serotonergic system, *Biochem Pharmacol* **38**, 4333-8.

Jonsson, E. G., Goldman, D., Spurlock, G., Gustavsson, J. P., Nielsen, D. A., Linnoila, M., Owen, M. J., Sedvall, G. C., 1997, Tryptophan hydroxylase and catechol-O-methyltransferase gene polymorphisms: relationships to monoamine metabolite concentrations in CSF of healthy volunteers, *Eur Arch Psychiatry Clin Neurosci* **247**, 297-302.

Kaplan, H. I., Sadock, B. J., 1995, Comprehensive Textbook of Psychiatry VI. 6th ed. Baltimore, Maryland: The Williams and Wilkins Company.

Kawanishi, Y., Tachikawa, H., Suzuki, T., 2000, Pharmacogenomics and schizophrenia, *Eur J Pharmacol* **410**, 227-241.

Kendler, K. S., Neale, M. C., Kessler, R. C., 1992, Heath A. C., Eaves L. J., Major depression and generalized anxiety disorder. Same genes, (partly) different environments?, *Arch Gen Psychiatry* **49**, 716-22.

Kendler, K. S., Walters, E. E., Neale, M. C., Kessler, R. C., 1995, Heath A. C., Eaves L. J., The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism, *Arch Gen Psychiatry* **52**, 374-83.

Kim, D. K., Lim, S. W., Lee, S., Sohn, S. E., Kim, S., Hahn, C. G., Carroll, B. J., 2000, Serotonin transporter gene polymorphism and antidepressant response, *Neuroreport* **11**, 215-9.

Kim, S. W., Park, S. Y., Hwang, O., 2002, Up-regulation of tryptophan hydroxylase expression and serotonin synthesis by sertraline, *Mol Pharmacol* **61**, 778-85.

Kohen, R., Metcalf, M. A., Khan, N., Druck, T., Huebner, K., Lachowicz, J. E., Meltzer, H. Y., Sibley, D. R., Roth, B. L., Hamblin, M. W., 1996, Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor, *J Neurochem* **66**, 47-56.

Kramer, M. S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J. J., Reines, S. A., Liu, G., Snavely, D., Wyatt-Knowles, E., Hale, J. J., Mills, S. G., MacCoss, M., Swain, C. J., Harrison, T., Hill, R. G., Hefti, F., Scolnick, E. M., Cascieri, M. A., Chicchi, G. G., Sadowski, S., Williams, A. R., Hewson, L., Smith, D., Rupniak, N. M., et al., 1998, Distinct mechanism for antidepressant activity by blockade of central substance P receptors, *Science* **281**, 1640-5.

Kubera, M., Holan, V., Mathison, R., Maes, M., 2000, The effect of repeated amitriptyline and desipramine administration on cytokine release in C57BL/6 mice, *Psychoneuroendocrinology* **25**, 785-97.

Kwok, P. Y., 2000, High-throughput genotyping assay approaches, *Pharmacogenomics* **1**, 95-100.

Lane, H. Y., Chang, Y. C., Chiu, C. C., Chen, M. L., Hsieh, M. H., Chang, W. H., 2002, Association of risperidone treatment response with a polymorphism in the 5-HT(2A) receptor gene, *Am J Psychiatry* **159**, 1593-5.

Le Beau, M. M., Rowley, J. D., 1986, Chromosomal abnormalities in leukemia and lymphoma: clinical and biological significance, *Adv Hum Genet* **15**, 1-54.

Leonhard, K., 1959, Aufteilung der Endopen psychosen. Berlin: Akademic Verlag.

Lesch, K. P., Balling, U., Gross, J., Strauss, K., Wolozin, B. L., Murphy, D. L., Riederer, P., 1994, Organization of the human serotonin transporter gene, *J Neural Transm Gen Sect* **95**, 157-62.

Lesch, K., Bengel, D., Heils, A., Sabol, S., Greenberg, B., Petri, S., Benjamin, J., Muller, C., Hamer, D., Murphy, D., 1996, Association of anxiety-related traits with a polymorphismin the serotonin transporter gene regulatory region., *Science* **274**, 1527-1530.

Licinio, J., Wong, M. L., 1999, The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection, *Mol Psychiatry* **4**, 317-27.

Lipshutz, R. J., Fodor, S. P., Gingeras, T. R., Lockhart, D. J., 1999, High density synthetic oligonucleotide arrays, *Nat Genet* **21**, 20-4.

Lomasney, J. W., Cotecchia, S., Lefkowitz, R. J., Caron, M. G., 1991, Molecular biology of alpha-adrenergic receptors: implications for receptor classification and for structure-function relationships, *Biochim Biophys Acta* **1095**, 127-39.

Lynch, S., Merson, S., Beshyah, S. A., Skinner, E., Sharp, P., Priest, R. G., Johnston, D. G., 1994, Psychiatric morbidity in adults with hypopituitarism, *J R Soc Med* **87**, 445-7.

Maes, M., 1999, Major depression and activation of the inflammatory response system, *Adv Exp Med Biol* **461**, 25-46.

Maes, M., Bosmans, E., Meltzer, H. Y., Scharpe, S., Suy, E., 1993, Interleukin-1 beta: a putative mediator of HPA axis hyperactivity in major depression?, *Am J Psychiatry* **150**, 1189-93.

Maes, M., Meltzer, H. Y., 1995, The serotonin hypothesis of major depression. In: Bloom FE, Kupfer DJ, eds: Psychopharmacology: The fourth generation of progress. New York: Raven Press. Pp. 933-944.

Maier, S. F., Goehler, L. E., Fleshner, M., Watkins, L. R., 1998, The role of the vagus nerve in cytokine-to-brain communication, *Ann N Y Acad Sci* **840**, 289-300.

Maier, W., Lichtermann, D., Minges, J., Hallmayer, J., Heun, R., Benkert, O., Levinson, D., 1993, Continuity and discontinuity of affective disorders and schizophrenia. Results of a controlled family study, *Arch Gen Psychiatry* **50**, 871-83.

Malhotra, S., Tesar, G. E., Franco, K., 2000, The relationship between depression and cardiovascular disorders, *Curr Psychiatry Rep* **2**, 241-6.

Mason, D. A., Moore, J. D., Green, S. A., Liggett, S. B., 1999, A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor*, J Biol Chem* **274**, 12670-4.

Massie, M. J., Holland, J. C., 1990, Depression and the cancer patient, *J Clin Psychiatry* **51** Suppl, 12-7.

Maziade, M., Roy, M. A., Martinez, M., Cliche, D., Fournier, J. P., Garneau, Y., Nicole, L., Montgrain, N., Dion, C., Ponton, A. M., 1995, Negative, psychoticism, and disorganized dimensions in patients with familial schizophrenia or bipolar disorder: continuity and discontinuity between the major psychoses, *Am J Psychiatry* **152**, 1458-63.

Meyer-Lindenberg, J., 1991, The Holocaust and German psychiatry, *Br J Psychiatry* **159**, 7-12 (1991).

Minov, C., Baghai, T. C., Schule, C., Zwanzger, P., Schwarz, M. J., Zill, P., Rupprecht, R., Bondy, B., 2001, Serotonin-2A-receptor and -transporter polymorphisms: lack of association in patients with major depression, *Neurosci Lett* **303**, 119-22.

Muller, D. J., Schulze, T. G., Macciardi, F., Ohlraun, S., Gross, M. M., Bauer, I., Scherk, H., Kischkel, E., Neidt, H., Syagailo, Y. V., Grassle, M., Nothen, M. M., Maier, W., Lesch, K. P., Rietschel, M., Moclobemide response in depressed patients: association study with a functional polymorphism in the monoamine oxidase-A promoter. Eighth World Congress on Psychiatric Genetics 2000, Versailles, France: 537.

Mundo, E., Walker, M., Cate, T., Macciardi, F., Kennedy, J. L., 2001, The Role of Serotonin Transporter Protein Gene in Antidepressant-Induced Mania in Bipolar Disorder, *Arch Gen Psychiatry* **58**, 539-544.

Murray, C. J., Lopez, A. D., 1996, Evidence-based health policy–lessons from the Global Burden of Disease Study, *Science* **274**, 740-3.

Nataraj, A. J., Olivos-Glander, I., Kusukawa, N., Highsmith, W. E., Jr., 1999, Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection, *Electrophoresis* **20**, 1177-85.

Neer, E. J., 1995, Heterotrimeric G proteins: organizers of transmembrane signals, *Cell* **80**, 249-57.

Nielsen, D. A., Jenkins, G. L., Stefanisko, K. M., Jefferson, K. K., Goldman, D., 1997, Sequence, splice site and population frequency distribution analyses of the polymorphic human tryptophan hydroxylase intron 7, *Brain Res Mol Brain Res* **45**, 145-8.

Ogilvie, A. D., Battersby, S., Bubb, V. J., Fink, G., Harmar, A. J., Goodwin, G. M., Smith, C. A. D., 1996, Polymorphism in serotonin transporter gene associated with susceptibility to major depression., *Lancet* **347**, 731-733.

O'Reilly, R. L., Bogue, L., Singh, S. M., 1994, Pharmacogenetic response to antidepressants in a multicase family with affective disorder, *Biol Psychiatry* **36**, 467-71.

Orr, M. S., Scherf, U., 2002, Large-scale gene expression analysis in molecular target discovery, *Leukemia* **16**, 473-7

Orsini, A., 1987, Antidepressant responses and segregation analyses in affective families. In: Racagni G, Smeraldi E, eds: Anxious Depression: assessment and treatment. New York: Raven Press.

Osby, U., Brandt, L., Correia, N., Ekbom, A., Sparen, P., 2001, Excess mortality in bipolar and unipolar disorder in Sweden, *Arch Gen Psychiatry* **58**, 844-50.

Owen, B., Eccleston, D., Ferrier, I., Young, A., 2000, Raised levels of plasma interleukin-1 in major and post viral depression., *Acta Psychiatr Scand* **103**, 226-228.

Pare, C. M., Mack, J. W., 1971, Differentiation of two genetically specific types of depression by the response to antidepressant drugs, *J Med Genet* **8**, 306-9.

Patterson, D., Jones, C., Hart, I., Bleskan, J., Berger, R., Geyer, D., Eisenberg, S. P., Smith, M. F., Jr., Arend, W. P., 1993, The human interleukin-1 receptor antagonist (IL1RN) gene is located in the chromosome 2q14 region, *Genomics* **15**, 173-6.

Paul, I. A., 2001, Antidepressant activity and calcium signaling cascades, Hum Psychopharmacol 16, 71-80.

Penninx, B. W., Geerlings, S. W., Deeg, D. J., van Eijk, J. T., van Tilburg, W., Beekman, A. T., 1999, Minor and major depression and the risk of death in older persons, *Arch Gen Psychiatry* **56**, 889-95.

Pickar, D., Rubinow, K., 2001, Pharmacogenomics of psychiatric disorders, *Trends Pharmacol Sci* **22**, 75-83.

Pincus, H. A., Pettit, A. R., 2001, The societal costs of chronic major depression, *J Clin Psychiatry* **62** Suppl 6, 5-9.

Pociot, F., Molvig, J., Wogensen, L., Worsaae, H., Nerup, J., 1992, A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro, *Eur J Clin Invest* **22**, 396-402.

Poirier, J., Delisle, M. C., Quirion, R., Aubert, I., Farlow, M., Lahiri, D., Hui, S., Bertrand, P., Nalbantoglu, J., Gilfix, B. M., et al., 1995, Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease, *Proc Natl Acad Sci U S A* **92**, 12260-4.

Pollock, B. G., Ferrell, R. E., Mulsant, B. H., Mazumdar, S., Miller, M., Sweet, R. A., Davis, S., Kirshner, M. A., Houck, P. R., Stack, J. A., Reynolds, C. F., Kupfer, D. J., 2000, Allelic Variation in the Serotonin Transporter Promoter Affects Onset of Paroxetine Treatment Response in Late-Life Depression, *Neuropsychopharmacology* **23**, 587-590.

Post, R. M., Weiss, S. R., 1995, The neurobiology of treatment-resistant mood disorders. In: Bloom FE, Kupfer DJ, eds: Psychopharmacology: the fourth generation of progress. New York: Raven press. pp. 1155-1170.

Price, C. J., Pittman, Q. J., 2001, Dopamine D4 receptor activation inhibits presynaptically glutamatergic neurotransmission in the rat supraoptic nucleus, *J Neurophysiol* **86**, 1149-55.

Ramamoorthy, S., Bauman, A. L., Moore, K. R., Han, H., Yang-Feng, T., Chang, A. S., Ganapathy, V., Blakely, R. D., 1993, Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization, *Proc Natl Acad Sci U S A* **90**, 2542-6.

Ramaraj, P., Kessler, S. P., Colmenares, C., Sen, G. C., 1998, Selective restoration of male fertility in mice lacking angiotensin-converting enzymes by sperm-specific expression of the testicular isozyme, *J Clin Invest* **102**, 371-8.

Ramsay, S., 1999, More developments in developing-world research ethics, *Lancet* **354**, 1405-6.

Rice, D. P., 1999, The economic impact of schizophrenia, *J Clin Psychiatry* **60** Suppl 1, 4-6; discussion 28-30.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., Soubrier, F., 1990, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels, *J Clin Invest* **86**, 1343-6.

Roses, A. D., 2000, Pharmacogenetics and the practice of medicine, *Nature* **405**, 857-65.

Rubinstein, M., Phillips, T. J., Bunzow, J. R., Falzone, T. L., Dziewczapolski, G., Zhang, G., Fang, Y., Larson, J. L., McDougall, J. A., Chester, J. A., Saez, C., Pugsley, T. A., Gershanik, O., Low, M. J., Grandy, D. K., 1997, Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine, *Cell* **90**, 991-1001.

Sabol, S. Z., Hu, S., Hamer, D., 1998, A functional polymorphism in the monoamine oxidase A gene promoter, *Human Genetic* **103**, 273-279.

Sato, K., Yoshida, K., Takahashi, H., Ito, K., Kamata, M., Higuchi, H., Shimizu, T., Itoh, K., Inoue, K., Tezuka, T., Suzuki, T., Ohkubo, T., Sugawara, K., Otani, K., 2002, Association

between -1438G/A promoter polymorphism in the 5-HT(2A) receptor gene and fluvoxamine response in Japanese patients with major depressive disorder, *Neuropsychobiology* **46**, 136-40.

Sederer, L. I., 1986, Depression. In: Sederer LI, ed: Inpatients psychiatry diagnosis and treatment. Baltimore: William & Wilkins, pp. 20.

Segman, R., Neeman, T., Heresco-Levy, U., Finkel, B., Karagichev, L., Schlafman, M., Dorevitch, A., Yakir, A., Lerner, A., Shelevoy, A., Lerer, B., 1999, Genotypic association between the dopamine D3 receptor and tardive dyskinesia in chronic schizophrenia, *Mol Psychiatry* **4**, 247-53.

Serretti, A., Artioli, P., From molecular biology to pharmacogenetics: a review of the literature on antidepressant treatment and suggestions of possible candidate genes, *Psychopharmacolgy*, (In press).

Serretti, A., Franchini, L., Gasperini, M., Rampoldi, R., Smeraldi, E., 1998, Mode of inheritance in mood disorders families according to fluvoxamine response, *Acta Psychiatr Scand* **98**, 443-450.

Serretti, A., Lilli, R., Smerladi, E., 2002, Pharmacogenetics in affective disorders, *European Journal of Pharmacology* **438**, 117-128.

Serretti, A., Lorenzi, C., Cusin, C., Zanardi, R., Lattuada, E., Rossini, D., Lilli, R., Pirovano, A., Catalano, M., Smeraldi, E., 2003, SSRIs antidepressant activity is influenced by Gbeta3 variants, *Eur Neuropsychopharmacol* **13**, 117-22.

Serretti, A., Zanardi, R., Cusin, C., Rossini, D., Lilli, R., Lorenzi, C., Lattuada, E., Smeraldi, E., 2001, No association between dopamine D2 and D4 receptor gene variants and antidepressant activity of two selective serotonin reuptake inhibitors, *Psychiatry Res* **104**, 195-203.

Serretti, A., Zanardi, R., Cusin, C., Rossini, D., Lorenzi, C., Smeraldi, E., 2001, Tryptophan hydroxylase gene associated with paroxetine antidepressant activity, *Eur Neuropsychopharmacol* **11**, 375-380.

Serretti, A., Zanardi, R., Rossini, D., Cusin, C., Lilli, R., Smeraldi, E., 2001, Influence of tryptophan hydroxylase and serotonin transporter genes on fluvoxamine antidepressant activity, *Mol Psychiatry* **6**, 586-592.

Service, R. F., 1998, Microchip arrays put DNA on the spot, *Science* **282**, 396-9.

Shoemaker, D. D., Schadt, E. E., Armour, C. D., He, Y. D., Garrett-Engele, P., McDonagh, P. D., Loerch, P. M., Leonardson, A., Lum, P. Y., Cavet, G., Wu, L. F., Altschuler, S. J., Edwards, S., King, J., Tsang, J. S., Schimmack, G., Schelter, J. M., Koch, J., Ziman, M., Marton, M. J., Li, B., Cundiff, P., Ward, T., Castle, J., Krolewski, M., Meyer, M. R., Mao, M., Burchard, J., Kidd, M. J., Dai, H., Phillips, J. W., Linsley, P. S., Stoughton, R., Scherer, S., Boguski, M. S., 2001, Experimental annotation of the human genome using microarray technology, *Nature* **409**, 922-7.

Siffert, W., Rosskopf, D., Moritz, A., Wieland, T., Kaldenberg-Stasch, S., Kettler, N., Hartung, K., Beckmann, S., Jakobs, K. H., 1995, Enhanced G protein activation in immortalized lymphoblasts from patients with essential hypertension, *J Clin Invest* **96**, 759-66.

Skrebuhhova, T., Allikmets, L., Matto, V., 1999, Effects of anxiogenic drugs in rat forced swimming test, *Methods Find Exp Clin Pharmacol* **21**, 173-8.

Smeraldi, E., Zanardi, R., Benedetti, F., Dibella, D., Perez, J., Catalano, M., 1998, Polymorphism Within the Promoter of the Serotonin Transporter Gene and Antidepressant Efficacy of Fluvoxamine, *Mol Psychiatry* **3**, 508-511.

Sparkes, R. S., Lan, N., Klisak, I., Mohandas, T., Diep, A., Kojis, T., Heinzmann, C., Shih, J. C., 1991, Assignment of a serotonin 5HT-2 receptor gene (HTR2) to human chromosome 13q14-q21 and mouse chromosome 14, *Genomics* **9**, 461-5.

Spurlock, G., Heils, A., Holmans, P., Williams, J., D'Souza, U. M., Cardno, A., Murphy, K. C., Jones, L., Buckland, P. R., McGuffin, P., Lesch, K. P., Owen, M. J., 1998, A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter, *Mol Psychiatry* **3**, 42-9.

Takahashi, H., Yoshida, K., Ito, K., Sato, K., Kamata, M., Higuchi, H., Shimizu, T., Inoue, K., Tezuka, T., Suzuki, T., Ohkubo, T., Sugawara, K., 2002, No association between the serotonergic polymorphisms and incidence of nausea induced by fluvoxamine treatment, *Eur Neuropsychopharmacol* **12**, 477.

Torgersen, S., 1990, Comorbidity of major depression and anxiety disorders in twin pairs, *Am J Psychiatry* **147**, 1199-202.

Tsuang, M., Faraone, S., 1990, The genetics of mood disorders. Baltimore: The Johns Hopkins University Press.

Ustun, T. B., 2001, The worldwide burden of depression in the 21st century. In: Weismann MM, ed: Treatment of depression: Bridging the 21st Century. Washington, DC: *American Psichiatric Press*. Pp. 35-45.

Van Tol, H., Caren, M., Guan, H., Ohara, K., Bunzow, J., Civelli, O., Kennedy, J., Seeman, P., Niznik, H., Jovanovic, V., 1992, Multiple dopamine D4 receptor variants in the human population., *Nature* **358**, 149-152.

Vuckovic, A., Cohen, B. M., Zubenko, G. S., 1991, The use of captopril in treatment-resistant depression: an open trial, *J Clin Psychopharmacol* **11**, 395-6.

Warren, J. T., Jr., Peacock, M. L., Rodriguez, L. C., Fink, J. K., 1993, An MspI polymorphism in the hyman serotonin receptor gene (HTR2): detection by DGGE and RFLP analysis, *Hum Mol Genet* **2**, 338.

Weber, M. M., 1997, Ernest Rubin 1874-1952: a German psychiatrist and geneticist, *Am J Med Gen* **67**, 323-331.

Weissman, M. M., Prusoff, B. A., Merikangas, K. R., 1984, Is delusional depression related to bipolar disorder?, *Am J Psychiatry* **141**, 892-893.

Winokur, G., Coryell, W., Endicott, J., Akiskal, H., 1993, Further distinctions between manic-depressive illness (bipolar disorder) and primary depressive disorder (unipolar depression), *Am J Psychiatry* **150**, 1176-81.

Winokur, G., Coryell, W., Keller, M., Endicott J., Leon A., 1995, A family study of manic-depressive (bipolar I) disease. Is it a distinct illness separable from primary unipolar depression?, *Arch Gen Psychiatry* **52**, 367-73.

Winokur, G., Coryell, W., Keller, M., Endicott, J., Akiskal, H., 1993, A prospective follow-up of patients with bipolar and primary unipolar affective disorder, *Arch Gen Psychiatry* **50**, 457-65.

Wu, W. H., Huo, S. J., Cheng, C. Y., Hong, C. J., Tsai, S. J., 2001, Association Study of the 5-HT(6) Receptor Polymorphism (C267T) and Symptomatology and Antidepressant Response in Major Depressive Disorders, *Neuropsychobiology* **44**, 172-5.

Yaffe, K., Blackwell, T., Gore, R., Sands, L., Reus, V., Browner, W. S., 1999, Depressive symptoms and cognitive decline in nondemented elderly women: a prospective study, *Arch Gen Psychiatry* **56**, 425-30.

Yoshida, K., Ito, K., Sato, K., Takahashi, H., Kamata, M., Higuchi, H., Shimizu, T., Itoh, K., Inoue, K., Tezuka, T., Suzuki, T., Ohkubo, T., Sugawara, K., Otani, K., 2002, Influence of the serotonin transporter gene-linked polymorphic region on the antidepressant response to fluvoxamine in Japanese depressed patients, *Prog Neuropsychopharmacol Biol Psychiatry* **26**, 383-6.

Yoshida, K., Naito, S., Takahashi, H., Sato, K., Ito, K., Kamata, M., Higuchi, H., Shimizu, T., Itoh, K., Inoue, K., Tezuka, T., Suzuki, T., Ohkubo, T., Sugawara, K., Otani, K., 2002, Monoamine oxidase: A gene polymorphism, tryptophan hydroxylase gene polymorphism and antidepressant response to fluvoxamine in Japanese patients with major depressive disorder, *Prog Neuropsychopharmacol Biol Psychiatry* **26**, 1279-83.

Yu, Y. W., Chen, T. J., Hong, C. J., Chen, H. M., Tsai, S. J., 2003, Association Study of the Interleukin-1beta (C-511T) Genetic Polymorphism with Major Depressive Disorder, Associated Symptomatology, and Antidepressant Response, *Neuropsychopharmacology* **28**, 1182-5.

Yu, Y. W., Chen, T. J., Wang, Y. C., Liou, Y. J., Hong, C. J., Tsai, S. J., 2003, Association analysis for neuronal nitric oxide synthase gene polymorphism with major depression and fluoxetine response, *Neuropsychobiology* **47**, 137-40.

Yu, Y. W., Tsai, S. J., Chen, T. J., Lin, C. H., Hong, C. J., 2002, Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders, *Mol Psychiatry* **7**, 1115-9.

Zanardi, R., Benedetti, F., DiBella, D., Catalano, M., Smeraldi, E., 2000, Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of serotonin transporter gene, *J Clin Psychopharmacol* **20**, 105-107.

Zanardi, R., Serretti, A., Rossini, D., Franchini, L., Cusin, C., Lattuada, E., Dotoli, D., Smeraldi, E., 2001, Factors affecting fluvoxamine antidepressant activity: influence of pindolol and 5-HTTLPR in delusional and nondelusional depression, *Biol Psychiatry* **50**, 323-330.

Ziegelstein, R. C., 2001, Depression after myocardial infarction, *Cardiol Rev* **9**, 45-51.

Ziegelstein, R. C., 2001, Depression in patients recovering from a myocardial infarction, *Jama* **286**, 1621-7.

Zill, P., Baghai, T. C., Engel, R., Zwanzger, P., Schule, C., Minov, C., Behrens, S., Bottlender, R., Jager, M., Rupprecht, R., Moller, H. J., Ackenheil, M., Bondy, B., 2003, Beta-1-adrenergic receptor gene in major depression: Influence on antidepressant treatment response, *Am J Med Genet* **120B**, 85-9.

Zill, P., Baghai, T. C., Zwanzger, P., Schule, C., Minov, C., Riedel, M., Neumeier, K., Rupprecht, R., Bondy, B., 2000, Evidence for an association between a G-protein beta3-gene variant with depression and response to antidepressant treatment, *Neuroreport* **11**, 1893-7.

4. PHARMACOGENETICS OF BIPOLAR DISORDER

Pierre Oswald, Daniel Souery, Julien Mendlewicz

1. INTRODUCTION

Writers of Ancient Greece already used the terms mania and melancholia to describe what we consider now as bipolar disorder, but in a broader sense (Gouveritch, 1994; Cookson, 2002). Mania was considered as a chronic disease with agitation and delusion, as opposed to frenzy ("*phrenitis*"), which is a more acute disease with fever. The concept of melancholia included two characteristics, sadness and fear. Hippocrates (5th century BC) argued that "if fear and sadness persist, it is a melancholic state". But the first author to consider that melancholia and mania could be two manifestations of the same disease is Aretaeus of Cappodocia, in the 2th century AC: "After a despondency phase, an improvement could sometimes happen: most of the people become joyful but the others become manic". In the Middle Ages, mental illness was more associated with supernatural forces but physicians still continued to use the same nosology as in the Ancient Greece. With the Renaissance and during the Age of Enlightenment, clinical science began to re-emerge. In the 19th century, considerable advances were made in the nosography of manic and depressive states. Falret described the so-called "folie circulaire" in 1854, recognizing that mania and depression could occur during the same episode. From 1893, Kraepelin introduced the concept of manic-depressive insanity ("*manisch-depressives irresein*"), distinguished from dementia praecox. Based on clinical observation, longitudinal course and family history, Kraepelin envisaged a real continuum between manic and depressive states. Bleuler (1920) used the term "affective illness" to describe the condition. This unitary vision dominated psychiatry until the late 1960s. Since then, following the works of Leonard and Angst, the bipolar-unipolar dichotomy replaced the traditional nosography of Kraepelin in formal classification systems, such as the *Diagnostic and Statistical Manual for Mental Disorders*, fourth edition (DSM-IV, American Psychiatric Association, 1994) and the *International Classification of Diseases*, tenth edition (ICD-10). Besides this evolution, there is a considerable need for improved treatments for the different phases of the disease. Lithium

discovery in the late 40s was considered as a revolution in the psychiatric field. For the first time, it was possible to stabilize bipolar patients and to allow them a social insertion. But the response remained difficult to predict. Some people were largely improved, but some others not. Until now, the response to the current mood stabilizers is not optimal in terms of recurrence or side effects. Pharmacogenetics represents thus a considerable hope for many clinicians and patients in its possibility to understand why some people are able to respond and why some others will never respond to one or another mood stabilizer.

2. THE CLASSIFICATIONS OF BIPOLAR DISORDERS

The DSM-IV distinguishes between Bipolar I Disorder (BPAD I), Bipolar II Disorder (BPAD II), Cyclothymic Disorder and Bipolar Disorder Not Otherwise Specified. BPAD I is defined as episodes of mania (with or without major depressive episode) and BPAD II as recurrent episodes of major depression with hypomanic episodes. Cyclothymic Disorder is defined as the presence, for at least 2 years, of numerous periods with hypomanic symptoms and numerous periods of depressive symptoms that do not meet criteria for major depression. The Bipolar Disorder Not Otherwise Specified includes disorders with bipolar features that do not meet criteria for any specific bipolar disorder. In addition to manic, hypomanic and major depressive episodes, the DSM-IV describes "mixed episodes" where the criteria are met both for a manic episode and for a major depressive episode. The term of "dysphoric mania" has been proposed since phenomenologic studies have shown that patients with mania experience irritability, depressed mood and mood lability, as often as euphoria (Goodwin and Jamison, 1990). Moreover, if the patient has four or more affective episodes in a year, they are considered as rapid-cycling patients. Ultra-rapid cycling describes four or more episodes in a month. Finally, ultradian cycling represents mood changes within the same day.

DSM-IV also proposes additional specifiers, describing severity, clinical type and the longitudinal course. BPAD may thus be: mild, moderate, severe without psychotic features, severe with psychotic features, in partial remission, in full remission; with catatonic features; with post-partum onset. If the most recent episode is depressive, it may be chronic, melancholic or with atypical features. The longitudinal course specifiers are: with or without full inter-episode recovery, with a seasonal pattern, or with rapid-cycling.

More recently, novel approaches have been proposed in the nosography of BPAD to take into account subthreshold syndromes in the so-called bipolar disorder spectrum from pure mania to depression (Zurich criteria). Angst distinguishes three subtypes of BPAD I: pure mania, predominantly manic bipolar I disorder and the nuclear form of bipolar I with severe mania and severe depression (Angst and Gamma, 2002). Besides the BPAD II group lies the group of minor bipolar disorder, defined by the combination of mild depression with hypomania or hypomanic symptoms. Applying the Zurich criteria, Angst suggests that approximately half of all patients suffering from major depression may have been misdiagnosed and are likely to be suffering from BPAD II. These findings have important consequences in the treatment and the outcome of

affective disorders. In the same effort to return to Kraepelin's broad concept of manic-depressive spectrum, Akiskal and Pinto (1999) have proposed further subtypes of bipolar disorder. In addition to bipolar I and II subtypes, Bipolar I½ is defined as depression with protracted hypomania (causing some trouble to the patient without the destructive potential of mania). Bipolar II½ is the alternance of major depressive states and short hypomanic symptoms (cyclothymic depressions). Bipolar III is represented by the antidepressant-associated hypomania. Bipolar III½ is the bipolarity masked by stimulant abuse. Finally, Bipolar IV subtype is the hyperthymic depression. One of the major finding from these approaches reveals that many patients considered as unipolar affective disorder (UPAD) patients are in reality part of the bipolar spectrum.

3. BURDEN AND OUTCOME OF BIPOLAR DISORDER

BPAD are chronic disorders with a high rate of recurrence and relapses. More than 90% of individuals who have a single manic episode will have future episodes (Hopkins and Gelenberg, 1994). Ten to 15% of patients will have more than 10 episodes in their life. Bipolar disorder is therefore one of the leading causes of disability which contributes to the important economic burden of bipolar disorder to society. Patients with BD suffer great losses in productivity, with more bed rest and absenteeism days (see Pini et al., 2005 for review). The economic burden was estimated in a cost-to-illness study around US$45 billion in 1991 in the USA, representing 70% of the annual cost of schizophrenia (Wyatt and Henter, 1995). Worldwide, bipolar disorder is listed as the sixth leading cause of disability (Murray and Lopez, 1996).

The lifetime prevalence of BPAD, based on the DSM-IV criteria, is ranging from 1,3% to 1,6% with a sex-ratio around 1 (Weissman et al., 1996). The peak age of onset seems to fall around 20 yo in Hungary (Szadoczky et al., 1998), from 18 to 23.8 yo in Germany (Wittchen et al., 2003) and 25 yo in Australia (Morgan et al., 2005). The age of onset of manic episodes is usually 6 to 8 years before depressive episodes. There is often a 5 to 10 years interval before correct diagnosis is obtained. After a first episode of mania, most of the patients show a low functional recovery. Although the overt symptoms are relatively well controlled, continued impairments in the overall quality remain. This fact concerns both BPAD I and BPAD II. BPAD II has been considered for a long time as a minor expression of the classic BPAD I. Recently, important studies have focused on the natural history of BPAD II. Judd et al. (2003) have followed a cohort of BPAD II patients during a mean of 13.4 years of prospective follow-up. They showed that patients were symptomatic 53.9% of all follow-up weeks. Most important, depressive symptoms (50,3% of the weeks) dominated the course over hypomanic (1,3% of the weeks) and cycling/mixed (2.3% of the weeks) symptoms. They concluded that BPAD II is a chronic disease with a high rate of major depressive episodes but also periods involving minor or subsyndromal symptoms, as already suggested by Angst and Akiskal (see above).

One of the major concerns about BPAD remains the suicide risk. According to epidemiologic studies, at least 25% of patients with BPAD attempt suicide and 10% to 15% will complete suicide, explaining in part the fact that the

mortality rate of the disease is two to three times higher than that of the general population (Jamison, 1998). Furthermore, the rate of suicidal behavior among BPAD patients (including BPAD I and BPAD II) is significantly higher than that of UPAD patients. Rihmer and Pestality (1999) have combined epidemiologic studies on the suicide risk in UPAD, BPAD I and BPAD II. They found that the lifetime history of suicide attempts is significantly higher in BPAD II patients than in BPAD I patients. When considering suicide completers among a population of BPAD and UPAD patients with primary major depression at the time of their suicide in two independent studies, Rihmer and Pestality found that 46% in the first study and 36% in the second study had a diagnosis of BPAD II (compared to 1%-8% of BPAD I patients and 53%-56% of UPAD patients). Given the fact that the lifetime prevalence rate of BPAD II is relatively lower than UPAD, the authors suggested that BPAD II represented a high risk of completed suicide among the population of primary major mood disorder. Because of the high risk of recurrence and suicide, long-term prophylactic treatment is indicated.

4. TREATMENT OF BIPOLAR DISORDERS

Ideally, successful treatment should treat both mania and depression, prevent recurrence, and improve quality of life between episodes. But, in the reality, the features of BPAD make it a challenging illness to manage. Pharmacologic treatment is further complicated by the risk of inducing mood changes ("switches"). We will here briefly review the current available strategies for the acute management of BPAD and the long-term treatment (maintenance treatment) (table 1 [modified]).

Table 1: Current Treatment Strategies in BPAD (based on international guidelines and expert consensus)

	First-line Treatment	***Adjunctive Treatment***	***Alternative Treatment***
(Hypo)Manic Episode	• Lithium • Valproate • Carbamazepine • Atypical Antipsychotic	• Atypical (or classical) Antipsychotics • Benzodiazepines	• ECT[1]
Maintenance Phase	• Lithium • Valproate • Carbamazepine • Lamotrigine	• Atypical antipsychotics	Atypical antipsychotic alone (olanzapine)
Depressive Episode	Lithium Lamotrigine	Antidepressants Atypical Antipsychotics	ECT[1]

[1] ECT: Electroconvulsivotherapy

4.1. Acute Episode: Manic and Mixed States

Lithium remains the golden standard treatment in acute mania. Data from randomized, controlled trials found lithium to be significantly more efficacious than placebo in the reduction of manic symptoms (for review see Keck et al., 2000a). In addition, when comparing lithium to typical antipsychotics, lithium showed comparable efficacy in reducing both manic and psychotic symptoms. More specifically, lithium as monotherapy is generally effective in mild to moderate mania and in euphoric mania (Prien et al., 1972; American Psychiatric Association, 2002). On the other side, patients with prominent depressive symptoms during mania (mixed state) have lower acute antimanic response rates to lithium than patients without these features (Swann et al., 1997). In acute manic or depressive episodes, lithium should be initiated at higher doses than in prophylactic treatment to reach serum concentrations of 1.0-1.2 mmol/L. In addition, benzodiazepines, such as clonazepam and lorazepam, are often required before a therapeutic effect of lithium is reached. In case of severe or psychotic mania, a combination with antipsychotics is required. Carbamazepine is also widely used in the treatment of mania. Efficacy of carbamazepine was assessed in several controlled trials (Ballenger and Post, 1978; Okuma et al., 1979; Grossi et al., 1984; Lerer et al., 1987; Small et al., 1991; Keck et al., 1992). It may cause ataxia and vertigo when high-dose treatment is initiated but the sedative effect might be an advantage. Valproate has been shown to be effective in the treatment of acute mania in 10 controlled trials (for review, see Keck et al. 2000). High-dose treatment is usually well tolerated and, unlike carbamazepine, this drug does not interact with other medications. Valproate seems to show a better efficacy in mixed and dysphoric manic states (Bowden et al., 1994). Finally, valproate is also recommended in rapid-cycling patients. In Europe, antipsychotics are traditionally preferred to lithium, carbamazepine and valproate and are considered as the cornerstone of treatment of acute mania. Newer ("atypical") antipsychotics, such as olanzapine, risperidone, quetiapine and ziprasidone (See Vieta and Goikolea, 2005 for review), have shown efficacy alone in the treatment of acute mania Olanzapine has been shown to be effective in acute mania on the pivotal Young Mania Rating Scale at 3 weeks (Tohen et al., 1999) and at 4 weeks (Tohen et al., 2000). Risperidone, in monotherapy or as adjunctive treatment, has well established antimanic properties with an early response (Hirschfeld et al., 2004; Khanna et al., 2005). Quetiapine was evaluated in mania and was found also to be effective (See Vieta and Goikolea, 2005 for review). Atypical antipsychotics are useful in combination with lithium or valproate (Goodwin, 2003). Finally, in refractory severe mania, electro-convulsivotherapy remains the treatment of choice (Keck et al., 2000).

4.2. Acute Episode: Bipolar Depression

The treatment of acute bipolar depression has been largely ignored during the last 20 years. Lithium is the most studied medication in this indication. Pooled results from the few controlled trials found than one third of patients had marked improvement in depressive symptoms with lithium and 80% had at least

partial improvement (Zornberg and Pope, 1993). Most studies investigating the efficacy of antidepressants have been found to be effective in the treatment of bipolar depression. Most trials, conducted in the 80s and the 90s, investigated the efficacy of tricyclic agents and monoamine oxidase inhibitor (Zornberg and Pope, 1993). More recently, fluoxetine and paroxetine have been reported to reduce depressive symptoms when administered in combination to lithium (Cohn et al., 1989; Nemeroff et al., 2001). However, controlled trials investigating the usefulness of antidepressants in bipolar depression remain sparse and neef further studies. Mood switches into mania, hypomania and mixed states are the major risk when administrating antidepressants. The extend of this complication remains unclear (Altshuler et al., 2003; Gijsman et al., 2004). Recent data have shown that the fluoxetine-olanzapine combination was more effective in bipolar depression than fluoxetine alone with a lower risk to switch into hypomanic, manic or mixed states (Tohen et al., 2003). If replicated this finding may confirm the benefit of combining an SSRI and an atypical antipsychotic in minimizing the risk of switch. More recently, interesting findings have raised questions on the antidepressant-induced mania or hypomania (Altshuler et al., 2003). In this study, the authors argued that early antidepressant treatment discontinuation is more associated with depressive relapse. Given the morbidity associated with subsyndromal or major depression, it is recommended to prolong the antidepressant treatment even if the risk of switches is present in some cases.

Encouraging data from large, multicenter randomized, placebo-controlled trials have shown that an antiepileptic agent, lamotrigine, was superior to placebo in the reduction of depressive symptoms in BPAD I patients (Calabrese et al., 1999; Goldsmith et al., 2003). In addition, there was no significant induction of mood switches. Lamotrigine has been recently recommended as a first-line treatment in bipolar depression by the American Psychiatric Association (APA). However, recent reports of pooled studies did not reach significance of superior efficacy of lamotrigine to placebo (Hirschfeld et al., 2005). Finally, quetiapine in doses of both 300mg and 600mg a day showed a significant separation from placebo from week one to week 8 on the pivotal MADRS scale in a population of bipolar I or II with a major depressive episode (Calabrese et al., 2005). The important data must be confirmed but may imply a putative specific antidepressive efficacy of quetiapine.

4.3. Maintenance Treatment

The prevention of mood episodes is the most important goal in the long-term treatment of BPAD. Currently, lithium is the only medication with a FDA-approved indication, although newer compounds are frequently used in this purpose. Evidences from randomized placebo-controlled trials in the 1960s and 1970s have shown that lithium reduced the rate of recurrence by 4-fold over placebo after one year of treatment (Keck et al., 2000b). Several meta-analyses confirmed these data later (Baldessarini et al., 2002; Geddes et al., 2004). Unfortunately, many patients do not respond completely to maintenance

treatment with lithium. One of the reasons seems to be the high rate of psychiatric comorbidity (substance abuse, psychotic features and medication-induced switches). The lack of compliance is also suggested. In addition, genetic component may also play a role in treatment response. The relatively high rate of unsatisfactory response to lithium has induced a broadening of treatment options and the emergence of anticonvulsivants. Carbamazepine is effective in long-term treatment, but interactions with other medications may limit its use (Denicoff et al., 1997; Greil et al., 1997). The efficacy of valproate in the maintenance treatment was investigated in one large, placebo-controlled, lithium comparison study (Bowden et al., 2000). No significant difference in time to relapse over 1 year was observed between valproate and lithium. Lithium, carbamazepine and valproate show a high rate of side effects which may limit their use (table 2). More recently, atypical antipsychotics have focused interest.

Table 2: Brief overview of side effects of lithium, valproate and carbamazepine

	Lithium	***Valproate***	***Carbamazepine***
Polyuria, Polydipsia	+	-	-
Hypothyroidism	++	-	-
Nausea, Vomiting, Diarrhoea	+++	+++	++
Hepatotoxicity	-	-	+
Sedation	++	+	++
Asthenia, Dizziness	-	++	++
Ataxia	+	+	++
Tremor, Parkinsonism	++	++	+
Decreased cognition, Incoordination	+	-	+
Weight Gain	+++	++	-
Oedema	++	-	-
Psoriasis, Acne, Allergic Reaction	++	-	+
Alopecia	-	++	-
Teratogenicity	+	+	+
Haematological abnormalities	+	+	+
Hyponatremia	-	-	+

+ rare; ++ frequent; +++ very frequent

Results from open, placebo-controlled studies on risperidone, olanzapine and clozapine suggest a prophylactic effect, when prescribed as adjunctive treatment to classical mood stabilizers or in monotherapy (Tohen et al., 2004; 2005). One of the major side effects remains the weight gain which can contribute to the lack of compliance and an early treatment discontinuation. Lamotrigine also showed encouraging results when compared to placebo in maintenance relapse prevention in patients with rapid cycling BPAD I and BPAD II in a 6-month trial (Calabrese et al., 2000) and more recently in larger trials in recently manic and depressed patients (Calabrese et al., 2003; Goodwin et al., 2004). These results suggest that lamotrigine may a have depression prevention effect, but not a manic prevention effect. Lamotrigine has a few side effects, does not increase weight and has no medication interactions (Yatham et al., 2002b). In clinical practice, it is often necessary to combine different mood stabilizers, mood stabilizers and antidepressants or mood stabilizers and atypical antipsychotics. This fact is consistent with the disappointment of many clinicians when facing the lack of response, the importance of side effects in the long-term treatment of bipolar disorder.

5. RESPONSE TO MOOD STABILIZERS IN FAMILY STUDIES

As it is now, it remains difficult to predict who will respond to lithium and who will not. At a clinical level, some predictors of favourable long-term response to lithium have been studied and recently reviewed by Serretti (2002a, table 3). Besides clinical features, there is evidence of a genetic component in

Table 3: Clinical predictors of favourable long-term response to lithium

1.	A typical symptomatology of mood disorders and the absence of comorbidity with other DSM-IV axis I disturbances (e.g., substance abuse or mental retardation)
2.	Presence of retarded-endogenous symptomatology profile, i.e., characterised by psychomotor inhibition, diurnal variation, neurovegetative symptomatology, though this is not an univocal finding
3.	Presence of psychotic features, such as auditory or (less frequently) visual hallucinations, that for their content may be considered mood-congruent (e.g., guilt)
4.	An initial response during the first 6 - 12 months of lithium, even if this evaluation should not be considered a predictor because it is not available at the beginning of treatment
5.	Female sex
6.	Absence of personality disorder
7.	Good social adjustment, even if social adjustment could influence directly the disease time course therefore producing a spurious association
8.	A peculiar sequence of episode characterised by the mania-depression normal interval
9.	Early start of treatment

(reproduced with kind permission of Ashley Publications) (Serretti, 2002a)

the response to mood stabilizers based on family studies. A substantial number of studies have focused on the role of genetic factors in the response of BPAD patients to prophylactic treatment with lithium. Lithium has been extensively studied compared to other medications in this type of studies because of the stable dose and the fact that many relatives received the same agent.

5.1. Family Studies of Lithium Response and Non Response

The first studies on the relationship between response to lithium and family history have been published in the 1970s, supporting an association between a family history of BPAD and satisfactory response to treatment. Mendlewicz et al. (1973) first reported a study of 36 patients through a double blind study of lithium prophylaxis. They found that 66% of the responders to lithium had at least one first-degree relative with BPAD, and that only 21% of the lithium non responders had a first-degree relative with BPAD. Several family studies have confirmed the initial results from Mendlewicz et al. (1973). Zvolsky et al. (1979) compared a sample of 26 responders to a sample of 17 non responders. They found higher rates of psychiatric disorders in first-degree relatives of responders compared with first-degree relatives of nonresponders. Mendlewicz et al. (1979) examined forty-two pairs of monozygotic twins (n = 25) and dizygotic (n = 17) with BPAD. Concordant twins as a group showed better lithium prophylaxis than do discordant twins. Grof et al. (1994) investigated morbidity risks of BPAD and schizophrenia in first-degree relatives of BPAD responders and nonresponders to lithium. They found an increased risk of BPAD in families of responders and an increased risk of schizophrenia in families of nonresponders. Some other studies were unable to find an association between family history and lithium response but they were based on indirect diagnostic information and not on interview with as many relatives as possible (Dunner et al., 1976; Misra et Burns, 1976; Strober et al., 1988; Alda, 2002). More recently, Engstrom et al. (1997) conducted a study on the frequency of episodes during lithium treatment. Interestingly, they found that the patients without a family history of BPAD had fewer episodes on lithium compared with those with a family history of BPAD. Coryell et al. (2000) studied psychiatric morbidity in relatives of probands subdivided according to their frequency of episodes in the course of prophylactic lithium treatment (low-medium-high). No difference was found between the three groups on the rates of BPAD in families of probands. Those last two results can not be considered as contradictory with previous studies. As argued by Alda (2002), it is impossible to compare 2 different phenotypes: frequency of episodes and response to lithium. Furthermore, BPAD is more largely diagnosed since the 1990s, increasing, maybe falsely, the morbidity risk in relatives (Grof et al., 1995; Alda, 2002). Those results can be interpreted in two ways. First, response to lithium can be considered as a specific phenotype, with a higher family loading. Therefore, linkage studies could be applied in these family samples. Second, we can consider that lithium responsiveness is a familial, pharmacogenetic trait.

5.2. Lithium Response as Phenotype

Rather than comparing the rate of BPAD in relatives of lithium responders and nonresponders, some studies have focused on lithium responsiveness alone as phenotype in genetic studies (Lerer and Macciardi, 2002). This type of study is difficult to perform, because many affected subjects taking the same mood stabilizer for a long time must be recruited within the same families. For example, Grof et al. (2000) reported a higher response rate to lithium (67%) in the relatives of bipolar probands considered as lithium responders than in a comparison group (30%). This finding confirms the earlier report from McKnew et al. (1981) suggesting that children of bipolar lithium responders have a response concordant with that of their parents.

5.3. Other Mood Stabilizers

Studies are lacking showing a family component in the response to carbamazepine and valproate. Coryell et al. (2000) investigated a small group of BPAD patients taking carbamazepine or valproate alone during 26 weeks. The presence of major depressive disorder among relatives was associated with slower improvement during acute treatment and with higher symptom levels during continuing treatment. But due to the small sample size, no definitive conclusion can be drawn. Further studies are needed to exclude that response to carbamazepine or valproate can be considered as a pharmacogenetic trait or that these groups are subphenotypes in the BPAD phenotype. More recently, Passmore et al. (2003) compared two groups of patients responsive to lithium and lamotrigine. Here also, samples were small but the authors suggested that lithium- and lamotrigine-responsive patients differ with respect to family history and may represent distinct subtypes of BPAD.

6. BIOCHEMICAL MECHANISM OF ACTION OF MOOD STABILIZERS: SEARCH FOR SUSCEPTIBILITY GENES

The mechanism of action of mood stabilizers, and lithium particularly, are not yet exactly elucidated. Recent progresses in the knowledge of their biochemical effects have confirmed that they are complex involving different systems, mainly first messengers, transduction pathways and gene expression mechanisms. The understanding of the mechanism of action is crucial to select candidate genes for genetic association studies.

6.1. Mechanisms of Action of Lithium, Carbamazepine et Valproate

6.1.1. Lithium Ratio

Lithium is distributed between extracellular and intracellular compartments. Dorus et al. (1974; 1975) brought evidence from twin studies that the extracellular/intracellular ratio concentration of lithium (“lithium ratio”) is

genetically determined. In addition to these findings, it is been argued that the lithium ratio could influence treatment response, but other studies refuted this suggestion (for review see Serretti, 2002a). More recently, the lithium ratio has been implicated in the occurrence of side effects (De Maio et al., 1994).

6.1.2. First messengers, pre- and postsynaptic receptors

The effects of mood stabilizers, and in particular lithium, have been studied on virtually every neurotransmitters: serotonin (5HT), dopamine (DA), noradrenaline (NA), acetylcholine (Ach), γ-aminobutyric acid (GABA) and glutamate (GLU). From early reports in the 1970s showing increased 5-hydroxyindolacetic acid (5-HIAA, main serotonin metabolite) levels in manic patients, the relationship between 5HT, BPAD and lithium has been extensively studied (Mendels, 1971). An exhaustive review by Serretti (2002a) shows that chronic lithium administration may enhance 5HT function at different levels: precursor uptake, synthesis, storage, catabolism, release, receptors and receptor-effector interactions. Lithium may thus act as a 5HT function corrector, function which is thought to be abnormal in BPAD. Other mood stabilizers seem to modulate 5HT function. Valproate and carbamazepine increased extracellular 5HT in animal models (Whitton et al., 1985; Dailey et al., 1997). Maes et al. (1997) investigated the putative action of valproate on 5HT neurotransmission by examining plasma cortisol response to L-5-hydroxytrophan (5HTP, precursor of 5HT) in manic patients before and after valproate treatment. They showed that L-5HTP-induced cortisol response was higher after valproate treatment than before, indicating a possible action of valproate on 5HT function. Lithium has not shown consistent results on a putative regulation of DA receptors D_1 and D_2 but classic works have demonstrated that lithium increases the DA turnover in a region-specific manner and may decrease DA formation (Ahluwalia et al., 1981). Valproate, but not carbamazepine, has induced increased DA turnover in several brain areas, according to some reports (Sokomba et al., 1988; Löscher and Hönack, 1996). A recent PET-study demonstrated a decreased presynaptic DA function in the basal ganglia after 3-5 weeks of valproate treatment (Yatham et al., 2002a). The effects of lithium on NA receptor binding remain inconclusive (El-Mallakh, 1996). But, by acting on the postsynaptic receptor sensitivity and on cyclic adenosine monophosphate (cAMP) accumulation, chronic administration of lithium may facilitate NA release, possibly via the effects on the presynaptic α_2 autoreceptors (Manji et al., 1991; Lenox and Hahn, 2000). Chronic lithium treatment modifies various behavioral responses that are mediated by Ach. Unfortunately, studies on muscarinic receptors regulation by lithium have shown contradictory results (Dilsaver and Coffman, 1989). GABA is the major inhibitory neurotransmitter in brain and has been implicated and studied for years in the mechanism of action of lithium, valproate and carbamazepine. Lithium increases GABA levels in cerebrospinal fluid (CSF) and $GABA_B$ receptors were found to be increased in hippocampus during chronic lithium treatment (Berrettini et al., 1986; Motohashi, 1992). GABA seems to be the key neurotransmitter in the mechanism of action of valproate. Valproate appears to modulate the effects of GABA by increasing its synthesis and its release, by inhibiting its breakdown, reducing its

reuptake into GABA neurons or augmenting its effects at GABA receptors (Stahl, 2000). These effects are related to an inhibition of calcium and sodium channels. The action of carbamazepine on GABA remains unknown but, like other anticonvulsivants and valproate, it may exert its effects by increasing GABA activity. Finally, lithium was implicated in glutamatergic neurotransmission regulation. For example, GLU release has been demonstrated to be increased in animal models during lithium treatment (Dixon et al., 1994). In conclusion, it appears that mood stabilizers exert their function in the majority of neurotransmitters systems, which may certainly interact. A lot of candidate genes emerge from this review and could be investigated in pharmacogenetic studies.

6.1.3. Transduction pathways

Extensive research has been carried on second messengers implicated in the mechanism of action of lithium, valproate and carbamazepine. We will here review briefly the current theories on phosphoinositol, protein kinase C, glycogen synthase kinase-3 and adenylyl cyclase, thought to be implicated in the psychopharmacology of mood stabilizers, in order to point out promising target proteins for future association studies. Excellent and exhaustive reviews are available elsewhere (Manji and Duman, 2001; Gould and Manji, 2002; Gray et al., 2003 Brunello and Tascedda, 2003). After combining at a postsynaptic receptor, lithium inhibits a small group of enzymes including inositol polyphosphate 1-phosphatase (IPPase) and inositol monophosphate phosphatase (IMPase), which are involved in recycling inositol polyphosphates to inositol. The mechanism of action of lithium could thus be related to a depletion of free inositol (Berridge et al., 1989). However, the extremely fast delay to inhibit IPPase and IMPase is not correlated to clinical effects of lithium, which need several days or weeks to be initiated. The inositol is thus not the direct responsible of lithium response but the first step of a cascade implicating ultimately gene expression factors. A recent important study confirmed the hypothesis that the therapeutic target of lithium in the treatment of BPAD depends on inositol depletion and has succeeded in extending these findings to valproate and carbamazepine (Williams et al., 2002). In addition, the authors suggested that a cytoplasmic protein, prolyl oligopeptidase (PREP), could regulate inositol metabolism. Two specific inhibitors of PREP activity have abolished *in vitro* the effects of lithium, valproate and carbamazepine, suggesting an important role of PREP in BPAD (Harwood and Agam, 2003).

Another important research field concerns the effects of mood stabilizers on protein kinase C (PKC) pathway (Jope, 1999; Manji and Lenox, 2000a). Reports have documented that chronic lithium administration decreases PKC levels, maybe via lithium's effect on IMPase (Manji et al., 1993; Manji et al., 1996; Manji et al., 1999). Further studies have also reported that a major PKC substrate, myristoylated alanaine-rich C kinase substrate (MARCKS), is depleted during chronic lithium treatment in rats (Lenox et al., 1992). Here also, IMPase and IPPase inhibitions may be causative factors. In culture cells, valproate has been shown to reduce PKC activity (Chen et al., 1994). And, similar to lithium, valproate decreases the levels of MARCKS (Watson et al., 1998).

Glycogen synthase-3 (GSK-3) is also a target for lithium and valproate. This enzyme is an important effector for several endogenous growth factor (such as BDNF, see below) and seems to play a crucial role in regulating neuronal survival and synaptic plasticity. GSK-3 is specifically inhibited by lithium and valproate (Klein and Melton, 1996), suggesting that mood stabilizers may have some neuroprotective effects, due to the inhibition of GSK-3 (Manji et al., 2000b). More largely, GSK-3 is a component of the Wnt signalling pathway. Recent evidences show that lithium and valproate exert effects on component of the Wnt signalling pathway, such as GSK-3 but also β-catenin protein (Gould and Manji, 2002). The authors strongly suggest that Wnt/GSK-3 regulation could be a target for further mood-stabilizing drugs.

Finally, some contradictory reports have implicated chronic administration of lithium in the modulation of G protein subunits, without changing the overall density of G protein coupled receptors (Casebolt et al., 1990). There is also evidence for an increase in basal cyclic AMP activity during lithium treatment suggesting that lithium could act in the interaction between an inhibition of adenylate cyclase, an upregulation of adenylate cyclase subtypes and different effects on the stimulatory and inhibitory G proteins (Gould and Manji, 2002). Carbamazepine has been shown to be a direct inhibitor of adenylate cyclase, thus attenuating cyclic AMP mediated signalling (Chen et al., 1996a). One report suggested that valproate may exert its effect on AMP signalling at different levels, but this hypothesis must be further tested (Chen et al., 1996b).

6.1.4. Gene expression

Previous paragraphs have attempted to point out some proteins of interest that could be tested in further genetic studies. It is clear that these different pathways interact and are the first step to a long-term neuroplastic modulation mediated by gene regulation, explaining the delayed therapeutic effect of mood stabilizers. The key finding of recent studies is the role of the ERK MAP kinase pathway (Gould et al., 2002). ERK MAP kinases are abundantly present in the brain and are involved in several neuroplastic processes. This pathway is activated by neurotrophins (such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) or neurotrophin 3 (NT3)). The activation of the ERK MAP kinase pathway is responsible for the increasing expression of an important antiapoptotic agent, B-cell lymphoma/leukaemia-2-gene (bcl-2) and an important transcription factor, cAMP responsive element binding protein (CREB). It has been recently demonstrated that both lithium and valproate increase bcl-2 in rodent frontal cortex, hippocampus and striatum. Further studies have recently shown that lithium and valproate activate not only bcl-2 but also the whole ERK MAP kinase pathway, including its upstream and downstream factors, CREB and BDNF (Yuan et al., 2001, Manji and Chen, 2002). For example, neurogenesis seems to be activated in the dentate gyrus in the hippocampus during lithium treatment (Chen et al., 2000). Based on *in vivo* and postmortem imaging studies on BPAD patients suggesting structural brain abnormalities, it has been suggested for years that lithium and valproate could act as real neuroprotective agents. Taking together recent findings on the ERK

MAP kinase pathway and previous imaging studies, Manji et al. (2000b) undertook quantitative three-dimensional magnetic resonance imaging studies in BPAD patients and demonstrated that the total gray matter volume increases during lithium treatment. Besides the fact that the elucidation of the mechanism of action of mood stabilizer have helped in the comprehension of the pathophysiology of BPAD, these finding have pointed out a huge amount of candidate genes for further pharmacogenetic studies.

6.2. Mechanisms of Action of Lamotrigine

As exposed above, lamotrigine has focused interest since it has been shown to have antidepressant effects but also a prevention efficacy in relapses and recurrences of depressive episodes. Further studies are needed to consider lamotrigine as a real mood stabilizer but growing evidences imply that this compound will be largely used in BPAD, as well in the acute depressive state and during maintenance phase. Lamotrigine exert effect by inhibiting the release of GLU and aspartate by blocking sodium channels (Grunze et al., 2002). An increased of extracellular 5HT has been observed in vitro (Southam et al., 1998). Lamotrigine may also exert part of its action by calcium antagonistic effects (Grunze et al., 2002). Recently, Vinod and Subhash (2002) have suggested that one mode of action of lamotrigine may be by the down regulation of cortical 5-HT_{1A} receptor-mediated adenylyl cyclase response. On the other side, it seems that lamotrigine did not affect BDNF-mediated signalling (Mai et al., 2002) even lamotrigine had neuroprotective effects in several animal models of ischemia (for review see Li et al., 2002). A recent review suggests that lamotrigine psychotropic effects are mainly due to antiglutamatergic and neuroprotective actions and that sodium channel blockade is more important in the antiseizure effect (Ketter et al., 2003).

7. MOLECULAR GENETIC STUDIES

7.1. Associations Studies

Candidate-gene association studies have become the appropriate strategy for studying genetic factors involved in complex diseases and treatment response in which the mode of inheritance is unknown (Souery et al., 2001). Association studies investigate gene polymorphisms thought to be implicated in the mechanism of action of a disease or which may modulate the action of a medication, lithium in our case. However, limitations in association studies exist and are largely discussed elsewhere (Craddock et al., 2001; Potash and DePaulo, 2000; Oswald et al., 2003). The major limitation, because of the difficulty to recruit cases and controls, is small sample sizes which do not provide enough statistical power for minor gene effects. Furthermore, spurious associations between a genetic marker and a disorder or a (sub)phenotype may result from variation in allele frequency between cases and controls if the two populations are ethnically different (population stratification). It is thus important in this case to test populations that are comparable in ethnic background. Finally, caution

must be used in the interpretation of the association observed. In fact, the result may be interpreted as linkage disequilibrium between the locus implicated in the studied phenotype and an associated marker allele, located near the tested gene. In the specific case of pharmacogenetic studies, the major issue remains the phenotype definition. In the treatment of BPAD, it is difficult to establish what is exactly response and efficacy to lithium. Environmental factors and concomitant treatment are relevant features which need to be monitored during treatment. Another issue is the definition of response itself. The dichotomous approach in association studies has implied the definition of a cut-off point (Serretti, 2002a). Therefore, response may be defined as the complete absence of manic or depressive episode during lithium treatment, considering an adequate blood level. In some papers, response is defined as the absence of recurrence during lithium treatment for >= 3 years (Lipp et al., 1997). Other authors tend to consider response as a quantitative variable, reflecting the frequency of episodes on and off lithium (Engstrom et al., 1997; Serretti et al., 2002a). These different approaches may explain the apparent contradictory results between studies.

Results from extensive research on the mechanism of action of lithium have provided a huge amount of candidate genes which could be implicated in treatment response. For now, genes coding for enzymes or receptors implicated in the first messengers pathways have been largely studied (table 4). A few markers implicated in the transduction pathways have been studied. Further studies will have to focus on gene expression markers, thought to be the ultimate site of action of mood stabilizers.

Two approaches have been applied in association studies on lithium response. The first uses the pharmacogenetic trait of lithium response as the phenotype. The rationale underlying this purpose is to consider that lithium-responsive BPAD is more likely to be a genetically distinct category of the illness. Evidences from family studies tend to show that response to lithium is a highly familial trait (see above). This finding may thus help define that response to lithium prophylaxis is a distinct bipolar phenotype with less genetic heterogeneity and stronger genetic effect (Turecki et al., 2001). An international collaborative group (International Group for Study of Lithium, IGSLI) has constituted a sample of “excellent” responders to lithium (Cavazzoni et al., 1996; Turecki et al., 1996; Turecki et al., 1998; Turecki et al., 1999; Alda et al., 2000; Duffy et al., 2000). Excellent responders to lithium must have demonstrated a high risk for recurrence before they began treatment with lithium and must have been maintained on lithium exclusively, with no further episodes for at least 3 years. In addition, all these patients had a family history of BPAD. Results from these studies are shown in table 4. In summary, a positive result has been found on phospholipase Cγ1 gene (PLCG1), implicated in the phosphoinositol pathway, with a modest odd-ratio (OR=1.88) (Turecki et al., 1998). It has thus been concluded that PLCG1 has a modest implication in the response to lithium, or in the disease itself. The second approach is to compare directly responders and nonresponders to lithium in a case-control design. Lipp et al. (1997) first reported an association between DRD2 and nonresponse to lithium (table 4). Several studies have followed, mainly from the University of

Table 4: Case-control association pharmacogenetic studies on response to lithium

Author	*Marker*	*Sample*	*Study Design*	*Findings*
Cavazzoni et al. (1996)	Tyrosine Hydroxylase gene (TH)	54 responders (48 BPAD; 6 UPAD); 94 controls	Comparison Responders / Controls	No Association
Turecki et al. (1996)	GTP binding protein gene (G_{olf}) Five chromosome-18 markers that map to the same region (D18S40, D18S53, D18S56, D18S59, D18S62)	55 responders (47 BPAD; 8 UPAD); 94 controls	Comparison Responders / Controls	No Association
Lipp et al. (1997)	D_2 receptor gene (DRD2) D_3 receptor gene (DRD3) D_4 receptor gene (DRD4) TH	56 BPAD (20 responders; 36 nonresponders); 112 controls	Comparison Responders / Nonresponders / Controls	Association for DRD2 allele frequency when comparing nonresponders and controls (p=.01)
Turecki et al. (1998)	Phospholipase Cγ1 gene (PLCG1)	136 BPAD responders; 163 controls	Comparison Responders / Controls	Association between PLCG1 allele frequency and response (p=.033)
Serretti et al. (1998)	DRD3	43 BPAD; 12 UPAD	Comparison Responders / Nonresponders	No Association
Steen et al. (1998)	Inositol polyphosphate 1-phosphatase gene (INPP1)	Norwegian sample: 23 BPAD Israeli sample: 54 BPAD	Comparison Responders / Nonresponders	No Association in the Israeli sample; Association in the Norwegian sample for C973A polymorphism.
Turecki et al. (1999)	Monoamine oxidase A gene (MAOA)	138 BPAD responders; 108 controls	Comparison Responders / Controls	No Association
Del Zompo et al. (1999)	Serotonin Transporter gene (5-HTT)	66 BPAD	Comparison Responders / Nonresponders	Association between 5-HTTLPR l-allele and non-response
Serretti et al. (1999a)	D4 receptor gene (DRD4) DRD2 GABA receptor 1 gene (GABRA1)	100 BPAD; 25 UPAD	Comparison Responders / Nonresponders	No Association
Serretti et al. (1999b)	Tryptophan Hydroxylase gene (TPH)	90 BPAD; 18 UPAD	Comparison Responders / Nonresponders	Association with worse response (p=.046) for TPH A218C polymorphism
Alda et al. (2000)	Corticotropin-releasing hormone (CRH) Proenkephalin (PENK)	138 BPAD responders; 108 controls	Comparison Responders / Controls	No Association
Duffy et al. (2000)	GABA receptor 3 gene (GABRA3) GABA receptor 5 gene (GABRA5) GABA receptor 3B gene (GABRB3)	138 BPAD responders; 108 controls	Comparison Responders / Controls	No Association

Serretti et al. (2000)	5-HT_{2A} receptor gene (HTR2A) 5-HT_{2C} receptor gene (HTR2C) 5-HT_{1A} receptor gene (HTR1A)	102 BPAD; 18 UPAD	Comparison Responders / Nonresponders	No Association. No HTR1A polymorphism detected in the sample
Serretti et al. (2001)	5-HTT	167 BPAD; 34 UPAD	Comparison Responders / Nonresponders	Association between 5-HTTLPR s-s genotype and poor response
Lovlie et al. (2001)	PLCG1	61 BPAD; 50 controls	Comparison complete responders (n=29) / partial responders (n=16) / nonresponders (n=16) / controls	Association when comparing responders and controls
Serretti et al. (2002b)	Catéchol-*o*-methyl Transferase gene (COMT) MAOA G-Protein β 3-subunit gene (G- β3)	160 BPAD; 41 UPAD	Comparison Responders / Nonresponders	No Association
Serretti et al. (2003)	5-HTT	83 BPAD	Comparison Responders / Nonresponders	Marginally better response to lithium significantly associated with 5HTT s/l genotype in subjects with a low number of manic episodes before lithium and in subjects with a high daily dose of lithium

Milan (Serretti et al., 1998; Serretti et al., 1999a; Serretti et al., 1999b; Serretti et al., 2000; Serretti et al., 2001; Serretti et al., 2002b). Several genes coding for receptors of neurotransmitters or enzymes implicated in their synthesis or degradation were tested in a sample of both BPAD and UPAD lithium-treated patients, considering the response to lithium as a continuous measure. Positive results were found in TPH and 5HTT, where the 5-HTTLPR s-s genotype was associated with poor response (Serretti et al., 1999; Serretti et al., 2001). This last result is comparable with previous findings showing a higher frequency of s-s genotype among nonresponders to fluvoxamine, fluoxetine or paroxetine in major depression (Smeraldi et al., 1998; Kim et al., 2000). On the other side, a previous study showed a higher frequency of s-allele among responders (Del Zompo et al., 1999). Serretti et al. (2003) recently confirmed that a marginally better response to lithium treatment was associated with 5-HTTLPR s/l genotype among subjects who had a low number of manic episodes before lithium administration and among subjects with a high daily dose of lithium (more than 1200 mg). The group of V.Steen has shown that one polymorphism within the inositol polyphosphate 1-phosphatase was associated with lithium response, but only in the Norwegian subsample (Steen et al., 1998)

7.2. Linkage Studies

Only a few linkage studies were performed in lithium responder patients. The majority were done in addition to association studies from the IGSLI. Turecki et al. (1998) added a linkage study to their positive results on PLCG1, found using a case-control design. PLCG1 was studied in 32 families ascertained through lithium-responsive bipolar probands. A modest involvement of this gene in the pathogenesis of BD was found when unilineal families were considered, but not in the whole sample. Additional linkage studies on MAOA, CRH, PENK, GABRA3, GABRA5, GABRB3 did not support an implication of these genes in the response to lithium and in the occurrence of BPAD (Turecki et al., 1999; Alda et al., 2000; Duffy et al., 2000).

7.3. Genome-Wide Scans

A complete genome scan was recently performed. Turecki et al. (2001) recruited 247 subjects in 31 families ascertained through excellent lithium responders. 378 markers were scanned spaced at an average distance of 10 cM. BPAD and UPAD were first considered as phenotypes. Evidences for linkage were found on 15q14 (lod=3.46; p=.000014) and 7q11.2 (lod=2.68; p=.00011). When considering treatment response as phenotype, the highest lod score (1.53; p=.003) was for the marker D7S1816 located on chromosome 7q11.2. As argued by the authors, it has been difficult to assess lithium response in the relatives, particularly among those who are unaffected, apparently nonresponders or who have not been treated with lithium. These issue has led to a considerable power decrease, compared to the analyse using BPAD and UPAD as phenotypes. However, these results, based on a genome scan approach, are promising and provide arguments for future genome wide scans.

8. CONCLUSION AND PERSPECTIVES FOR FUTURE RESEARCH

Different approaches are available to identify genetic markers that could contribute to the response to mood stabilizers. The priority is a considerable need for improved methodology in case-control association studies. The different ways to improve it are discussed elsewhere (Lerer and Macciardi, 2002; Oswald et al., 2003). In the specific field of pharmacogenetics of BPAD, we will have to improve the specific design of mood stabilizers pharmacogenetic studies. In the "classical" studies, responsiveness is dichotomously defined (response or non-response). More recent studies use treatment response as a continuous measure allowing multivariate statistical analyses, including a large number of factors (Cusin et al., 2002).

Another way to consider future research is to focus on new candidate genes, believed to be the ultimate sites of action of mood stabilizers (see above). This must also be considered in parallel to a better understanding of the mode of action of "newer" mood stabilizers, i.e. atypical antipsychotics and lamotrigine. Some studies tend to show that lamotrigine may have neuroprotective effects, thus implicating gene regulation.

New lab techniques, such as expression and proteomic array technologies, will also help in defining new candidate genes.

Finally, it seems more and more obvious that, in addition to single-gene pharmacogenetic studies, it will be crucial to take into account gene-gene interactions, and certainly interactions with environment, which need to be incorporated in statistical models.

Many ethical issues need also to be resolved (Mancinelli et al., 2000). It has been shown that the cost of developing a new drug could be reduced to about 60% using pharmacogenetics, mainly by reducing the number of needed participants in clinical trials (Lipton, 2003). Pharmacogenetic studies will be therefore developed in the future with many stored genetic data for each participant. Obviously, information of this type must be carefully safeguarded to ensure privacy. In addition, there also questions about feedback to be given to the patient. For example, it may be possible to make some predictions on the propensity for a patient to respond to a medication and to classify him as a "bad responder", with all the psychological consequences. Another concern is that individuals may find it more difficult to find affordable health insurance as a consequence pharmacogenetic test. Many legal and economic issues will thus need to be resolved.

In the future, after refining the methodology and the investigated phenotypes of lithium pharmacogenetic studies, the prediction of drug efficacy and side effects, using a pharmacogenetic tool, could be considered as a helpful assistance in selection and monitoring of treatment in BPAD. In parallel to a better understanding of the neurobiology of BPAD, progress in pharmaco-genomics could improve the outcome of a still chronic disease, with a high rate of mortality.

REFERENCES

Ahluwalia P, Singhal RL, 1981, Monoamine uptake into synaptosomes from various regions of rat brain following lithium administration and withdrawal. *Neuropharmacology* **2 :** 483-487.

Akiskal HS and Pinto O, 1999, The evolving bipolar spectrum. Prototypes I, II, III, and IV. *Psychiatr Clin North Am* **22:** 517-534.

Alda M, Turecki G, Grof P, Cavazzoni P, Duffy A, Grof E, Ahrens B, Berghofer A, Muller-Oerlinghausen B, Dvorakova M, Libigerova E, Vojtechovsky M, Zvolsky P, Joober R, Nilsson A, Prochazka H, Licht RW, Rasmussen NA, Schou M, Vestergaard P, Holzinger A, Schumann C, Thau K, Rouleau GA, 2000, Association and linkage studies of CRH and PENK genes in bipolar disorder: a collaborative IGSLI study. *Am J Med Genet* **9 :** 178-181.

Alda M, 2002, Genetic factors and long-term prophylaxis, in: *Pharmacogenetics of psychotropic drugs*, Lerer B, ed, Cambridge University Press, Cambridge, pp 320-332.

Altshuler L, Suppes T, Black D, Nolen WA, Keck PE Jr, Frye MA, McElroy S, Kupka R, Grunze H, Walden J, Leverich G, Denicoff K, Luckenbaugh D, Post R, 2003, Impact of antidepressant discontinuation after acute bipolar depression remission on rates of depressive relapse at 1-year follow-up. *Am J Psychiatry* **1 :** 1252-1262.

American Psychiatric Association, 1994, Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), Washington, DC, American Psychiatric Press.

American Psychiatric Association, 2002, Practice guideline for the treatment of patients with bipolar disorder (revision). *Am J Psychiatry* **159** (4 Suppl)**:** 1-50.

Angst J. and Gamma A., 2002, Prevalence of bipolar disorders: traditional and novel approaches, *Clin Approaches Bipol Disord* **1:** 10-14.

Baldessarini RJ, Tondo L, Hennen J, Viguera AC. Is lithium still worth using? An update of selected recent research. *Harv Rev Psychiatry*. 2002 Mar-Apr;10(**2**)**:** 59-75.

Ballenger JC, Post RM, 1978, Therapeutic effects of carbamazepine in affective illness: a preliminary report. *Commun Psychopharmacol* **2:** 159-175.

Berrettini WH, Nurnberger JI Jr, Hare TA, Simmons-Alling S, Gershon ES, 1986, CSF GABA in euthymic manic-depressive patients and controls. *Biol Psychiatry* **21:** 844-846.

Berridge MJ, Irvine RF, 1989, Inositol phosphates and cell signalling. *Nature* **3 1:** 197-205.

Bowden CL, Brugger AM, Swann AC, Calabrese JR, Janicak PG, Petty F, Dilsaver SC, Davis JM, Rush AJ, Small JG, 1994, Efficacy of divalproex vs lithium and placebo in the treatment of mania. The Depakote Mania Study Group. *JAMA* **271:** 918-924.

Bowden CL, Calabrese JR, McElroy SL, Gyulai L, Wassef A, Petty F, Pope HG Jr, Chou JC, Keck PE Jr, Rhodes LJ, Swann AC, Hirschfeld RM, Wozniak PJ, 2000, A randomized, placebo-controlled 12-month trial of divalproex and lithium in treatment of outpatients with bipolar I disorder. *Arch Gen Psychiatry* **57:** 481-489.

Brunello N, Tascedda F, 2003, Cellular mechanisms and second messengers: relevance to the psychopharmacology of bipolar disorders. *Int J Neuropsychopharmacol* **6:** 181-189.

Calabrese JR, Bowden CL, Sachs GS, Ascher JA, Monaghan E, Rudd GD. A double-blind placebo-controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. Lamictal 602 Study Group. *J Clin Psychiatry*. 1999 Feb;60(**2**)**:** 79-88.

Calabrese JR, Suppes T, Bowden CL, Sachs GS, Swann AC, McElroy SL, Kusumakar V, Ascher JA, Earl NL, Greene PL, Monaghan ET, 2000, A double-blind, placebo-controlled, prophylaxis study of lamotrigine in rapid-cycling bipolar disorder. Lamictal 614 Study Group. *J Clin Psychiatry* **61:** 841-850.

Calabrese JR, Bowden CL, Sachs G, Yatham LN, Behnke K, Mehtonen OP, Montgomery P, Ascher J, Paska W, Earl N, DeVeaugh-Geiss J. A placebo-controlled 18-month trial of lamotrigine and lithium maintenance treatment in recently depressed patients with bipolar I disorder. *J Clin Psychiatry*. 2003 Sep;64(**9**)**:** 1013-24.

Calabrese JR, Keck PE Jr, Macfadden W, Minkwitz M, Ketter TA, Weisler RH, Cutler AJ, McCoy R, Wilson E, Mullen J. A randomized, double-blind, placebo-controlled trial of quetiapine in the treatment of bipolar I or II depression. *Am J Psychiatry*. 2005 Jul;162(**7**)**:** 1351-60.

Casebolt TL, Li XH, Jope RS, 1990, Alpha-1 adrenergic receptor binding and adrenergic-stimulated cyclic AMP production in rat cerebral cortex after chronic lithium treatment. *J Neural Transm Gen Sect* **82**: 197-204.

Cavazzoni P, Alda M, Turecki G, Rouleau G, Grof E, Martin R, Duffy A, Grof P, 1996, Lithium-responsive affective disorders: no association with the tyrosine hydroxylase gene. *Psychiatry Res* **64:** 91-96.

Chen G, Manji HK, Hawver DB, Wright CB, Potter WZ, 1994, Chronic sodium valproate selectively decreases protein kinase C alpha and epsilon in vitro. *J Neurochem* **63:** 2361-2364.

Chen G, Pan B, Hawver DB, Wright CB, Potter WZ, Manji HK, 1996a, Attenuation of cyclic AMP production by carbamazepine. *J Neurochem* **67:** 2079-2086;

Chen G, Manji HK, Wright CB, Hawver DB, Potter WZ, 1996b, Effects of valproic acid on beta-adrenergic receptors, G-proteins, and adenylyl cyclase in rat C6 glioma cells. *Neuropsychopharmacology* **15:** 271-280.

Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK, 2000, Enhancement of hippocampal neurogenesis by lithium. *J Neurochem* **75:** 1729-1734.

Cohn JB, Collins G, Ashbrook E, Wernicke JF, 1989, A comparison of fluoxetine imipramine and placebo in patients with bipolar depressive disorder. *Int Clin Psychopharmacol* **4:** 313-322.

Cookson J, 2002, The classification of Bipolar Disorders, in: *Seizures, Affective Disorders and Anticonvulsant Drugs*, M.R. Trimble, B. Schmitz, eds., Clarius Press Ltd, Guildford, pp. 35-51.

Coryell W, Akiskal H, Leon AC, Turvey C, Solomon D, Endicott J, 2000, Family history and symptom levels during treatment for bipolar I affective disorder. *Biol Psychiatry* **47:** 1034-1042.

Craddock N, Dave S, Greening J, 2001, Association studies of bipolar disorder. *Bipolar Disord* **3:** 284-298.

Cusin C, Serretti A, Zanardi R, Lattuada E, Rossini D, Lilli R, Lorenzi C, Smeraldi E, 2002, Influence of monoamine oxidase A and serotonin receptor 2A polymorphisms in SSRI antidepressant activity. *Int J Neuropsychopharmacol* **5:** 27-35.

Dailey JW, Reith ME, Yan QS, Li MY, Jobe PC, 1997, Anticonvulsant doses of carbamazepine increase hippocampal extracellular serotonin in genetically epilepsy-prone rats: dose response relationships. *Neurosci Lett* **227:** 13-16.

Del Zompo M, Ardau R, Palmas MA, Bocchetta A, Reina A, Piccardi MP, 1999, Lithium response: association study with two candidate genes. *Mol Psychiatry* **4:** 66-67.

De Maio D, Buffa G, Riva M, Laviani M, Levi-Minzi A, 1994, Lithium ratio, phospholipids and the incidence of side effects. *Prog Neuropsychopharmacol Biol Psychiatry* **18:** 285-293.

Denicoff KD, Smith-Jackson EE, Disney ER, Ali SO, Leverich GS, Post RM, 1997, Comparative prophylactic efficacy of lithium, carbamazepine, and the combination in bipolar disorder. *J Clin Psychiatry* **58:** 470-478.

Dilsaver SC, Coffman JA, 1989, Cholinergic hypothesis of depression: a reappraisal. *J Clin Psychopharmacol* **9:** 173-179.

Dixon JF, Los GV, Hokin LE, 1994, Lithium stimulates glutamate release and inositol 1,4, 5-trisphosphate accumulation via activation of the N-methyl-D-aspartate receptor in monkey and mouse cerebral cortex slices. *Proc Natl Acad Sci U S A* **91:** 8358-8362.

Dorus E, Pandey GN, Frazer A, Mendels J, 1974, Genetic determinant of lithium ion distribution. I. An in vitro monozygotic-dizygotic twin study. *Arch Gen Psychiatry* **31:** 463-465.

Dorus E, Pandey GN, Davis JM, 1975, Genetic determinant of lithium ion distribution. An in vitro and in vivo monozygotic-dizygotic twin study. *Arch Gen Psychiatry* **32:** 1097-1102.

Duffy A, Turecki G, Grof P, Cavazzoni P, Grof E, Joober R, Ahrens B, Berghofer A, Muller-Oerlinghausen B, Dvorakova M, Libigerova E, Vojtechovsky M, Zvolsky P, Nilsson A, Licht RW, Rasmussen NA, Schou M, Vestergaard P, Holzinger A, Schumann C, Thau K, Robertson C, Rouleau GA, Alda M, 2000, Association and linkage studies of candidate genes involved in GABAergic neurotransmission in lithium-responsive bipolar disorder. *J Psychiatry Neurosci* **25:** 353-358.

Dunner DL, Fleiss JL, Fieve RR, 1976, Lithium carbonate prophylaxis failure. *Br J Psychiatry* **129:** 40-44.

El-Mallakh RS, 1996, *Lithium: actions and mechanisms*. American Psychiatric Press, Washington DC.

Engstrom C, Astrom M, Nordqvist-Karlsson B, Adolfsson R, Nylander PO, 1997, Relationship between prophylactic effect of lithium therapy and family history of affective disorders. *Biol Psychiatry* **42:** 425-433.

Geddes JR, Burgess S, Hawton K, Jamison K, Goodwin GM. Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. Am J Psychiatry. 2004 Feb;161(**2**)**:** 217-22. Review.

Gijsman HJ, Geddes JR, Rendell JM, Nolen WA, Goodwin GM. Antidepressants for bipolar depression: a systematic review of randomized, controlled trials. Am J Psychiatry. 2004 Sep;161(**9**)**:** 1537-47.

Goldsmith D, Wagstaff A, Ibbotson T, Perry C, 2003, Lamotrigine: A Review of its Use in Bipolar Disorder. *Drugs* **63:** 2029-2050.

Goodwin FK, 2003, Rationale for using lithium in combination with other mood stabilizers in the management of bipolar disorder. *J Clin Psychiatry* **64** (Suppl 5)**:** 18-24.

Goodwin GM, Bowden CL, Calabrese JR, Grunze H, Kasper S, White R, Greene P, Leadbetter R. A pooled analysis of 2 placebo-controlled 18-month trials of lamotrigine and lithium maintenance in bipolar I disorder. J Clin Psychiatry. 2004 Mar;65(**3**)**:** 432-41.

Goodwin FK, Jamison KR, 1990, *Manic-Depressive Illness.* Oxford University Press, New York, NY.

Gould TD, Manji HK, 2002, Signaling networks in the pathophysiology and treatment of mood disorders. J Psychosom Res **53:** 687-697.

Gourevitch D, 1994, La psychiatrie de l'antiquité gréco-romaine, in: *Nouvelle histoire de la psychiatrie*, J.Postel, C.Quétel, eds, Dunod, Paris, pp 3-18.

Gray NA, Zhou R, Du J, Moore GJ, Manji HK, 2003, The use of mood stabilizers as plasticity enhancers in the treatment of neuropsychiatric disorders*. J Clin Psychiatry* 64 Suppl **5:** 3-17.

Greil W, Ludwig-Mayerhofer W, Erazo N, Schochlin C, Schmidt S, Engel RR, Czernik A, Giedke H, Muller-Oerlinghausen B, Osterheider M, Rudolf GA, Sauer H, Tegeler J, Wetterling T, 1997, Lithium versus carbamazepine in the maintenance treatment of bipolar disorders — a randomised study. *J Affect Disord* **43:** 151-161.

Grof P, Alda M, Grof E et al., 1994, Lithium response and genetics of affective disorders. *J Affect Disord* **32:** 85-95.

Grof P, Alda M, Ahrens B, 1995, Clinical course of affective disorders: were Emil Kraepelin and Jules Angst wrong? *Psychopathology* 28 Suppl **1:** 73-80.

Grof P, Duffy A, Cavazzoni P et al., 2000, Is response to prpohylactic lithium a familial trait? *Int J Neuropsychopharmacol* **3** (suppl 1)**:** 338-339.

Grossi E, Sacchetti E, Vita A, 1984, Carbamazepine vs chlorpromazine in mania: a double-blind trial. In: *Anticonvulsants in Affective Disorders*, Emrich HM, Okuma T Muller AA, eds. Elsevier Science Publishers, Amsterdam, pp. 177-187.

Grunze H, Amann B, Walden J, 2002, How might anticonvulsivants drug in bipolar affective disorder? in: *Seizures, Affective Disorders and Anticonvulsant Drugs*, M.R. Trimble, B. Schmitz, eds., Clarius Press Ltd, Guildford, pp. 117-130.

Harwood AJ, Agam G, 2003, Search for a common mechanism of mood stabilizers. *Biochem Pharmacol* **66:** 179-189.

Hirschfeld RM, Keck PE Jr, Kramer M, Karcher K, Canuso C, Eerdekens M, Grossman F. Rapid antimanic effect of risperidone monotherapy: a 3-week multicenter, double-blind, placebo-controlled trial. Am J Psychiatry. 2004 Jun;161(**6**)**:** 1057-65.

Hirschfeld RMA, Sachs G, Calabrese et al. The impact of lamotrigine on core symptoms in the acute treatment of bipolar depresión. In: Abstract of the 45th NCDEU Annual Meeting, Boca Raton, FL, June 6-9, 2005.

Hopkins HS, Gelenberg AJ, 1994, Treatment of bipolar disorder: how far have we come? *Psychopharmacol Bull* **30:** 27-38.

Jamison K, 2000, Suicide and bipolar disorder. *J Clin Psychiatry* **9:** 47-51.

Jope RS, 1999, Anti-bipolar therapy: mechanism of action of lithium. *Mol Psychiatry* **4:** 117-128.

Judd LL, Akiskal HS, Schettler PJ, Coryell W, Endicott J, Maser JD, Solomon DA, Leon AC, Keller MB, 2003, A prospective investigation of the natural history of the long-term weekly symptomatic status of bipolar II disorder. *Arch Gen Psychiatry* **60:** 261-269.

Keck PE Jr, McElroy SL, Nemeroff CB, 1992, Anticonvulsants in the treatment of bipolar disorder. *J Neuropsychiatry Clin Neurosci* **4:** 395-405.

Keck PE Jr, Mendlewicz J, Calabrese JR, Fawcett J, Suppes T, Vestergaard PA, Carbonell C, 2000a, A review of randomized, controlled clinical trials in acute mania*. J Affect Disord* **59** (Suppl 1)**:** 31-37.

Keck PE Jr, Welge JA, Strakowski SM, Arnold LM, McElroy SL, 2000b, Placebo effect in randomized, controlled maintenance studies of patients with bipolar disorder. *Biol Psychiatry* **47:** 756-765.

Ketter TA, Manji HK, Post RM, 2003, Potential mechanisms of action of lamotrigine in the treatment of bipolar disorders. *J Clin Psychopharmacol* **23:** 484-495.

Khanna S, Vieta E, Lyons B, Grossman F, Eerdekens M, Kramer M. Risperidone in the treatment of acute mania: double-blind, placebo-controlled study. Br J Psychiatry. 2005 Sep;**187:** 229-34.

Kim DK, Lim SW, Lee S, Sohn SE, Kim S, Hahn CG, Carroll BJ, 2000, Serotonin transporter gene polymorphism and antidepressant response. *Neuroreport* **11:** 215-219.

Klein PS, Melton DA, 1996, A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci U S A* **93:** 8455-8459.

Lenox RH, Watson DG, Patel J, Ellis J, 1992, Chronic lithium administration alters a prominent PKC substrate in rat hippocampus. *Brain Res* **570:** 333-340.

Lenox RH, Hahn CG, 2000, Overview of the mechanism of action of lithium in the brain: fifty-year update. *J Clin Psychiatry* **61**(Suppl 9)**:** 5-15.

Lerer B, Moore N, Meyendorff E, Cho SR, Gershon S, 1987, Carbamazepine versus lithium in mania: a double-blind study. *J Clin Psychiatry* **48:** 89-93.

Lerer B, Macciardi F, 2002, Pharmacogenetics of antidepressant and mood-stabilizing drugs: a review of candidate-gene studies and future research directions. *Int J Neuropsychopharmacol* **5:** 255-275.

Li X, Bijur GN, Jope RS, 2002, Glycogen synthase kinase-3beta, mood stabilizers, and neuroprotection. *Bipolar Disord* **4:** 137-144.

Lipp O, Mahieu B, Souery D, Serretti A, Cavallini C, Verga M, Van Broeckhoven C, Macciardi F, Mendlewicz J, 1997, Molecular genetics of bipolar disorders: Implication for psychotropic drugs. *Eur Neuropsychopharmacology* **7** (suppl 2)**:** 112-113.

Lipton P, 2003, Pharmacogenetics: the ethical issues. *Pharmacogenomics J* **3:** 14-16.

Loscher W, Honack D, 1996, Valproate and its major metabolite E-2-en-valproate induce different effects on behaviour and brain monoamine metabolism in rats. *Eur J Pharmacol* **299:** 61-67.

Maes M, Calabrese J, Jayathilake K, Meltzer HY, 1997, Effects of subchronic treatment with valproate on L-5-HTP-induced cortisol responses in mania: evidence for increased central serotonergic neurotransmission *Psychiatry Res* **71:** 67-76.

Mai L, Jope RS, Li X, 2002, BDNF-mediated signal transduction is modulated by GSK3beta and mood stabilizing agents. *J Neurochem* **82:** 75-83.

Mancinelli L, Cronin M, Sadee W, 2000, Pharmacogenomics: the promise of personalized medicine. *AAPS PharmSci* 2: E4.

Manji HK, Hsiao JK, Risby ED, Oliver J, Rudorfer MV, Potter WZ, 1991, The mechanisms of action of lithium. I. Effects on serotoninergic and noradrenergic systems in normal subjects. *Arch Gen Psychiatry* **48:** 505-512.

Manji HK, Etcheberrigaray R, Chen G, Olds JL, 1993, Lithium decreases membrane-associated protein kinase C in hippocampus: selectivity for the alpha isozyme. *J Neurochem* **61:** 2303-2310.

Manji HK, Bersudsky Y, Chen G, Belmaker RH, Potter WZ, 1996, Modulation of protein kinase C isozymes and substrates by lithium: the role of myo-inositol. *Neuropsychopharmacology* **15:** 370-381.

Manji HK, Bebchuk JM, Moore GJ, Glitz D, Hasanat KA, Chen G, 1999, Modulation of CNS signal transduction pathways and gene expression by mood-stabilizing agents: therapeutic implications. *J Clin Psychiatry* **60** (Suppl 2)**:** 27-39.

Manji HK, Lenox RH, 2000a, Signaling: cellular insights into the pathophysiology of bipolar disorder. *Biol Psychiatry* **48:** 518-530.

Manji HK, Moore GJ, Chen G, 2000b, Clinical and preclinical evidence for the neurotrophic effects of mood stabilizers: implications for the pathophysiology and treatment of manic-depressive illness. *Biol Psychiatry* **48:** 740-754.

Manji HK, Duman RS, 2001, Impairments of neuroplasticity and cellular resilience in severe mood disorders: implications for the development of novel therapeutics. *Psychopharmacol Bull* **35:** 5-49.

Manji HK, Chen G, 2002, PKC, MAP kinases and the bcl-2 family of proteins as long-term targets for mood stabilizers. *Mol Psychiatry* **7** (Suppl 1)**:** 46-56.

McKnew DH, Cytryn L, Buchsbaum MS, Hamovit J, Lamour M, Rapoport JL, Gershon ES, 198, Lithium in children of lithium-responding parents. *Psychiatry Res* **4:** 171-180.

Mendels J, 1971, Relationship between depression and mania. *Lancet* 1 (7694)**:** 342.

Mendlewicz J, Fieve RR, Stallone F, 1973, Relationship between the effectiveness of lithium therapy and family history. *Am J Psychiatry* **130:** 1011-1013.

Mendlewicz J, 1979, Prediction of treatment outcome: family and twin studies in lithium prophylaxis and the question of lithium red blood cell/plasma ratios in: *Lithium: controversies and unresolved issues*, Cooper TB, Gershon S, Kline NS, Schou M, eds, Excerpta Medica, Amsterdam, pp 226-240.

Misra PC, Burns BH, 1976, "Lithium nonresponders" in a lithium clinic. *Acta Psychiatr Scand* **55:** 32-38.

Morgan VA, Mitchell PB, Jablensky AV. The epidemiology of bipolar disorder: sociodemographic, disability and service utilization data from the Australian National Study of Low Prevalence (Psychotic) Disorders. Bipolar Disord. 2005 Aug;7(**4**)**:**326-37.

Motohashi N, 1992, GABA receptor alterations after chronic lithium administration. Comparison with carbamazepine and sodium valproate. *Prog Neuropsychopharmacol Biol Psychiatry* **16:** 571-579.

Murray CJ and Lopez AD, 1996, Evidence-based health policy--lessons from the Global Burden of Disease Study. *Science* **274**(5288) :740-743.

Nemeroff CB, Evans DL, Gyulai L, Sachs GS, Bowden CL, Gergel IP, Oakes R, Pitts CD, 2001, Double-blind, placebo-controlled comparison of imipramine and paroxetine in the treatment of bipolar depression. *Am J Psychiatry* **158:** 906-912.

Okuma T, Inanaga K, Otsuki S, Sarai K, Takahashi R, Hazama H, Mori A, Watanabe M, 1979, Comparison of the antimanic efficacy of carbamazepine and chlorpromazine: a double-blind controlled study. *Psychopharmacol* **66:** 211-217.

Oswald P, Souery D, Mendlewicz J, 2003, Molecular genetics of affective disorders. *Int J Neuropsychopharmacol* **6:** 155-169.

Passmore MJ, Garnham J, Duffy A, MacDougall M, Munro A, Slaney C, Teehan A, Alda M. Phenotypic spectra of bipolar disorder in responders to lithium versus lamotrigine. Bipolar Disord. 2003 Apr;5(**2**)**:** 110-4.

Pini S, de Queiroz V, Pagnin D, Pezawas L, Angst J, Cassano GB, Wittchen HU. Prevalence and burden of bipolar disorders in European countries. Eur Neuropsychopharmacol. 2005 Aug;15(**4**)**:** 425-34.

Potash JB, DePaulo JR Jr, 2000, Searching high and low: a review of the genetics of bipolar disorder. *Bipolar Disord* **2:** 8-26.

Prien RF, Caffey EM Jr, Klett CJ, 1972, Comparison of lithium carbonate and chlorpromazine in the treatment of mania. Report of the Veterans Administration and National Institute of Mental Health Collaborative Study Group. *Arch Gen Psychiatry* **26:** 146-153.

Rihmer Z, Pestality P, 1999, Bipolar II disorder and suicidal behavior. *Psychiatr Clin North Am* **22:** 667-673.

Szadoczky E, Papp Z, Vitrai J, Rihmer Z, Furedi J. The prevalence of major depressive and bipolar disorders in Hungary. Results from a national epidemiologic survey. J Affect Disord. 1998 Sep;**50**(2-3)**:**153-62.

Serretti A, Lilli R, Lorenzi C, Franchini L, Smeraldi E, 1998, Dopamine receptor D3 gene and response to lithium prophylaxis in mood disorders. *Int J Neuropsychopharmacol* **1:** 125-129.

Serretti A, Lilli R, Lorenzi C, Franchini L, Di Bella D, Catalano M, Smeraldi E, 1999a, Dopamine receptor D2 and D4 genes, GABA(A) alpha-1 subunit genes and response to lithium prophylaxis in mood disorders. *Psychiatry Res* **87:** 7-19.

Serretti A, Lilli R, Lorenzi C, Gasperini M, Smeraldi E, 1999b, Tryptophan hydroxylase gene and response to lithium prophylaxis in mood disorders. *J Psychiatr Res* **33:** 371-377.

Serretti A, Lorenzi C, Lilli R, Smeraldi E, 2000, Serotonin receptor 2A, 2C, 1A genes and response to lithium prophylaxis in mood disorders. *J Psychiatr Res* **34:** 89-98.

Serretti A, Lilli R, Mandelli L, Lorenzi C, Smeraldi E, 2001, Serotonin transporter gene associated with lithium prophylaxis in mood disorders. *Pharmacogenomics J* **1:** 71-77.

Serretti A, 2002a, Lithium long-term treatment in mood disorders: clinical and genetic predictors. *Pharmacogenomics* **3:** 117-129.

Serretti A, Lorenzi C, Lilli R, Mandelli L, Pirovano A, Smeraldi E, 2002b, Pharmacogenetics of lithium prophylaxis in mood disorders: analysis of COMT, MAO-A, and Gbeta3 variants. *Am J Med Genet* **114:** 370-379.

Serretti A, Malitas PN, Mandelli L, Lorenzi C, Alevizos B, Nikolaou C, Boufidou F, Christodoulou GN, Smeraldi E, 2003, SERTPR gene variants and lithium efficacy: a multicenter study. ISPG congress, Québec, 2003.

Small JG, Klapper MH, Milstein V, Kellams JJ, Miller MJ, Marhenke JD, Small IF, 1991, Carbamazepine compared with lithium in the treatment of mania. *Arch Gen Psychiatry* **48:** 915-921.

Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M, 1998, Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol Psychiatry* **3:** 508-511.

Sokomba EN, Patsalos PN, Lolin YI, Curzon G, 1988, Concurrent monitoring of central carbamazepine and transmitter amine metabolism and motor activity in individual unrestrained rats using repetitive withdrawal of cerebrospinal fluid. *Neuropharmacology* **27:** 409-415.

Souery D, Rivelli SK, Mendlewicz J, 2001, Molecular genetic and family studies in affective disorders: state of the art. *J Affect Disord* **62:** 45-55.

Southam E, Kirkby D, Higgins GA, Hagan RM, 1998, Lamotrigine inhibits monoamine uptake in vitro and modulates 5-hydroxytryptamine uptake in rats. *Eur J Pharmacol* **358:** 19-24.

Stahl SM, 2000, Newer antidepressants and mood stabilizers, in: *Essential Pharmacology Second Edition*, Cambridge University Press, Cambridge, pp 245-295.

Steen VM, Lovlie R, Osher Y, Belmaker RH, Berle JO, Gulbrandsen AK, 1998, The polymorphic inositol polyphosphate 1-phosphatase gene as a candidate for pharmacogenetic prediction of lithium-responsive manic-depressive illness. *Pharmacogenetics* **8:** 259-268.

Strakowski SM, Del Bello MP, Adler CM, Keck PE Jr, 2003, Atypical antipsychotics in the treatment of bipolar disorder. *Expert Opin Pharmacother* **4:** 751-760.

Strober M, Morrell W, Burroughs J, Lampert C, Danforth H, Freeman R, 1988, A family study of bipolar I disorder in adolescence. Early onset of symptoms linked to increased familial loading and lithium resistance. *J Affect Disord* **15:** 255-268.

Swann AC, Bowden CL, Morris D, Calabrese JR, Petty F, Small J, Dilsaver SC, Davis JM, 1997, Depression during mania. Treatment response to lithium or divalproex. *Arch Gen Psychiatry* **54:** 37-42.

Thase ME, Bhargava M, Sachs GS, 2003, Treatment of bipolar depression: current status, continued challenges, and the STEP-BD approach. *Psychiatr Clin North Am* **26:** 495-518.

Tohen M, Sanger TM, McElroy SL, Tollefson GD, Chengappa KN, Daniel DG, Petty F, Centorrino F, Wang R, Grundy SL, Greaney MG, Jacobs TG, David SR, Toma V. Olanzapine versus placebo in the treatment of acute mania. Olanzapine HGEH Study Group. Am J Psychiatry. 1999 May;156(**5**)**:** 702-9.

Tohen M, Jacobs TG, Grundy SL, McElroy SL, Banov MC, Janicak PG, Sanger T, Risser R, Zhang F, Toma V, Francis J, Tollefson GD, Breier A. Efficacy of olanzapine in acute bipolar mania: a double-blind, placebo-controlled study. The Olanzapine HGGW Study Group. Arch Gen Psychiatry. 2000 Sep;57(**9**)**:** 841-9.

Tohen M, Baker RW, Altshuler LL, et al, 2001, Olanzapine versus divalproex sodium for bipolar mania: a 47-week study. Program and abstracts of the Annual Meeting of the American College of Neuropsychopharmacology; December 12, 2001; San Juan, Puerto Rico.

Tohen M, Vieta E, Calabrese J, Ketter TA, Sachs G, Bowden C, Mitchell PB, Centorrino F, Risser R, Baker RW, Evans AR, Beymer K, Dube S, Tollefson GD, Breier A. Efficacy of olanzapine and olanzapine-fluoxetine combination in the treatment of bipolar I depression. Arch Gen Psychiatry. 2003 Nov;60(**11**)**:** 1079-88.

Tohen M, Chengappa KN, Suppes T, Baker RW, Zarate CA, Bowden CL, Sachs GS, Kupfer DJ, Ghaemi SN, Feldman PD, Risser RC, Evans AR, Calabrese JR. Relapse prevention in bipolar I disorder: 18-month comparison of olanzapine plus mood stabiliser v. mood stabiliser alone. Br J Psychiatry. 2004 Apr;**184:** 337-45.

Tohen M, Greil W, Calabrese JR, Sachs GS, Yatham LN, Oerlinghausen BM, Koukopoulos A, Cassano GB, Grunze H, Licht RW, Dell'Osso L, Evans AR, Risser R, Baker RW, Crane H, Dossenbach MR, Bowden CL. Olanzapine versus lithium in the maintenance treatment of bipolar disorder: a 12-month, randomized, double-blind, controlled clinical trial. Am J Psychiatry. 2005 Jul;162(**7**)**:** 1281-90.

Turecki G, Alda M, Grof P, Martin R, Cavazzoni PA, Duffy A, Maciel P, Rouleau GA, 1996, No association between chromosome-18 markers and lithium-responsive affective disorders. *Psychiatry Res* **63:** 17-23.

Turecki G, Grof P, Cavazzoni P, Duffy A, Grof E, Ahrens B, Berghofer A, Muller-Oerlinghausen B, Dvorakova M, Libigerova E, Vojtechovsky M, Zvolsky P, Joober R, Nilsson A, Prochazka H, Licht RW, Rasmussen NA, Schou M, Vestergaard P, Holzinger A, Schumann C, Thau K, Rouleau GA, Alda M, 1998, Evidence for a role of phospholipase C-gamma1 in the pathogenesis of bipolar disorder. *Mol Psychiatry* **3:** 534-538.

Turecki G, Grof P, Cavazzoni P, Duffy A, Grof E, Ahrens B, Berghofer A, Muller-Oerlinghausen B, Dvorakova M, Libigerova E, Vojtechovsky M, Zvolsky P, Joober R, Nilsson A, Prochazka H, Licht RW, Rasmussen NA, Schou M, Vestergaard P, Holzinger A, Schumann C, Thau K, Rouleau GA, Alda M, 1999, MAOA: association and linkage studies with lithium responsive bipolar disorder. *Psychiatr Genet* **9:** 13-16.

Turecki G, Grof P, Grof E, D'Souza V, Lebuis L, Marineau C, Cavazzoni P, Duffy A, Betard C, Zvolsky P, Robertson C, Brewer C, Hudson TJ, Rouleau GA, Alda M, 2001, Mapping susceptibility genes for bipolar disorder: a pharmacogenetic approach based on excellent response to lithium. *Mol Psychiatry* **6:** 570-578.

Vieta E, Goikolea JM. Atypical antipsychotics: newer options for mania and maintenance therapy. Bipolar Disord. 2005;7 Suppl **4:** 21-33.

Watson DG, Watterson JM, Lenox RH, 1998, Sodium valproate down-regulates the myristoylated alanine-rich C kinase substrate (MARCKS) in immortalized hippocampal cells: a property of protein kinase C-mediated mood stabilizers. *J Pharmacol Exp Ther* **285:** 307-316.

Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG, Joyce PR, Karam EG, Lee CK, Lellouch J, Lepine JP, Newman SC, Rubio-Stipec M, Wells JE, Wickramaratne PJ, Wittchen H, Yeh EK, 1996, Cross-national epidemiology of major depression and bipolar disorder. *JAMA* **276:** 293-299.

Whitton PS, Oreskovic D, Jernej B, Bulat M, 1985, Effect of valproic acid on 5-hydroxytryptamine turnover in mouse brain. *J Pharm Pharmacol* **37:** 199-200.

Williams RS, Cheng L, Mudge AW, Harwood AJ, 2002, A common mechanism of action for three mood-stabilizing drugs. *Nature* **417:** 292-295.

Wittchen HU, Mhlig S, Pezawas L. Natural course and burden of bipolar disorders. Int J Neuropsychopharmacol. 2003 Jun;6(**2):** 145-54.

Wyatt RJ, Henter I, 1995, An economic evaluation of manic-depressive illness. *Soc Psychiatry Psychiatr Epidemiol* **30:** 213-219.

Yatham LN, Liddle PF, Shiah IS, Lam RW, Ngan E, Scarrow G, Imperial M, Stoessl J, Sossi V, Ruth TJ, 2002a, PET study of [(18)F]6-fluoro-L-dopa uptake in neuroleptic- and mood-stabilizer-naive first-episode nonpsychotic mania: effects of treatment with divalproex sodium.*Am J Psychiatry* **159:** 768-774.

Yatham LN, Kusumakar V, Calabrese JR, Rao R, Scarrow G, Kroeker G, 2002b, Third generation anticonvulsants in bipolar disorder: a review of efficacy and summary of clinical recommendations. *J Clin Psychiatry* **63:** 275-283.

Yuan PX, Huang LD, Jiang YM, Gutkind JS, Manji HK, Chen G, 2001, The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. *J Biol Chem* **276:** 31674-31683.

Zornberg G, Pope HG, Jr, 1993, Treatment of depression in bipolar disorder: new directions for research. *J Clin Psychopharmacol* **13:** 397-408.

Zvolsky P, Dvorakova M, Soucek K et al., 1979, Clinical use of lithium salts from the genetic viewpoint, in: *Lithium: controversies and unresolved issues*, Cooper TB, Gershon S, Kline NS, Schou M, eds, Excerpta Medica, Amsterdam, pp 152-154.

5. PSYCHOPHARMACOGENETICS OF SCHIZOPHRENIA AND PSYCHOSIS

Dr. Joachim Scharfetter*

1. INTRODUCTION

Psychotic disorders are severe psychiatric conditions, frequent, disabling, with patients at high risk for suicide, for addiction and social decline. The most prominent of psychotic disorders is schizophrenia with a lifetime prevalence of about 1%. Almost all insights into the pharmacogenetics of psychosis are derived from schizophrenic patients therefore all the following comments will generally refer to this disorder.

Until the development of antipsychotic medication means for therapy have been sparse and the course of the disease frustrating, often leading to chronic illness and social decline. The situation improved profoundly with the advent of antipsychotic medication in the 1950's, later called the typical antipsychotics or neuroleptics. These pharmacological substances are very potent in their antipsychotic efficacy but often inducing severe side effects, for the most part extrapyramidal motor disorders like akathisia, parkinsonism, and early and late onset dyskinesias, beyond else.

The next milestone in pharmacological antipsychotic treatment was the development of the so called atypical antipsychotics (second generation antipsychotics) with Clozapine as their prototype in the 1970's. The term atypical was coined for pharmacological agents (almost) without extrapyramidal side effects that generally have a higher affinity to the 5-HT2A receptor than to the Dopamine D2 receptor. Clozapine was a big success, improving psychotic conditions in patients that were hitherto refractory to treatment. Nevertheless Clozapine was withdrawn from the market due to the severe side effect of agranulozytosis. Owing to its unique and indispensable efficacy in treatment refractoriness the substance was reintroduced in the late 1980's under stringent safety regulations. The 1990's saw the advent of further atypical antipsychotics and today we are equipped with a multitude of pharmacological treatment options.

* Joachim Scharfetter, Universitatsklinik fur Psychiatrie, Abteilung fur Allgemeine Psychiatrie, Wahringer Gurtel 18-20, A-1090 Wien, Austria. joachim.scharfetter@akh-wien.ac.at.

There are various strategies for the clinical decision on what substances to prescribe for which patient. These strategies are partially dependent on pharmacodynamic properties of the substances, on the specific symptomatology of the patients, side effect profiles and treatment history. Substances like Clozapine or Quetiapine are preferred when extrapyramidal motor side effects are to be omitted, Amisulpride is administered in patients with predominantly negative symptomatology, Ziprasidone is renown for causing less weight gain, Clozapine is the substance for treatment resistance, to name a few.

Nevertheless a considerable portion of patients still fail to respond to treatment, despite adequate dosage and duration and moreover patients are often suffering from severe side effects. Consequently psychosis itself and its treatment remains a cost intensive and sometimes frustrating endeavor. Despite considerable efforts there has been no success so far in determining predictive factors for treatment response and relapse. Antipsychotic therapy still remains try and error to a considerable degree.

In this context pharmacogenetics of schizophrenia have gained much attention recently. Targeting treatment more efficiently on the basis of a simple genetic test would have a considerable economic and not the least ethical impact. There have even been proposals for presymptomatic genetic diagnosis and prophylactic treatment [Tsuang et al. 2000, Hurko 2001], after all with considerable ethical caveats of such a proposal to be considered.

Many considerations on methodology and study design in the following have been proposed by Rietschel et al. [Rietschel et al. 1999] in a consensus conference on the application of pharmacogenetics to psychotic disorders, valuable input comes from Masellis et al. [Masellis et al. 2000] in their publication about pharmacogenetics of Clozapine.

2. METHODOLOGY

Dealing with pharmacogenetic studies in psychosis we are almost exclusively confronted with case-control association study designs where it is investigated if the presence (case) or absence (control) of a specific phenotype can be paralleled to a specific genotype. A methodological prerogative for such studies is the fact that the genes whose genetic polymorphisms are investigated have to have an a priori evidence of being involved into the pathogenesis of the phenotype.

It has recently been proposed to preferentially investigate only polymorphisms with functional consequences (that is alteration of the transcribed proteins or changing gene expression by being located in regulatory regions) with the aim to increase the prior probability of detecting valid associations. Silent mutations in the “degenerated” third position of codon triplets, in introns or in the vast areas of non-coding “junk” DNA can nevertheless not be completely disregarded. Seen apart from the possibility that they might be in linkage disequilibrium with functionally relevant sites nearby, they might introduce alternative splicing sites or affect stability or accessibility of DNA. The functional significance of non-coding DNA regions is being intensively discussed just recently. “Junk-DNA”, as these non-coding regions have been termed due to their seemingly uselessness, seem to be highly

conserved throughout evolution and they would not be if really useless [Bejerano et al. 2004]. Many of these non-coding regions are after all transcribed into non-protein-coding RNA with so far unknown purpose [Cawley et al. 2004].

Another methodological issue controversially discussed recently in medical statistics is correction for multiple testing. The latter has often been demanded for reasons of methodological rigor. Authors as for example Dettling et al. [2001b] and Illi et al. [2003] refrained from applying a multiple hypotheses testing adjustment referring to recently published critical comments on this issue [Pernegger 1998].

3. PHENOTYPE

The phenotype definition for pharmacogenetic studies in psychosis is complex since the phenotype is trait- (psychosis) as well as state- (being under pharmacological treatment) dependent. All patients enrolled must be pharmacologically treated with antipsychotics and must have a diagnosis of psychosis that is schizophrenia or schizophrenia and schizoaffective disorder respectively.

Schizophrenia is most likely a complex trait with multiple genetic, developmental and environmental factors contributing to the liability to develop the disease. Consequently there is heterogeneity in symptomatology and pathogenetic pathways probably leading to individual peculiarities in the accessibility to pharmacological treatment. A stringent phenotypic definition of schizophrenia therefore seems to be a prerogative for informative study designs.

Diagnostic subtypes of schizophrenia however could not be related to variability in treatment response and individual psychotic symptoms could not be specifically targeted by different antipsychotic drugs [Nimgaonkar et al. 1988]. Almost all pharmacogenetic studies cited in the following do not take schizophrenia subtypes into account. Schizophrenia and schizoaffective disorder in these studies are primarily diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition) [American Psychiatric Association 1994] or ICD-10 (International Classification of Disease, Tenth Edition) [World Health Organization 1992]. Sometimes detailed diagnostic manuals like SCID (Structured Clinical Interview for DSM-IV Diagnoses) [Spitzer et al. 1990], DIGS (Diagnostic Interview for Genetic Studies) [Nurnberger et al. 1994] or SADS (Schedule for Affective Disorders and Schizophrenia) are applied [Endicot and Spitzer 1978].

Another confounding variable in phenotype definition is ethnic heterogeneity in patients that has in fact been found to influence antipsychotic response [Frackiewicz et al. 1997]. This latter observation is imperatively asking for ethnically homogenous samples or better for methods to control for ethnic heterogeneity [Rosenberg et al. 2002].

On the other hand a stringent phenotype definition asks for patients that are uniformly treated with the same antipsychotic, a prerequisite that is not always met by study designs. Since molecular targets and metabolic pathways of different antipsychotic agents are quite divers, genotypes putatively influencing their efficacy are most likely divers too.

Based on this universal set of schizophrenic/schizoaffective/psychotic patients treated with an antipsychotic drug, the phenotype under investigation in the stricter sense is the clinical effect of treatment. In the desired form this effect is defined as amelioration or disappearance of symptoms, in the following called response, and in the undesired variant it is called side effects.

Assessment of response is preferentially done by applying scales, response is then defined either by proposing a cut-off or as a continuous measure. It has been demanded that the scales applied should be able to differentiate between psychopathological dimensions as positive symptoms (hallucinations, delusions) and negative symptoms (avolition, affective flattening). Widely used scales are the PANNS (Positive and Negative Syndrome Scale) [Kay et al. 1987], BPRS (Brief psychiatric Rating Scale) [Overall and Gorham 1962], GAS (Global Assessment Scale) [Endicott et al. 1976], SANS [Andreasen 1989] and CGI [Guy 1976].

The definition of the phenotype response has to take the dimension of time into account since a minimal period of pharmacological treatment is required for symptoms improvement. An evaluation of treatment response after four to six weeks is commonly applied, a continuation up to six month is recommendable.

An elegant means to circumvent problems of response definition could be the use of endophenotypes. Endophenotypes are assessed by measuring physiological parameters of pharmacological effects as for example neuro-endocrine responses, receptor occupancy or changes in cerebral blood flow.

Basically the same issues hold true for the definition of side effects. The most prominent of antipsychotics treatments side effects are the extrapyramidal motor side effects, these are dealt with in a chapter of their own. Weight gain as another prominent side effect is commonly defined by a cut off of >7%, sometimes treated as a continuous variable. The Neuroleptic Malignant Syndrome has been investigated using the clinical diagnosis criteria by Pope et al. [Pope et al. 1986]. A treatment regimen with Clozapine apparently is a prerequisite for developing Clozapine induced agranulozytosis (CA). The latter is consistently defined as less than 500 neutrophil counts per mm^3 of whole blood.

Besides these more prominent side effects there are only a few studies on secondary adverse events like urinary incontinence and hypotension. No studies to my knowledge are existing on the pharmacogenetics of neuroleptic induced QT syndrome and diabetes.

4. GENOTYPES

In the following polymorphisms of genes will be discussed that have been targeted in pharmacogenetic studies. These will be presented with special emphasis on their functional importance.

4.1. Overview with respect to specific phenotypes

Since the methodological basis for pharmacogenetic studies in psychosis is a candidate gene association approach, all genetic polymorphisms investigated have to have an a priori evidence for their putative impact on the phenotype under investigation. In other words, the choice of genes whose polymorphisms should be investigated has to be hypothesis driven.

4.1.1 Response (see table 2-6)

One of the first and most prominent hypotheses about the efficacy of antipsychotic medication has been the dopamine hypothesis (renewed and modified by the "fast-off D2 hypothesis" by Kapur [2000] for the quality of neuroleptics to be atypical). Dopamine receptor genes are consequently a prime target for studies investigating the pharmacogenetics of antipsychotic response. Nevertheless the serotonin receptor genes have been the first to be intensively investigated. This is apparently due to the fact that most samples investigated in the beginning of pharmacogenetic studies in psychosis have been in patients treated with the "atypical" drug Clozapine. One of the first hypotheses for the quality of being atypical has been the preferential efficacy of such antipsychotic drugs in the serotonin neurotransmitter system.

Variation in antipsychotics treatment efficacy has repeatedly been assumed to be connected to plasma levels of the prescribed substances. Even though a simple concentration-effect relationship for antipsychotic drugs has never been substantiated [see Bengtsson 2004] the most prominent drug metabolizing enzyme system, the Cytochrome P450, has been focused on in early pharmacogenetic response studies.

Furthermore a variety of neurotransmitter receptor genes have been investigated, namely adrenergic, histaminergic, cholinergic and glutamatergic receptors, due to their putative involvement into the pathophysiology of psychosis and the fact that they are part of the target spectrum of antipsychotic drugs.

The rationale for investigating the HLA system is to be found in the fact that the HLA genes are located on chromosome 6 which has been a positive finding in genetic linkage studies of schizophrenia [see Lahdelma et al. 1998]. Apolipoprotein E ε4 has been associated with less severity of negative symptoms [Hong et al. 2000]. Neurotensin is a neuroregulatory peptide involved into the regulation of neurotransmitter circuits. Catechol-O-methyltransferase (COMT) and monoamine-oxidase (MAO) are enzymes participating in the inactivation of biogenic amines as the neurotransmitters dopamine, serotonin and norepinephrine. The brain derived neurotrophic factor (BDNF) is an important member of the nerve growth factor family involved into neurodevelopment. Brain development abnormalities are one of several paradigms for schizophreniform disorders. Namely dopaminergic systems in the brain and dopamine receptor expression are affected by BDNF [Krebs et al. 2000]. The methylenetetrahydrofolate reductase (THFR) is an enzyme contributing to the folate metabolism and thereby involved into homocysteine regulation. High plasma levels of homocysteine have been paralleled to developmental abnormalities. Schizophreniform symptoms have been described in several homocysteinuric patients and case reports about positive outcome of folate treatment of psychotic patients have been published [Joober et al. 2000b].

Table 1. Antipsychotic Response Cytochrome P450 - CYP2D6

referenc	polymorphism	notes	sample	treatment	phenotype	result
Arranz 1995b	PM(CYP2D6-A and -B) and EM	PCR	123 schizophrenics	Clozapine	GAS 20-point improvement	n.s.
Lane 1997	PM, EM	dextrometho rphan phenotyping	18 chinese schizophrenics	Haloperidol	BPRS improvement >35%	n.s.
Aitchiso n 1999	PM (CYP2D6*3, -*4, -*5), EM, UM (duplications)	PCR	235 treatment resistant schizophrenics/sc hizoaffectives vs. 73 schizophrenic controls	miscellaneous typical	treatment resistance defined as by Kane et al. 1988	n.s., trend for ultrafast metabolizers in response group
Hamelin 1999	EM (genotypes containing of at least 1 CYP2D6*1), PM (all other enotypes with CYP2D6*3, -4, -5, -6, -7)	PCR	39 schizophrenics	miscellaneous NL	BPRS continous	n.s.
Brockm öller 2002	PM, IM, EM, UM (for specific genotypes see publication)	PCR	172 acute psychotics	Haloperidol	PANSS continous	n.s., trend for fast metabolizers in non-response

n.s. not significant

Table 2. Antipsychotic response and combined genotypes?

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 2000b	5HT2A: His452Tyr, Thr25Asp, -1438G/A, 102T/C, 516C/T 5HT2C: -330GT/-244CT, Cys23Ser 5HT3A: 178C/T, 1596G/A 5HT5A: -12A/T, -19G/C 5HTT: 5-HTTLPR, VNTR DRD3: Ser9Gly ADRA1A: Arg492Cys ADRA2A: -1291G/C, -261G/A H1: Leu449Ser H2: -1018G/A	no correction for multiple testing treatment response retrospective ly assessed	200 schizophrenic patients	Clozapine	response: GAS	combination of 6 polymorphisms predict clozapin response: 5HT2A: 102T/C His452Tyr 5HT2C: -330GT/-244CT Cys23Ser 5HTT: 5-HTTLPR H2: -1018G/A
Schumacher 2000	Same combination of 6 response predicting genotypes as Arranz 2000b	an attemt to replicate Arranz 2000b	163 schizophrenic patients	Clozapine	4 response groups, group 3 and 4 corresponding to 20 point GAS improvement	n.s. Only H2: -1018 G/A associated with response on the allelic level

n.s. not significant

4.1.2 Agranulozytosis

Table 3. Side Effect: Clozapine-induced Agranulozytosis (CA)
HLA-System

reference	polymorphism	notes	sample	treatment	phenotype	result
Lieberman 1990	HLA-A, -B, -C, -DR, -DQ (HLA-class I and II antigens)	serotyped	schizophrenic and schizoaffective Ashkenazi patients 5 patients with Clozapine induced Agranulozytosis (CA), 26 controls	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with a haplotype consisting of HLA-B38, -DR4, -Dqw3
Claas 1992	HLA-class I and II antigens	serotyped	103 patients with CA, 95 matched controls	Clozapine	granulozytopenia: less than 1500 granulozytes/ml	n.s. after correction for multiple testing
Yunis 1992	HLA-class I and II antigens	serotyped	11 CA patients, 31 controls, most of jewish ancestry	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with HLA-B38, -DR4 and –DQw3 haplotype in jewish patients; with HLA-DR2 and –DQw1 in non-jewish
Abt 1992	HLA-class I and II antigens 48 HLA antigens	serotyped diagosis=?	72 patients with granulozytopenia/CA, 74 controls	Clozapine	definition of CA=?	no significant model of HLA subsets
Yunis 1995	HLA-class I and II antigens	serotyped	Ashkenazi and non-Ashkenazi schizophrenics and schizoaffectives, 31 CA, 52 controls extended Yunis 1992 sample	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	Markers for jewish CA patients: B38, DRB1*0402, DRB4*0101, DQB1*0302, DQA1*0301 “protecting alleles”: DR11, DQB1*0301 Markers for non-jewish CA patients: DRB*1601, DRB5*02, DQB1*0502, DQA1*0102, DR2, DQw1
Theodoropoulou 1997	HLA-class I and II antigens	serotyped	43 schizophrenics, 3 of them developing agranulocytosis	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s.

Amar 1998	HLA-class I and II antigens	serotyped	18 schizophrenics, 5 of them with granulozytopenia/CA	Clozapine	granulozytopenia: less than 1000 and agranulozytosis: less than 500 neutrophils per mm3	granulozytopenia/agranulozytosis associated with HLA-DQB1*0201
Valevski 1998	HLA-class I and II antigens	serotyped	61 jewish schizophrenics, 11 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	HLA-B38 associated with agranulozytosis
Meged 1999	HLA-class I	serotyped	88 Jewish schizophrenics, 3 of them with CA	Haloperidol, Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s. trend for HLA-B38 to be associated with agranulozytosis
Lahdema 2001	HLA-A, -B	serotyped, partially genotyped	26 schizophrenic patients with Granulozytopenia/Agranulozytosis and 19 schizophrenic controls	Clozapine	granulozytopenia: less than 1,5x109/L agranulozytosis: less than 0.5x109/L	granulozytopenia/agranulozytosis associated with absence of HLA-A1
Dettling 2001a	HLA-class I and II antigens	genotyped no correction for multiple testing	107 caucasian paranoid schizophrenics, 30 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-DQB1*0502, -DRB5*02, trend for –DQB1*0201
Dettling 2001b	HLA-class I and II antigens	genotyped; no correction for multiple testing	108 caucasian paranoid schizophrenics, 31 of them with CA sample seems to be identical with Dettling 2001a	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-Cw*7, -DQB*0502, -DRB1*0101, -DRB3*0202

Heat Shock Protein

reference	polymorphism	notes	sample	treatment	phenotype	result
Corzo 1995	HSP70-1 HSP70-2	HSP70 is part of the HLA-class III cluster	75 schizophrenic and schizoaffektive patients, 32 of them with CA	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	HSP70-1 A and HSP70-2 9.0kb in linkage disequilibrium with each other and associated to CA in jewish patients

Tumor Necrosis Factor

reference	polymorphism	notes	sample	treatment	phenotype	result
Turbay 1997	TNF microsattelites	a-b, d-e	12 jewish, 21 non-jewish schizophrenics vs 33 controls	Clozapine	agranulozytosis: less than 500 neutrophils per ml	CA associated with d3 and b4, inversely associated with b5

NQO2

reference	polymorphism	notes	sample	treatment	phenotype	result
Ostrousky 2003	NQO2: 1536C/T, 1541G/A, 372C/T (Phe/Leu), 202G/A, -367A/G, -394G/C	Sample seems to be partially identical to Valevski 1998	98 schizophrenics 18 of these with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	CA patients predominantly heterozygous for several exon and intron SNP's

n.s. not significant

The basis for the development of Clozapine induced agranulozytosis/granulozytosis/granulozytopenia (CA) is still hypothetically. Theories are ranging from immune-mediated toxicity, induction of apoptosis unto direct toxicity of Clozapine via certain degradation products acting as free radicals [see Ostousky et al. 2003]. All association studies done to date are dealing either with HLA polymorphisms or polymorphisms of genes mapping to the MHC III region like TNF, HSP70 or NQO2. The latter gene seems to be involved into the degradation or detoxification of Clozapine.

4.1.3 Weight gain

Regarding weight gain the serotonin system is a prime target. The 5HT2C receptor seems to be involved in the appetite regulation propensities of leptin, a peptide secreted by adipozytes and acting in the hypothalamus as a catabolic regulator. 5HT2A seems to mediate the effect of neuropeptide Y (NPY) another regulatory peptide in the hypothalamus with anabolic effects. 5HT1A agonists have been shown to induce hyperphagia in rats. Further genotypic targets out of the serotonergic system have been 5HT6 and the serotonin transporter 5HTTLPR.

Histamine H1 receptor antagonism seems to increase food intake in rats making this receptor gene a candidate. Adrenergic receptors as part of the sympathetic nervous system are involved into the body's energy management probably via a mitochondrial pathway. The cytochrome P450 enzyme CYP1A2 has been investigated in the pharmacogenetics of antipsychotics induced weight gain due to its involvement in the degradation of Clozapine, an antipsychotic drug with pronounced weight increasing properties. The tumor necrosis factor TNF-α too has been implicated in the regulation of metabolic regulatory processes. See Basile et al. [2001] for a synopsis.

Table 4. Side Effect: Weight Gain

reference	polymorphism	notes	sample	treatment	phenotype	result
Rietschel 1997	5-HT2C: Cys23Ser		152 schizophrenics	Clozapine	?	n.s.
Hong 2001b	5-HTTLPR, 5-HT2A 102T/C, 5-HT2C 68G/C, 5-HT6 267C/T		93 schizophrenic	Clozapine	weight gain continous	n.s.
Basile 2001	5HT2C: Cys23Ser, 5HT1A: CAn repeat, 5HT 2A: 102T/C and His452Tyr, H1:?, H2: -1018G/A, Cyp1A2: Intron1 C/A, ADRA1A: Arg347Cys, ADRB3: Trp64Arg, TNFa -308G/A		80 schizophrenics	Clozapine	weight gain continous	trends for ADRB3, ADRA1A, TNFa, 5HT2C
Reynolds 2002	5-HT2C: -759C/T		123 chinese schizophrenics	miscellaneous NL	cut off: weight gain >7%	-759C associated with weight gain
Tsai 2002	5-HT2C: -759C/T		80 chinese schizophrenics	Clozapine	weight gain: BMI continous and cut off >7%	n.s.
Basile 2002a	5-HT2C: -759C/T		80 schizophrenics	Clozapine	Cut off: weight gain >7%	n.s.
Reynolds 2003	5-HT2C: -759C/T	subsample of Reynolds 2002	32 chinese schizophrenics	Clozapine	cut off: weight gain >7%	-759T associated with less weight gain
Theisen 2004	5-HT2C: -759C/T		97 german schizophrenic patients	Clozapine	Cut off: weight gain >7%	n.s.

n.s. not significant

4.1.4 NMS

Regarding neuroleptic malignant syndrome (NMS) two candidate genes have been targeted, the cytochrome P450 CYP2D6 and the dopamine receptor D2. The rationale for the first is the fact that many antipsychotic drugs are metabolized by CYP2D6 and a poor metabolizer state with increased plasma levels has been hypothesized as a risk factor for developing NMS. The latter is due to the fact that predominantly D2 blocking agents are increasing the risk for NMS and discontinuation of this medication improved this condition.

Table 5. Side Effect: Neuroleptic Malignant Syndrome

reference	polymorphism	notes	sample	treatment	phenotype	result
Ueno 1996	CYP2D6: 1795T del, 1934G/A, Arg296Cys (Hha I)		9 NMS patients	miscellaneous NL ?	?	n.s.
Iwahashi 1997	CYP2D6: HhaI	identical to Iwahashi 1999	56 japanese schizophrenics, 8 of them with NMS	miscellaneous NL ?		n.s.
Kawanishi 2000	Cyp2D6: Pro34Ser		36 patients with NMS, 107 schizophrenic controls	miscellaneous NL ?	NMS diagnosis according to the criteria of Pope et al. 1986	n.s.
Suzuki 2001c	DRD2: TaqI A		153 schizophrenic patients, 15 with NMS	miscellaneous NL ?	NMS criteria by Pope et al. 1986	A1 allele associated with NMS
Kishida 2003	DRD2: TaqI A		49 patients with NMS, 123 schizophrenic controls	miscellaneous NL ?	NMS criteria Pope et al. 1986	n.s.
Kishida 2004	DRD2: TaqI A, -141C Ins/Del, Ser311Cys		164 japanese schizophrenics, 32 with NMS	miscellaneous NL ?	criteria by Pope et al. 1986	-141C Del more frequent in NMS

n.s. not significant

4.2. Specific genotypes

4.2.1. Pharmacokinetic phase

The traditional pharmacogenetic paradigm has been genetic variability in major drug metabolizing enzymes [Massellis et al. 1998] consequently scientists have almost exclusively focussed on drug metabolizing enzymes in the advent of pharmacogenetics. There are several pharmacokinetic enzyme systems involved in drug metabolism recently described as ADME (absorption, distribution, metabolism and excretion) [Ring and Kroetz 2002]. The most prominent of these are the phase I and II reactions as delineated in the following.

4.2.1a. Cytochrome P450

Oxidative reactions as the most prominent of the phase I group of drug metabolizing reactions are mediated by the Cytochrome P450 system (CYP), situated in the liver as a group of heme-containing enzymes. There are over 30 different CYP enzymes in humans, organized in 14 families. Specific pharmacological agents are preferentially detoxified by specific CYP enzymes. Clozapine for example appears to be metabolized by CYP*1A2,* CYP*3A4*, CYP*2C19* and CYP*2D6*, it inhibits CYP*2C9* and CYP*2C19*, induces CYP*1A*,

CYP*3A* and CYP*2B*; Risperidone seems to be metabolized by CYP*2D6*; Olanzapine by CYP*1A2* andCYP*2D6*; Quetiapine by CYP*3A4*; Sertindole by CYP*2D6* (see table x). Further information on CYP enzymes and their antipsychotic drug substrates can be found in Dahl [2002] and in Scordo and Spina [2002].

Table 6.

Isoenzyme	Substrates	Inhibitors	Inducers
CYP-1A2	Chlorpromazine, Clozapine, Haloperidol, Olanzapine, Perphenazine, Thioridazine, Zotepine		
CYP-2C9	Perazine	Clozapine	
CYP-2C19	Clozapine, Perphenazine, Thioridazine	Clozapine	
CYP-2D6	Bromperidol, Chlorpromazine, Clozapine, Fluphenazine, Haloperidol, Olanzapine, Perphenazine, Risperidone, Thioridazine, Zotepine, Zuclopenthixol	Clozapine, Haloperidol, Perphenazine, Thioridazine	
CYP-3A4	Bromperidol, Clozapine, Haloperidol, Olanzapine, Perazine, Perphenazine, Quetiapine, Risperidone, Ziprasidone, Zotepine		Clozapine

adapted from Prior et al. [1999], Scordo and Spina [2002], Dahl [2002]

Traditionally low and high metabolizing phenotypes have been defined by probe reactions like the debrisoquine/sparteine reaction of the cytochrome P450 enzyme CYP2D6, more recently it has become the practice to identify specific alleles by genotyping genetic polymorphisms.

For CYP2D6, the most polymorphic of the P450 isoenzyme, 4 different phenotypes have been described: “poor”, “intermediate”, “extensive” and “ultra rapid metabolizer” [Arranz et al. 2001]. These are defined by varying combinations of different active/inactive alleles, differentiated by point mutations, deletions, duplications and conversions [Sachse et al. 1997]. A unified nomenclature of these alleles was developed by Daly et al. [1996].

Allele CYP2D6*1 is the wild type allele. The most frequent inactivating mutation among caucasians is the CYP2D6*4 allele, a 1934G/A splice-site mutation, former known as type-B mutation. Duplications are described for the 1, 2 and 4 allele, in case of the 2 allele resulting in an ultra rapid metabolizing state [Sachse et al. 1997]. The CYP2D6*2 allele is, beyond else, resulting from a 2938C/T point mutation, detected by a HhaI RFLP, leading to an Arg296Cys amino acid substitution. CYP2D6*6 is another inactivating mutation, alternatively known as type-A mutation, a 1795Tdel mutation [Ueno et al. 1996]. An 188C/T mutation resulting in a Pro24Ser substitution is found in allele CYP2D6*10 and constitutes a further poor metabolizing variant [Kawanishi 2000]. The CYP1A2 genotype investigated by Basile et al. [2001] in neuroleptic induced weight gain is a C/A polymorphism in the first intron of the gene, with the C/C genotype being less inducible by smoking [Sachse et al. 1999].

4.2.1b. NQO2

Dihydronicotinamide riboside (NRH) quinone oxidoreductase 2 (NQO2) has been deemed a candidate gene for Clozapine induced agranulozytosis

(AGR) for several reasons. Starting from MHC association findings for AGR by different groups [Lieberman et al. 1990, Corzo et al. 1997] Ostrousky et al. [2003] found evidence for a gene or genes mapped telomeric to the MHC complex to be associated to AGR in experiments with microsatellite markers spanning the entire MHC region (unpublished data). The gene for NQO2 is mapping to this latter region of the chromosome 6, 6p25, its transcript is an enzyme catalyzing reduction of quinones and quinoid compounds. It has been suggested that NQO2 should play an important role in detoxification of chemicals and in the protection of cells against drug-induced oxidative and electrophilic stress. Clozapine has been hypothesized to be oxidized on the membranes of activated neutrophils to chemical reactive nitrenium ions acting as free radicals leading to apoptosis [Ostrousky et al. 2003].

Of the polymorphisms investigated 1536C/T and 1541G/A are situated in the first intron and the mutation of either is leading to the disruption of a myeloid zinc finger protein (MZF1) binding site. MZF1 is specifically expressed in myeloid cells lineages; it may have a general role in the regulation of hematopoietic gene expression. The -367A/G and -394G/C polymorphisms are situated in the promoter region of the NQO2 gene implying gene expression regulatory functionality, 202G/A is a silent mutation in exon 5 and 372C/T in exon 3 is conferring a Phenylalanin to Leucin substitution in position 47 with yet undetermined functionality [Ostrousky 2003].

4.2.1c. COMT

The catechol O-methyl-transferase (COMT) is a phase II reaction (conjugation) enzyme involved in the degradation and inactivation of dopamine and norepinephrine. Other prominent phase II enzymes are for example N-acetyltransferase and glutathione-S-transferase. There are low, intermediate and high activity variants of the enzyme due to a common polymorphism consisting of a G to A transition at codon 158 of the membrane-bound form of COMT (codon 108 of the soluble form). This transition results in a valine (Val) to methionine (Met) substitution with the Met/Met genotype being 3 to 4 fold lower in enzyme activity than the wild type Val/Val, the Met/Val genotype being in between. [Illi 2003]

4.2.1d. MAOA

The enzyme monoamine oxidase consists of 2 isoenzymes, MAOA and MAOB, involved in the degradation of biological amines with different substrate affinities. The former is involved in the metabolic inactivation of dopamine, norepinephrine and serotonin. A polymorphism in the promoter region 1.5kb upstream of the coding sequence consisting of a 30-bp repeat in 3, 3.5, 4 or 5 copies (30bp VNTR) has been shown to affect transcriptional activity of the MAOA promoter. [Sabol et al. 1998]

4.2.2. Pharmacodynamic phase

With growing knowledge about the action of pharmacological agents at target structures like neurotransmitter receptors, pharmacogenetics of the pharmacodynamic aspect of drug actions have increasingly been focussed on.

4.2.2a. Serotonergic System

5-HT1A: The rationale for 5-HT1A to be investigated in neuroleptic induced weight gain is derived from the fact that agonists of this receptor have been shown to increase food intake in rats. Furthermore 5-HT1A receptors are localized in high density in the brains satiety control centers as shown by autoradiographic studies. A (CA)n dinucleotide repeat polymorphism has been utilized to detect association of 5-HT1A with weight gain. [Basile 2001]

5-HT2A: Of the 5-HT2A polymorphisms the most frequently investigated is the 102T/C substitution. It is a silent mutation and might consequently be considered a weak candidate for association studies. However it is in complete linkage disequilibrium with the -1438G/A polymorphism residing in the promoter region of the 5-HT2A gene and therefore most likely of greater functional importance [Ellingrod et al. 2003].

Another frequently investigated single nucleotide polymorphism (SNP) is the one encoding a His452Tyr substitution. The 452Tyr variant has been shown to have a reduced ability to activate phospholipase C and D through the G-protein signaling pathway [Hazelwood and Sanders-Bush 2004].

Regarding the functional relevance of Thr25Asn and 516T/C a literature search yielded no specific information.

5-HT2C: The most frequently investigated 5-HT2C polymorphism in response, a 68G/C substitution resulting in a Cys23Ser amino acid substitution, seems to have no specific functional importance of its own and might only be relevant as being in linkage disequilibrium with a relevant site [Fentress et al. 2005].

The -759C/T SNP in the promoter region of the 5-HT2C gene seems to be functionally relevant by reducing transcriptional activity through its -759T allele [Buckland et al. 2005].

No information could be found about the -330GT/-244CT repeat as reported by Arranz et al. [2000b]

5-HT3A/B: The potent antagonism of clozapine on the 5HT3 receptors has been hypothesized to contribute to the antipsychotic properties of atypical antipsychotics. Of the polymorphisms investigated by Gutierrez et al. [2002] 5HT3A 178C/T and 1596A/G and a CA-repeat of 5HT3B no information about functional importance could be found.

5-HT5A: The rationale for an investigation of 5-HT2A receptors in psychosis is not as stringent as for the afore mentioned 5-HT receptors. The polymorphisms investigated are a -19G/C substitution in the promoter region and a silent 12A/T substitution without further information on functional impact to be found [Birkett et al. 2000].

5-HT6: The 267T/C polymorphism of 5-HT6 is a silent mutation situated in exon 1. There is no further information on its functional importance.

5-HTT: Two polymorphisms of the serotonin transporter (SERT) gene have been pharmacogenetically investigated. The 5-HTTLPR is a variable number tandem repeat (VNTR) polymorphism with 14 to 16 copies of a 22 bp repeat sequence. The most common alleles are the 16 repeat or long allele and the 14 repeat or short allele. Consequently the polymorphism is alternatively termed 44 bp Ins/Del. The short allele predicts lower levels of 5-HTT mRNA and HTT activity in vitro [Heils et al. 1996, Lesch et al. 1996]. A second VNTR polymorphism of 17bp is situated in intron 2 of the 5-HTT gene and seems to influence gene transcription in an allele dependant manner [Hranilovic et al. 2004, De Luca et al. 2005].

4.2.2b. Dopaminergic System

DRD1: The D1/D2 receptor balance is a major hypothesis for the mode of antipsychotic action of clozapine. The DdeI polymorphism investigated in DRD1 is a restriction fragment length polymorphism (RFLP) situated in the 5' untranslated region of the DRD1 gene [Basile 2002b, Potkin 2003].

DRD2: There is convincing evidence for the -141C Ins/Del polymorphism in the 5' flanking region of the DRD2 gene to be functionally relevant. It seems to affect DRD2 expression [Arinami et al. 1997] and in vivo PET studies [Jönsson et al. 1999] showed an increased striatal DRD2 density in subjects with the Del allele, a result however that is not supported by a second PET study [Pohjalainen et al. 1999].

A further clue to functionality might be derived from a remarkable study on synonymous (or silent) polymorphisms in the DRD2 gene influencing mRNA folding, stability and consequently DRD2 expression in a complex manner. One of the discussed polymorphisms was even found to be in linkage disequilibrium with -141C Ins/Del and Taq1 A [Duan et al. 2003].

In post mortem studies on the functional consequences of the Taq1 A polymorphism the DRD2 receptor density in the striatum has been demonstrated to be lower in subjects with A1 alleles [Tompson et al. 1997] and in vivo PET studies revealed a decreased binding potential in individuals with the A1 allele [Pohjalainen et al. 1998]. The latter result however has not been replicated by [Laruelle et al. 1998].

For the Ser311Cys polymorphism in the 3rd cytoplasmatic loop it has been shown that the 311Cys allele was markedly less effective in inhibiting cAMP synthesis than the 311Ser allele, probably due to the reduced ability of those variant receptors to activate the receptor coupled G protein [Cravchik et al. 1996].

DRD3: Ser9Gly is a SNP causing a serine to glycine amino acid substitution in the N-terminal extracellular part of DRD3. Mutations in this region could disturb membrane insertion as has been demonstrated for similar mutations in other receptors [Lundstrom and Turpin 1996]. Furthermore a higher binding affinity for D3 selective ligands and dopamine, but not for D2 selective ligands, such as Haloperidol, has been demonstrated for the Gly-9 homozygote [Lundstrom and Turpin 1996].

It has been demonstrated that DRD3 receptors form oligodimers and moreover even heterodimers with DRD2 [Scarselli et al. 2001] giving ample opportunity to speculate about functional implications. Since typical and atypical antipsychotics differ in their potency to bind to D2 and D3 receptors the formation of heterodimers might be responsible for the above discussed genetic association findings pattern.

The 5'-leader SNP's investigated by Sivagnanasundaram et al. [2000] and the -205A/G polymorphism were found to be in linkage disequilibrium with the Ser9Gly polymorphism.

DRD4: There is sparse evidence that long alleles should be functionally different from short alleles of the 48bp VNTR [Asghari et al. 1995]. A twofold reduction of dopamin potency to reduce forscolin stimulated cAMP formation was observed for the allele 7 variant. However in his study only 3 out of 10 alleles have been tested and consequently results can not easily be generalized to short versus long.

The 12bp repeat polymorphism is located in exon 1, it is occurring 1 to 3 fold and coding for 4 amino acids at the N-terminal part of the receptor protein. The 13bp deletion in the first exon causes a frameshift mutation and is probably a complete loss of function mutation. [Rietschel et al. 1996]

4.2.2c. Adrenergic, Cholinergic and Histaminergic System

Different polymorphisms of the adrenergic system (ADRA1A: Arg492Cys, Arg347Cys, RsaI; ADRA2A: -1291C/G, -261G/A; ADRB3: Trp64Arg), the cholinergic system (CHRM1: 267C/A, 1044G/A, 1221C/T, 1353C/T; CHRM3: 193G/A; CHRM4: 1338C/T) the histaminergic system (H1: -17C/T, -974C/A, -1023A/G, -1536C/G; H2: -294A/G, -592A/G, -1018G/A, -1077G/A, Leu449Ser) and the glutamatergic system (NMDA-GRIN2B: 1664C/T) have been investigated but there is only sparse evidence about functional importance of these polymorphisms.

4.2.2d. HLA System

HLA alleles and haplotypes have found to be associated with various immune- and non-immune mediated diseases. An overview on their functional importance would considerably exceed the scope of this chapter and I would therefore recommend the consultation of a review like Wright et al. [2001].

4.2.2e. Neurotensin

The VNTR polymorphism of the neurotensin receptor 1 (NTSR1) and a 3020T/C polymorphism have both been found to resides in the untranslated regions of the neurotensin gene and seems therefore to be without functional impact [Huezo-Diaz 2004, Austin et al. 2000].

4.2.2f. TNF

The TNFα polymorphism 308G/A does not seem to be functionally relevant [Baylay et al. 2004]

4.2.2g. BDNF

The Val66Met polymorphism in the 5'region of the BDNF gene has been shown to affect intracellular storage and secretion of BDNF [Egan et al. 2003].

4.2.2h. THFR

The 677C/T point mutation of the THFR gene results in a valine substitution for an alanine. Heterozygotes (CT) and homozygotes (TT) have 71% and 33%, respectively, of the activity of the wild-type THFR (CC). [Chiuve et al. 2005]

5. FINDINGS

5.1. Response

5.1.1. Cytochrome P450 (see table 2)

The pharmacogenetic studies of genes of importance for the pharmacokinetic phase of drug action have almost exclusively focused on

Cytochrome P450 and its CYP2D6 variant. All but one study [Lane et al. 1997] utilized PCR techniques for identification of allele carrier status, the latter utilized a probe drug approach with dextromethorphan.

Arranz et al. [1995b] reported no association of CYP2D6 metabolizer status with response to Clozapine, the same as Lane et al. [1997] in a Haloperidol sample and Hamelin et al. [1999] in a miscellaneous neuroleptics sample.

Aitchison et al. [1999] and Brockmöller et al. [2002] too found no significant association with miscellaneous neuroleptics and Haloperidol respectively. Both however report a trend, the first of ultra fast metabolizing status to be associated with response, the second of fast metabolizer genotypes with non-response. This latter finding after all seems more apt to meet expectations.

5.1.2. Serotonergic System

5.1.2a. 5-HT2A

All the earlier studies with the 5-HT2A polymorphism 102T/C dealt with Clozapine treated patients. Arranz et al. [1995a] reported a significant association between 102C homozygotes and treatment non-response, a finding that was not replicated by Masellis et al. [1995], Nöthen et al. [1995] and Malhotra et al. [1996a].

In a meta-analysis of the above mentioned studies Arranz et al. [1998a] reported an overall significant association between the 102C allele and treatment non-response. Subsequent Clozapine studies by Masellis et al. [1998] and Lin et al. [1999] once again yielded insignificant results, as well as studies by Nimgaonkar et al. [1996a] and Jönsson et al. [1996], the latter treating patients with varying neuroleptics.

Significant results have then been reported by Joober et al. [1999], who, treating patients with typical neuroleptics, found an association of the 102C/102C genotype with male poor responders. Ellingrod et al. [2002] with an Olanzapine design reported an association of the 102T/102T genotype with improvement in negative symptoms. Both studies are in accordance with Arranz et al. [1998a]'s meta-analysis report of the 102C allele being associated with non-response. Lane et al. [2002] however, in a Risperidone sample, reported the 102C/102C genotype to be associated with better outcome. Yamanouchi et al. [2003] reported a trend for a diplotype containing a C allele to be associated with better response in a small Risperidone sample.

102C/T however is a silent mutation and might be considered a weak candidate for association studies. -1438G/A is a polymorphism residing in the promoter region of the 5-HT2A gene and therefore most likely of greater functional importance. This polymorphism is in strong linkage disequilibrium with 102C/T. Arranz et al. [1998b] reported an association of –1438G with non response, however in a sample that is identical to their 1995 sample with the significant 102C association result. In a second sample they could only see a trend in the same direction. Two other studies by Masellis et al. [1998] and Ellingrod et al. [2003] yielded insignificant results.

Another candidate polymorphism with promising association results is His452Tyr. Whilst there is no significant result reported by Nöthen et al. [1995]

and Malhotra et al. [1996a] with Clozapine treated patients, Arranz et al. [1996] reported association of Tyr452 with non-response and Arranz et al. [1998b] a trend in the same direction. A meta analysis of the above cited studies was done by Arranz et al. [1998a] and the association result was upheld. In the meantime the association of Tyr452 with non-response has been replicated by Masellis et al. [1998] with Clozapine treated patients, Ellingrod et al. [2002] were not able to do so in an Olanzapine sample.

Finally there are two studies with the polymorphism Thr25Asn both with insignificant results: Nöthen et al. [1995] and Ellingrod et al. [2002]. The latter was additionally investigating the 516T/C polymorphism with insignificant results too.

To summarize: all studies on 5-HT2A 102C/T genotype report either insignificant findings or association of 102C with non-response, except one that reports association of 102C with response and a second that reports a trend in the same direction, both of the latter with an atypical antipsychotics sample. All studies about His452Tyr that are not reporting insignificant findings, demonstrate association of Tyr452 with non-response. However these results have to be interpreted with caution: positive and negative predictive values based on the meta analyses remain moderate, haplotype analyses do not show stronger association with response [Masellis et al. 1998] and the predicted population frequency of the Tyr452 allele is very low [Veenstra-VanderWeele et al. 2000].

Table 7. Antipsychotic Response and Serotonergic System, the 5HT2A studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 1995a	102T/C	retrospective	149 schizophrenics resistent to typical NL	Clozapine	response: 20 point improvement in GAS	102C homozygotes more frequent in non-responders
Masellis 1995	102T/C		126 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.
Nöthen 1995	102T/C	retrospective	146 schizophrenics	Clozapine	response: clinical rating	n.s.
Malhotra 1996a	102T/C		70 typical neuroleptics responder	Clozapine	response: 20% improvement in BPRS	n.s.
Arranz 1998a	102T/C	meta-analysis	Masellis 1995, Arranz 1995, Nöthen 1995, Nimgaonkar 1996a, Malhotra 1996a	Clozapine	response	102C associated with non-response
Masellis 1998	102T/C		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.

Lin 1999	102T/C	sample identical with Tsai 2000, 2001 and Yu 1999, Hong 2000	97 chinese schizophrenics	Clozapine	response: BPRS continous	n.s.
Nimgaonkar 1996a	102T/C		174 patients	miscellaneous NL	response: clinical rating	n.s.
Jönsson 1996	102T/C		118 schizophrenics	miscellaneous NL	response: clinical ratings	n.s.
Joober 1999	102T/C		schizophrenics: 39 responders, 63 nonresponders	Typical neuroleptics	response: clinical rating according to the criteria by Brenner 1990	102C homozygotes more frequent in male non-responders
Lane 2002	102T/C		100 Chinese schizophrenics	Risperidone	response: PANS subscales continous	102C homozygotes associated with better outcome
Ellingrod 2002	102T/C		41 schizophrenics	Olanzapine	response: BPRS and SANS continous	Trend for 102T homozygotes to be associated with reduction of negative symtoms
Arranz 1998b	-1438G/A,	"in strong linkage disequilibrium with 102T/C"	Sample I: 160 schizophrenics (same sample as Arranz 1995); Sample II: 114 patients	Clozapine	response: 20 point improvement in GAS	-1438G associated with non-response
Masellis 1998	-1438G/A		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.
Ellingrod 2003	-1438G/A	sample identical to Ellingrod 2002	41 schizophrenics	Olanzapine	response: BPRS and SANS continous	n.s.
Arranz 1996	His452Tyr		153 schizophrenics, 178 normal controls	Clozapine	response: 20 point improvement in GAS	Tyr452 associated with poor response
Nöthen 1995	His452Tyr		146 schizophrenics	Clozapine	response: clinical rating	n.s.
Malhotra 1996a	His452Tyr		70 typical neuroleptics responder	Clozapine	response: 20% improvement in BPRS	n.s.
Arranz 1998b	His452Tyr		Sample I: 160 schizophrenics (same sample as Arranz 1995); Sample II: 114 patients	Clozapine	response: 20 point improvement in GAS	Tyr452 trend to be assoziated with non-response

Arranz 1998a	His452Tyr	meta-analysis	Nöthen 1995, Malhotra 1996a, Arranz 1998b	Clozapine	response	Tyr452 associated with poor response
Masellis 1998	His452Tyr		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	Tyr452 associated with non-response
Ellingrod 2002	His452Tyr		41 schizophrenics	Olamzapine	response: BPRS and SANS continous	n.s.
Nöthen 1995	Thr25Asn,		146 schizophrenics	Clozapine	response: clinical rating	n.s.
Ellingrod 2002	Thr25Asn		41 schizophrenics	Olamzapine	response: BPRS and SANS continous	n.s.
Ellingrod 2002	516T/C		41 schizophrenics	Olamzapine	response: BPRS and SANS continous	n.s.
Masellis 1998	102T/C, -1438A/G, His452Tyr	haplotype	185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.
Yamanou chi 2003	102T/C, -1438G/A	haplotype	73 japanese schizophrenics	Risperidone	response: PANS subscales continous	n.s. but diplotype A-T/A-T trend to worse outcome than A-T/G-C

5.1.2b. 5-HT2C

In several studies with Clozapine treated patients only Sodhi et al. [1995] were able to report a significant finding for the Cys23Ser polymorphism, they found 23Ser being associated with better response. The publications by Malhotra et al. [1996b] and Rietschel et al. [1997] yielded insignificant results, as well as Masellis et al. [1998], the latter two at least reported a trend for 23Ser and better response, the former a contradictory trend of 23Ser and association with non-response.

An exploratory meta analysis of the above cited studies was undertaken by Veenstra-VanderWeele et al. [2000] with a preliminary affirmation of the 23Ser/response association result. A more recent study by Ellingrod et al. [2002] with Olanzapine treated patients did not yield a significant result.

Table 8. Antipsychotic Response and Serotonergic System, the 5HT2C studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Sodhi 1995	Cys23Ser		162 (168?) treatment resistent Schizophrenics	Clozapine	response: 20 point improvement in GAS	23Ser associated with response
Malhotra 1996b	Cys23Ser		66 schizophrenic and schizoaffective	Clozapine	response: 20% improvement in BPRS	n.s., trend for 23Ser to be associated with non-response

Rietschel 1997	Cys23Ser		152 schizophrenics	Clozapine	?	n.s., trend for 23Ser to be associated with response
Masellis 1998	Cys23Ser		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s. trend for 23Ser to be associated with response
Veenstra-VanderWeele 2000	Cys23Ser	meta-analysis	Sodhi 1995, Malhotra 1996b, Rietschel 1997, Masellis 1998	Clozapine	response	23Ser associated with response
Ellingrod 2002	Cys23Ser		41 schizophrenics	Olanzapine	response: BPRS and SANS	n.s.

5.1.2c. 5-HT3A/B

There is only one study by Gutierrez et al. [2002] with a Clozapine design investigating the 178C/T and the 1596A/G polymorphism of 5-HT3A and a CA repeat polymorphism of 5-HT3B with insignificant results.

Table 9. Antipsychotic Response and Serotonergic System, the 5HT3A/B studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Gutierrez 2002	5HT3A: 178C/T, 1596A/G; 5HT3B: CA-repeat		266 british caucasian schizophrenics	Clozapine	response: 20 point improvement in GAS-scale 3 month after initiation of treatment	n.s.

5.1.2d. 5-HT5A

Birkett et al. [2000] investigated the –19G/C and the 12A/T polymorphism in Clozapine treated patients without significant findings.

Table 10. Antipsychotic Response and Serotonergic System, the 5HT5A studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Birkett 2000	-19G/C, 12A/T		269 schizophrenic	Clozapine	GAS, response definition = ?	n.s.

5.1.2e. 5-HT6

Masellis et al. [2001] could not replicate the finding by Yu et al. [1999], who saw homozygous T/T 267 genotypes of the 267T/C polymorphism being associated with response. Masellis et al. speculated on methodological differences in statistics as possible cause for this divergence but consequently dismissed this possibility again by recalculating their data with Yu et al.'s statistical approach, again yielding insignificant results. Ethnic heterogeneity of their sample and genetic heterogeneity of the phenotype "Clozapine response" were alternative explanations given.

Table 11. Antipsychotic Response and Serotonergic System, the 5HT6 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Yu 1999	267T/C	sample identical with Tsai 2000, 2001, Lin 1999, Hong 2000	99 chinese schizophrenics	Clozapine	response: BPRS continous	Better response for 267T homozygotes
Masellis 2001	267T/C		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.

5.1.2f. 5-HTT

Arranz et al. [2000a] found a VNTR polymorphism in the 2nd intron and a VNTR in the promoter region not associated with Clozapine response, Tsai et al. [2000] reported insignificance for the VNTR in the promoter region.

Table 12. Antipsychotic Response and Serotonergic System, the 5HTT studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 2000a	VNTR in Intron2, 5HTTLPR-VNTR in promoter region		268 treatment resistant schizophrenics	Clozapine	rsponse: 20 point improvement in GAS	n.s.
Tsai 2000	5HTTLPR - VNTR in the promoter region	sample identical with Tsai 2001, Lin 1999 and Yu 1999, Hong 2000	90 chinese schizophrenics	Clozapine	response: BPRS continous	n.s.

n.s. not significant

5.1.3 Dopaminergic System

5.1.3a. DRD1

The 2/2 genotype of DdeI, an upstream DRD1 polymorphism, has been found to be associated with better response to Clozapine and decrease in frontal and cortical metabolism as assessed by FDG-PET [Potkin et al. 2003]. The same polymorphism has been reported to be significantly associated with change in scores on the Wisconsin card sort test assessed before and after treatment with Clozapine in a pilot study by Basile et al. [2002b].

Table 13. Antipsychotic Response and Dopaminergic System, the DRD1 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Potkin 2003	DdeI		15 schizophrenics	Clozapine	response: BPRS, SANS, CGI: continous; PET scan: metabolism continous	Homozygotes for allele2 better improvement in BPRS and decrease in PET frontal and temporal cortical metabolism.
Basile 2002b	DdeI	pilot study	35 schizophrenic patients	Clozapine	response: changes in score of Wisconsin card sort test, continous	Association with changes in score of Wisconsin card sort test

5.1.3b. DRD2

The –141C Ins/Del polymorphism was primarily investigated by Arranz et al. [1998c] in a mixed and partially Clozapine treated sample yielding insignificant results. Ohara et al. [1998], giving no information about treatment regimes, reported insignificance too. Malhotra et al. [1999] however, in a Clozapine treated sample, found the –141C Ins allele being associated with better treatment response. This latter result was supported by Suzuki et al. [2001a] who investigated schizophrenic patients treated with Bromperidol, a close structural analogue to Haloperidol, and Nemonapride and found –141C Ins allele carriers more responsive to treatment as determined by the anxiety/depression subscale of the BPRS (Brief Psychiatric Rating Scale).

There are two studies combining the –141C Ins/Del polymorphism with the TaqI A polymorphism into a diplotype analysis. Kondo et al. [2003] reported genotypes carrying the –141C Del allele to be associated with worse outcome in the anxiety/depression subscales of the BPRS, a fact that is not surprising, given that their sample seems to be identical with Suzuki et al. [2001a]. Yamanouchi et al. [2003] however reported better response for diplotypes with the –141C Del allele.

The TaqI A polymorphism has been investigated in several samples, all with different regimes of neuroleptics prescribed. Only the study by Suzuki et al. [2001b] reported an insignificant finding. All other studies found either the allele 1 associated with better response (Suzuki et al. [2000], Dahmen et al. [2001] in a diplotype analysis with DRD3 Ser9Gly and Yamanouchi et al. [2003] in a diplotype analysis with –141C Ins/Del) or the allele 2 associated with non-response (Schäfer et al. [2001] and Kondo et al. [2003] in a diplotype analysis with –141C Ins/Del).

Two studies investigating the Ser311Cys polymorphisms reported no significant results (Shaikh et al. [1994] and Ohara et al. [1996]).

To summarize: two studies reported no association of the –141C Ins/Del polymorphism with treatment response, two (three if we include Kondo et al. [2003]) studies report the –141C Ins allele to be associated with better treatment response and one with worse. For TaqI A the situation seems to be clearly in favor of the allele 1 to be associated with better response. The samples of Suzuki et al. [2000, 2001b] and Kondo et al. [2003] however seem to be at least partially identical thus somehow blurring the picture.

Table 14. Antipsychotic Response and Dopaminergic System, the DRD2 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 1998c	-141C Ins/Del		Sample 1: "white british caucasians " 94 responders vs. 57 non-responders Sample 2: "chinese`" 85 responders vs. 65 non-responders	Sample 1: Clozapine Sample 2: ?	response: Sample 1: GAS: 20 point improvement Sample 2: "personal interview"	n.s.
Ohara 1998	-141C Ins/Del		170 schizophrenics	?	Response: PANSS	n.s.

Malhotra 1999	-141C Ins/Del		72 patients	Clozapine	response: BPRS: 20% improvement	-141C Ins carriers greater reduction in psychotic symptoms
Suzuki 2001a	-141C Ins/Del		49 acutely exacerbated schizophrenics	Bromperidol, Nemonapride	response: BPRS (continous) in subgrouped symptoms	-141C Ins carriers better improvement in anxiety/depression
Suzuki 2000	TaqI A		25 schizophrenics	Nemonapride	response: BPRS (continuous)	A1 allele associated with better response
Dahmen 2001	TaqI A	diplotype analysis with DRD3: Ser9Gly	18 patients	miscellaneous NL	response: BPRS (continous)	DRD3-Ser9 homozygozity and at least one allele of DRD2-A1 better improvement
Schäfer 2001	TaqI A		57 patients with “acute psychosis”	Haloperidol	response: PANSS (continuous)	allele 2 homozygotes display less improvement in positive symptoms
Suzuki 2001b	TaqI A		“japanese”: 30 acutely exacerbated schizophrenics	Bromperidol	response: BPRS (continous)	n.s.
Kondo 2003	TaqI A, -141C Ins/Del	sample seems to be identical with Suzuki et al. 2000, 2001a; polymorphisms 250kb apart; diplotype analysis	49 schizophrenics	Bromperidol, Nemonapride	response: BPRS continous	A2/Del poorer improvement in anxiety/depression
Yamanouchi 2003	-141C Ins/Del, Taq1 A;	diplotype analysis	73 japanese schizophrenics	Risperidone	response: PANS subscales continous	Ins-A2/Del-A1 diplotype better response than Ins-A2/Ins-A2
Shaikh 1994	Ser311Cys		“Caucasians”: 87 “treatment-resistant” vs. 100 controls	Clozapine	response: ?	n.s.
Ohara 1996	Ser311Cys		“japanese?”: 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.

5.1.3c. DRD3

For DRD3 exclusively the Ser9Gly genotype has been investigated, with the exception of Sivagnanasundaram et al [2000] who tried to associate several 5' leader SNP's with Clozapine response and reported no significant results.

The situation for Ser9Gly firstly remains complex. There are several studies with no significant results and even more studies with contradictory ones. If the studies are grouped according to the type of dispensed neuroleptics however there is a remarkable pattern to be observed.

With only the studies with a Clozapine treatment regime taken into account, we find three studies with insignificant findings [Gaitonde et al. 1996, Malhotra et al. 1998, Shaikh et al. 1996], the latter after all with a trend for the genotype Ser9/Ser9 to be associated with non-response. One study reported association of the Gly9 allele and of genotypes including the Gly9 allele with response [Scharfetter et al. 1999]. A meta-analysis incorporating the results of Malhotra et al. [1998], Shaikh et al. [1996] and Scharfetter et al. [1999] substantiated this latter result [Scharfetter et al. 1999].

There are further studies with atypical antipsychotics treatment regimes that reported corresponding findings. Szekeres et al. [2004] with "atypical antipsychotics" reported association of treatment non-response with Ser9 alleles and Ser9/Ser9 genotypes and Staddon et al. [2002] in an analysis combining Ser9Gly with -205A/G and an Olanzapine sample reported the Gly9/-205G diplotype to be associated with better positive symptoms improvement.

All the other studies about Ser9Gly are either administering typical neuroleptics [Kennedy et al. 1995], "mixed" neuroleptics [Dahmen et al. 2001] or give no clue at all about which neuroleptics were administered [Nimgaonkar et al. 1993, Jönsson et al. 1993, Yang et al. 1993, Mant et al. 1994, Ohara et al. 1996, Nimgaonkar et al. 1996B, Durany et al. 1996, Ebstein et al. 1997, Krebs et al. 1998, Joober et al. 2000a, Jönnson et al. 2003]. In most of the latter studies the pharmacogenetic part is just an addition to a classical schizophrenia association study design and it's justifiable to assume that most patients have been treated with typical neuroleptics.

While a majority of these latter studies reported no significant results, there are two studies that reported an association of homozygotes with response [Jönsson et al. 1993, Mant et al. 1994] and one study by Krebs et al. [1998] that reports an association of homozygotes with response and of Gly9/Gly9 genotypes with response, the latter result in accordance with the "atypical neuroleptics studies" by Scharfetter et al. [1999], Szekeres et al. [2004] and Staddon et al. [2002]. Three other studies however associate response with the Ser9 allele or non-response with Gly9 respectively [Jönsson et al. 2003, Dahmen et al. 2001 (in a diplotype analysis with DRD2 TaqI A) and Ebstein et al. 1997].

The above characterized trend was first delineated by Jönssen et al. [2003], incorporating genotypic data from studies where such were available into a meta analysis: Mant et al. 1994, Durany et al. 1996, Krebs et al. 1998, Joober et al. 2000a, Jönnson et al. 2003, Malhotra et al. 1998, Shaikh et al. 1996 and

Scharfetter et al. 1999. Jönsson et al. were parting the studies with typical antipsychotics from the studies with Clozapine and ascertained an association of Gly9 with response in Clozapine treated patients and an association of Ser9 with response in patients treated with traditional antipsychotics. Discussing this finding however Jönnson et al. speculated that this should be due to the fact that patients treated with Clozapine have been recruited mainly from former nonresponders to typical antipsychotics.

Table 15. Antipsychotic Response and Dopaminergic System, the DRD3 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Shaikh 1996	Ser9Gly		"white european caucasian " : 79 responder vs. 54 non responder	Clozapine	response: GAS: 20 point improvement	trend for Ser9 homozygotes to be associated with non-response
Gaitonde 1996	Ser9Gly		84 "caucasians, except 3 ": ? vs. ?	Clozapine	response: clinical rating	n.s.
Malhotra 1998	Ser9Gly		68 schizophrenics: 19 responders vs. 49 non-responders	Clozapine	response: BPRS: discrete – 20% reduction; continuous	n.s.
Scharfetter 1999	Ser9Gly		"pakistani patients ": 21 responders vs. 11 non-responders	Clozapine	response: BPRS: 50% improvement after 6 month of treatment	Gly9 allele and Gly9 homozygotes as well as Ser9/Gly9 genotypes more frequent in responders
Nimgaonkar 1993	Ser9Gly		53 "caucasian " schizophrenics: ? vs. ?	?	response: clinical rating from hospital records	n.s.
Jönsson 1993	Ser9Gly		76 "caucasian" schizophrenics: ? response present vs. ? response absent	?	response: ?, hospital records	n.s., association between homozygosity and response before corrected for multiple testing
Yang 1993	Ser9Gly		"Han-chinese": 45 responders vs. 98 controls	?	response: clinical rating	n.s.
Mant 1994	Ser9Gly		"western european Caucasians": 68 good response vs. 63 no response	?	response: clinical rating	better response in homozygotes
Kennedy 1995	Ser9Gly		"north American": 38 treatment resistant vs. 38 controls	traditional neuroleptics	response: clinical rating	n.s.
Ohara 1996	Ser9Gly		"japanese?": 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.

Nimgaonkar 1996b	Ser9Gly		"caucasians" and "afro-americans": ? vs. ?	?	response: clinical rating	n.s.
Durany 1996	Ser9Gly		61 patients with good vs. 43 with bad response	?	response: ?	n.s.
Ebstein 1997	Ser9Gly		"israeli sample": "41 Ashkenazi": 10 good vs. 31 poor responders; "46 non-Ashkenazi": 9 good vs. 37 poor responders "italian sample": 39 good vs. 21 poor responders	?	response: clinical rating	association of homozygosity with poor response in the non-Ashkenazi and the combined sample
Krebs 1998	Ser9Gly		70 responders vs. 19 non-responders	?	response: PANSS, GAS, CGI	significant difference in genotype distribution (fewer Gly9 homozygotes in non-responders); higher frequency of homozygosity in responders
Joober 2000a	Ser9Gly		42 responders vs. Controls 64 non-responders vs. controls	?	response: clinical rating BPRS, CGI	n.s.
Dahmen 2001	Ser9Gly	Diplotype analysis with DRD2: TaqIA	18 patients	miscellaneous NL	response: BPRS (continous)	DRD3-Ser9 homozygotes and at least one allele of DRD2-A1 better improvement
Jönsson 2003	Ser9Gly		153 patients Sample in part identical to Jönnson et al. 1993	?	response: clinical rating	more Ser9 alleles and homozygous genotypes in responders
Szekeres 2004	Ser9Gly		caucasian: 28 non-reponder vs. 47 responders	atypical antipsychotics	response: GAF: 20 points improvement	Ser9 alleles and Ser9 homozygotes more frequent in non responders
Staddon 2002	Ser9Gly, -205A/G	diplotype analysis	50 Basque schizophrenics	Olanzapine	response: PANSS positive and negative symptome scale, continous, after 3 month of treatment	Gly9/-205G associated with better positive symptomes improvement
Sivagnanasundaram 2000	5'-leader SNP's		49 patients	Clozapine	response: "symptoms rated on ordinal scale"	n.s.

5.1.3d. DRD4

Several genotypes have been investigated regarding DRD4. A 12bp repeat polymorphism could not be associated with treatment response in studies by Ohara et al. [1996], Rietschel et al [1996], Kohn et al. [1997] and Ozdemir et al. [1999].

Rietschel et al. [1996] additionally investigated a 13bp deletion and a Gly11Arg polymorphism, both without association with response. Ozdemir et al. [1999] investigated a (G)n repeat polymorphism and a SmaI RFLP, both without association with response too.

The most frequently investigated polymorphism in the DRD4 gene is a 48bp VNTR polymorphism with several alleles differing in repeat length from 2 ("short") to 8 ("long"). There are 6 studies with Clozapine treated patients, all of them yielding insignificant results [Shaikh et al. 1993, Kerwin et al. 1994, Rao et al. 1994, Shaikh et al. 1995, Rietschel et al. 1996, Kohn et al. 1997]. The same holds true for Kaiser et al. [2000] with a typical neuroleptics and a Clozapine cohort, for Zalsman et al. [2003] with a Risperidone sample and for Ohara et al. [1996] with a sample of unknown therapeutic regime. Only in the study by Cohen et al. [1999] it was reported that responders to typical neuroleptics carried the 7 repeat allele more often than Clozapine responders or controls and in a study by Hwu et al. [1998] it was reported that allele 4 homozygotes responded better than all others in a sample where there is no clue about what neuroleptics were administered.

To summarize: results of association studies of DRD4 48bp VNTR with response remain insignificant or conflicting. Due to the diversity of allelic distribution between the samples and of statistical approaches the individual studies can not be readily compared.

Table 16. Antipsychotic Response and Dopaminergic System, the DRD4 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Shaikh 1993	48bp VNTR	?	41 responders vs. 23 non responders	Clozapine	response: ?	n.s.
Kerwin 1994	48bp VNTR	regression analysis between repeat number and response	124 "european caucasian " ; 42 "taiwanese"	Clozapine	response: GAS continuous and discrete (20 points improvement)	n.s.
Rao 1994	48bp VNTR	Chi2/fishers exact test of each allele against all others	29 patients: 13 responders vs. 6 intermediate vs. 10 non responders	Clozapine	response: BPRS (20% decrease)	n.s.
Shaikh 1995	48bp VNTR	ANOVA	147 "european caucasian";42 "chinese"	Clozapine	response: GAS continuous	n.s.

Rietschel 1996	48bp VNTR	Chi2/fishers exact test Pooled: alleles 2 and 3; alleles 4 and 5; alleles 6,7,8 and 9 rare genotypes dismissed	149 "german schizophrenics and schizoaffective": 40 no- vs. 32 slight- vs. 45 marked- vs. 32 total-response	Clozapine	response: clinical rating	n.s.
Kohn 1997	48bp VNTR	Chi2/fishers exact test;rare alleles/ genotypes collapsed into "others"	37 "Ashkenazi"; 27 "non Ashkenazi"	Clozapine	response: clinical rating	n.s.
Cohen 1999	48bp VNTR	allele 4 vs. allele 7	"european caucasian": 28 patients with typical neuroleptics vs. 32 with clozapine vs. 57 controls	typical neuroleptics/ Clozapine	response: clinical rating	typical neuroleptic group significantly lower frequency of 7 repeat allele vs. 4 repeat allele
Kaiser 2000	48bp VNTR	Genotypes with alleles 4 and shorter vs. 5 and longer	"german caucasian": typical antipsychotics: 360 responder vs. 67 non-responder; clozapine: 136 responder vs. 36 non-responder	typical neuroleptics/ Clozapine	response: clinical rating and PANSS	n.s.
Zalsman 2003	48bp VNTR	alleles collapsed into short (<7) and long	10 responders, 14 non-responders	Risperidone	response: BPRS: 40% improvement	n.s.
Ohara 1996	48bp VNTR	Chi2/fishers exact test	"japanese?": 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.
Hwu 1998	48bp VNTR	Genotype 4/4 against all others	80 "chinese": 39 good vs. 41 poor responders	?	response: clinical rating	genotypes homozygous for allele 4 have significantly better response than others
Ohara 1996	12bp repeat		"japanese?": 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.

Rietschel 1996	12bp repeat	149 “german schizophrenics and schizoaffective” : 40 no- vs. 32 slight- vs. 45 marked- vs. 32 total-response	Clozapine	response: clinical rating	n.s.
Kohn 1997	12bp repeat	37 “Ashkenazi”; 27 “non Ashkenazi”	Clozapine	response: clinical rating	n.s.
Ozdemir 1999	12bp repeat	50 “treatment refractory” schizophrenics	Clozapine	response: BPRS continuous	n.s.
Rietschel 1996	13bp deletion Gly11Arg	149 “german schizophrenics and schizoaffective” : 40 no- vs. 32 slight- vs. 45 marked- vs. 32 total-response	Clozapine	response: clinical rating	n.s.
Ozdemir 1999	(G)n repeat SmaI RFLP	50 “treatment refractory” schizophrenics	Clozapine	response: BPRS continuous	n.s.

n.s. not significant

5.1.4 Other Genotypes

Bolonna et al. [2000] investigated polymorphisms of the adrenergic system, Arg492Cys of the ARDA1A receptor and -1291C/G as well as -261G/A of the ARDA2A receptor and found no association with Clozapine response. The -1291C/G polymorphism of the ARDA2A receptor was investigated too by Tsai et al. [2001] in a Clozapine sample identical with Tsai et al. [2000], Yu et al. [1999] and Lin et al. [1999] without significant findings.

Mancama et al. [2000] found among different polymorphisms of different muscarinergic receptors only the 1044A allele of the CHRM1 1044G/A polymorphism associated with non response.

In another study Mancama et al. [2002] analyzed histamine H1 and H2 receptor polymorphisms and found the H2 1018G/A polymorphism associated with good response, a finding that did not endure correction for multiple testing and was only replicated as a trend in a second independent sample.

A polymorphism of the glutamatergic NMDA-GRIND2B receptor was studied by Hong et al. [2001b] and no association of 2664C/T with treatment response was reported.

The HLA system was examined regarding association with Clozapine treatment response by Lahdelma et al. [1998]. They typed HLA-A, -B and -DR genotypes utilizing the lymphotoxicity method and found the A1 allele to be associated with treatment response. In a second study Meged et al. [1999] investigated HLA class I alleles (-A, -B, -C) and found no significant association.

The Apolipoprotein E ε4 allele was neither associated with treatment response in a japanese sample of Ohara et al. [1997] nor in a sample with Clozapine treated patients of Hong et al. [2000].

Two Neurotensin receptor polymorphisms, NTSR1 3020T/C and a VNTR, were not associated with treatment response [Huezo-Diaz et al. 2004].

Illi et al. [2003] found no association with treatment response for the 30bp VNTR of the MAOA gene, they additionally investigated the COMT Val158Met polymorphism and report the Met/Met genotype (low enzymatic activity phenotype) associated with non-response. The latter result could not be replicated by Yamanouchi et al. [2003].

Regarding BDNF 2 studies have been performed to date. Krebs et al. [2000] report an excess of long alleles (172-176bp) of a dinucleotide repeat polymorphism in responders. Hong et al. [2003] found the Val/Val genotype of a Val66Met polymorphism more frequent in responders to Clozapine.

A 677C/T polymorphism of the methylenetetrahydrofolate reductase was investigated by Joober et al. [2000b] who reported the T/T genotype and the T allele associated with response to conventional neuroleptics.

Table 17. Antipsychotic Response and Other Genotypes

Adrenergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Bolonna 2000a	ADRA1A: Arg492Cys; ADRA2A: -1291C/G, -261G/A		289 schizophrenics	Clozapine	response: 20 points GAS improvement	n.s.
Tsai 2001	ADRA2A: -1291C/G	sample identical with Tsai 2000, Lin 1999 and Yu 1999, Hong 2000	97 Chinese schizophrenic patients	Clozapine	BPRS continous	n.s.

Cholinergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Mancama 2000	CHRM1: 267C/A, 1044G/A, 1221C/T, 1353C/T;CHRM3: 193G/A;CHRM4: 1338C/T	abstract	?	?	response: ?	marginal significance for CHRM-1 1044A to be associated with non-response

Histaminergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Mancama 2002	H1: -17C/T, -974C/A, -1023A/G, -1536C/G H2: -294A/G, -592A/G, -1018G/A, -1077G/A		Sample 1:158 schizophrenics Sample 2: 164 schizophrenics	Clozapine	response: GAS 20point improvement	H2 -1018G/A associated with better responsebefore correction for multiple testing; not replicated in a 2nd sample

Glutamatergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Hong 2001a	NMDA-GRIN2B: 2664C/T		100 Chinese schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.

HLA System

reference	polymorphism	notes	sample	treatment	phenotype	result
Lahdelma 1998	HLA-A, -B, -DR Alleles	serotyping	19 responders to conventional antipsychotics vs. 19 clozapine responders	Clozapine	response: 20% improvement in BPRS	HLA-A1 associated with clozapine response
Meged 1999	HLA-class I (-A, -B, -C)	serotyping	88 Jewish schizophrenics	Haloperidol, Clozapine	response: CGI rating of at least much improved	n.s.

Apolipoprotein E

reference	polymorphism	notes	sample	treatment	phenotype	Result
Ohara 1997	ε4 allele present/absent		87 japanese schizophrenics	miscellaneous NL	response: PANSS continous	n.s.
Hong 2000	ε4		95 schizophrenics	Clozapine	response: PANSS continous	n.s.

Neurotensin

reference	polymorphism	notes	sample	treatment	phenotype	result
Huezo-Diaz 2004	NTSR1: 3020T/C, VNTR		196 british caucasian schizophrneics	Clozapine	response: GAS 20point improvement	n.s.

COMT/MAO

reference	polymorphism	notes	sample	treatment	phenotype	result
Illi 2003	COMT: Val 158Met MAOA: 30bp VNTR		94 finnish schizophrenics	conventional neuroleptics	CGI clinical	COMT: Met/Met associated with non response MAOA: n.s.
Yamanouchi 2003	COMT: Val158Met		73 japanese schizophrenics	Risperidone	response: PANSS subscales continous	n.s.

BDNF

reference	polymorphism	notes	sample	treatment	phenotype	result
Krebs 2000	a dinucleotide repeat polymorphism		88 schizophrenic/ schizoaffective patients	?	PANSS ?; categorical	excess of long alleles (172-176bp) in responders
Hong 2003	Val66Met		93 schizophrenic patients	Clozapine	20% decrease in BPRS	Val homozygotes more frequent in responders

Methylenetetrahydrofolate Reductase (THFR)

reference	polymorphism	notes	sample	treatment	phenotype	result
Joober 2000b	677C/T		105 schizophrenic patients	conventional neuroleptics	response: treatment resistance defined as by Kane et al. 1988	T alleles and T homozygote genotypes more frequent in responders

5.1.5. Combined Analyses (see table 6)

In an attempt to depict the assumable complexity of the genetic background of Clozapine response and with the goal to constitute a pattern of multiple

genotypes associated with response Arranz et al. [2000b] analyzed 19 polymorphisms of 5HT1A, -2A, -3A, -5A, 5-HTTLPR, DRD3, ADRA and Histamine-receptor H1 and H2. They reported a combination of 6 polymorphisms: 5HT2A 102T/C and His452Tyr, 5HT2C -330GT/-244CT and Cys23Ser, 5-HTTLPR and H2 -1018G/A to predict response to Clozapine with a positive predictive value of 0.82.

This finding however could not be replicated by Schumacher et al. [2000] who investigated the same 6 polymorphisms and reported only H2 -1018G/A to be associated with response.

Table 18. Antipsychotic Response and Combined Genotypes

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 2000b	5HT2A: His452Tyr, Thr25Asp, -1438G/A, 102T/C, 516C/T 5HT2C: -330GT/-244CT, Cys23 Ser 5HT3A: 178C/T, 1596G/A 5HT5A: -12A/T, -19G/C 5HTT: 5-HTTLPR, VNTR DRD3: Ser9Gly ADRA1A: Arg492Cys ADRA2A: -1291G/C, -261G/A H1: Leu449Ser H2: -1018G/A	no correction for multiple testing treatment response retrospectively assessed	200 schizophrenic patients	Clozapine	response: GAS	combination of 6 polymorphisms predict clozapin response: 5HT2A: 102T/C His452Tyr 5HT2C: -330GT/ -244CT Cys23Ser 5HTT: 5 -HTTLPR H2:-1018G/A
Schumacher 2000	Same combination of 6 response predicting genotypes as Arranz 2000b	an attemt to replicate Arranz 2000b	163 schizophrenic patients	Clozapine	4 response groups, group 3 and 4 corresponding to 20 point GAS improvement	n.s. Only H2: -1018G/A associated with response on the allelic level

n.s. not significant

5.2. Agranulozytosis

The majority of association studies regarding Clozapine induced agranulozytosis have been investigating the HLA system. The interpretation of the results is very problematic due to several methodological shortcomings. One of those is ethnic background: several of the studies are investigating jewish, mostly Ashkenazi patients, partially discriminating results into jewish/non-jewish subgroups partly not discriminating them at all. Sample sizes are often painfully small, 3 to 5 patients with Clozapine induced agranulozytosis (CA) versus 13 to 80 controls is not really a basis for a valid statistical analysis (see Abt et al. [1992] for more detailed statistical critique). Correction for multiple testing is not always done (as in this case should be done since the study designs are exploratory and not hypothesis based). A lot of results do not bear up against

correction for multiple testing when in fact applied or are reported as trends firsthand.

Besides more optimistic results reported by Lieberman et al. [1990] and Yunis et al. [1992, 1995], the two early studies with considerable sample size by Claas et al. [1992] and Abt et al. [1992] both report no significant results.

Theodoropoulou et al. [1997], Amar et al. [1998], Meged et al. [1999], Valevski et al. [1998], Lahdelma et al. [2001] all those studies suffer from small sample sizes. The most robust finding from these studies after all, or at least the one most often reported, is the association of HLA-B38 with CA in jewish patients (HlA-B16 in Lahdelma et al. [2001], but B38 is a splice variant of B16).

Dettling et al. [2001a, 2001b] in a more extended non-jewish sample report a complex association pattern with several predominantly class II antigens.

For further details on HLA association findings see table F.

In succession to their 1995 paper [Yunis et al. 1995], the Yunis group published further studies about a heat shock protein (HSP70) gene and the tumor necrosis factor (TNF) gene, both located in the class III region of the major histocompatibility complex (MHC). In Corzo et al. [1995] they report the HSP70-1 A allele and the HSP70-2 9.0kb allele in linkage disequilibrium with each other and associated to CA in jewish patients. Regarding TNF Turbay et al. [1997] report an association of the TNF microsattelites d3 and b4 with CA, b5 with non-CA controls.

Ostrousky et al. [2003] report several SNP's in the first intron and exon 3 and 5 of the dihydronicotineamide riboside quinone oxidoreductase 2 (NQO2) gene to be associated with CA. The paper seems to be an extension of the Valevski et al. [1998] study. The NQO2 gene maps to a region telomeric to the MHC complex.

Table 19. Side Effect: Clozapine-induced Agranulozytosis (CA)
HLA-System

reference	polymorphism	notes	sample	treatment	phenotype	result
Lieberman 1990	HLA-A, -B, -C, -DR, -DQ (HLA-class I and II antigens)	serotyped	schizophrenic and schizoaffective Ashkenazi patients 5 patients with Clozapine induced Agranulozytosis (CA), 26 controls	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with a haplotype consisting of HLA-B38, -DR4, -Dqw3
Claas 1992	HLA-class I and II antigens	serotyped	103 patients with CA, 95 matched controls	Clozapine	granulozytopenia: less than 1500 granulozytes/ml	n.s. after correction for multiple testing
Yunis 1992	HLA-class I and II antigens	serotyped	11 CA patients, 31 controls, most of jewish ancestry	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with HLA-B38, -DR4 and -DQw3 haplotype in jewish patients; with HLA-DR2 and -DQw1 in non-jewish

Abt 1992	HLA-class I and II antigens 48 HLA antigens	serotyped diagosis=?	72 patients with granulozytopenia/CA, 74 controls	Clozapine	definition of CA=?	no significant model of HLA subsets
Yunis 1995	HLA-class I and II antigens	serotyped	Ashkenazi and non-Ashkenazi schizophrenics and schizoaffectives, 31 CA, 52 controls extended Yunis 1992 sample	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	Markers for jewish CA patients: B38, DRB1*0402, DRB4*0101, DQB1*0302, DQA1*0301 "protecting alleles": DR11, DQB1*0301 Markers for non-jewish CA patients: DRB*1601, DRB5*02, DQB1*0502, DQA1*0102, DR2, DQw1
Theodoropoulou 1997	HLA-class I and II antigens	serotyped	43 schizophrenics, 3 of them developing agranulocytosis	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s.
Amar 1998	HLA-class I and II antigens	serotyped	18 schizophrenics, 5 of them with granulozytopenia/CA	Clozapine	granulozytopenia: less than 1000 and agranulozytosis: less than 500 neutrophils per mm3	granulozytopenia/ agranulozytosis associated with HLA-DQB1*0201
Valevski 1998	HLA-class I and II antigens	serotyped	61 jewish schizophrenics, 11 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	HLA-B38 associated with agranulozytosis
Meged 1999	HLA-class I	serotyped	88 Jewish schizophrenics, 3 of them with CA	Haloperidol, Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s. trend for HLA-B38 to be associated with agranulozytosis
Lahdema 2001	HLA-A, -B	serotyped, partially genotyped	26 schizophrenic patients with Granulozytopenia/Agranulozytosis and 19 schizophrenic controls	Clozapine	granulozytopenia: less than 1,5x109/L agranulozytosis: less than 0.5x109/L	granulozytopenia/ agranulozytosis associated with absence of HLA-A1
Dettling 2001a	HLA-class I and II antigens	genotyped ;no correction for multiple testing	107 caucasian paranoid schizophrenics, 30 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-DQB1*0502, -DRB5*02, trend for -DQB1*0201

Dettling 2001b	HLA-class I and II antigens	genotyped; no correction for multiple testing	108 caucasian paranoid schizophrenics, 31 of them with CA sample seems to be identical with Dettling 2001a	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-Cw*7, -DQB*0502, -DRB1*0101, -DRB3*0202

Heat Shock Protein

reference	polymorphism	notes	sample	treatment	phenotype	result
Corzo 1995	HSP70-1 HSP70-2	HSP70 is part of the HLA-class III cluster	75 schizophrenic and schizoaffektive patients, 32 of them with CA	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	HSP70-1 A and HSP70-2 9.0kb in linkage disequilibrium with each other and associated to CA in jewish patients

Tumor Necrosis Factor

reference	polymorphism	notes	sample	treatment	phenotype	result
Turbay 1997	TNF microsattelites a-b, d-e		12 jewish, 21 non-jewish schizophrenics vs 33 controls	Clozapine	agranulozytosis: less than 500 neutrophils per ml	CA associated with d3 and b4, inversely associated with b5

NQO2

reference	polymorphism	notes	sample	treatment	phenotype	result
Ostrousky 2003	NQO2: 1536C/T, 1541G/A, 372C/T (Phe/Leu), 202G/A, -367A/G, -394G/C	Sample seems to be partially identical to Valevski 1998	98 schizophrenics 18 of these with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	CA patients predominantly heterozygous for several exon and intron SNP's

n.s. not significant

5.3. Weight Gain

Weight gain is an issue in neuroleptics treatment side effects that has gained increasing attention recently. All but one study on this topic are dealing with Clozapine treated patients. The 5-HT2C receptor was predominantly investigated.

Rietschel et al. [1997] reported no significant result for the 5-HT2C Cys23Ser polymorphism, Hong et al. [2001b] investigated the 5-HT2C 68G/C polymorphism among other serotonin receptor and transporter gene polymorphisms without significance too. The 5-HT2C –759C/T polymorphism was investigated in several more recent publications without significant findings by Tsai et al. [2002], Basile et al. [2002a] and Theisen et al. [2004]. Reynolds et al. [2002] however report an association of the 5-HT2C –759C allele with

weight gain in patients treated with a variety of neuroleptics and in the Clozapine treated subgroup of this sample [Reynolds et al. 2003].

Basile et al. [2001] investigated 10 genetic polymorphisms in 9 candidate genes and found trends of association for adrenergic receptor gene polymorphisms (ADRB3, ADRA1A), for 5-HT2C Cys23Ser and for TNFα 308G/A.

Table 20. Side Effect: Weight Gain

reference	polymorphism	notes	sample	treatment	phenotype	result
Rietschel 1997	5-HT2C: Cys23Ser		152 schizophrenics	Clozapine	?	n.s.
Hong 2001b	5-HTTLPR, 5-HT2A 102T/C, 5-HT2C 68G/C, 5-HT6 267C/T		93 schizophrenic	Clozapine	weight gain continous	n.s.
Basile 2001	5HT2C: Cys23Ser, 5HT1A: CAn repeat, 5HT 2A: 102T/C and His452Tyr, H1:?, H2: -1018G/A, Cyp1A2: Intron1 C/A, ADRA1A: Arg347Cys, ADRB3: Trp64Arg, TNFa -308G/A		80 schizophrenics	Clozapine	weight gain continous	trends for DRB3, ADRA1A, TNFa, 5HT2C
Reynolds 2002	5-HT2C: -759C/T		123 chinese schizophrenics	miscellaneous NL	cut off: weight gain >7%	-759C ssociated with weight gain
Tsai 2002	5-HT2C: -759C/T		80 chinese schizophrenics	Clozapine	weight gain: BMI continous and cut off >7%	n.s.
Basile 2002a	5-HT2C: -759C/T		80 schizophrenics	Clozapine	Cut off: weight gain >7%	n.s.
Reynolds 2003	5-HT2C: -759C/T	subsample of Reynolds 2002	32 chinese schizophrenics	Clozapine	cut off: weight gain >7%	-759T associated with less weight gain
Theisen 2004	5-HT2C: -759C/T		97 german schizophrenic patients	Clozapine	Cut off: weight gain >7%	n.s.

n.s. not significant

5.4. NMS

The neuroleptic malignant syndrome (NMS) is a potentially fatal side effect of neuroleptic treatment with extrapyramidal symptoms, hyperpyrexia and elevated serum creatine phosphokinase levels. Trying to elucidate the putative genetic background of this severe condition cytochrome P450 2D6 (CYP2D6) polymorphisms and Dopamine D2 receptor gene polymorphisms have been investigated.

Regarding CYP2D6 no significant associations have been reported by Ueno et al. [1996], Iwahashi et al. [1997] and Kawanishi et al. [2000]. Suzuki et al. [2001c] found the DRD2 TaqI A1 allele associated with NMS, Kishida et al. [2004] the DRD2 –141C Del allele. The DRD2 TaqI A result could not be replicated by Kishida et al. [2003].

Table 21. Side Effect: Neuroleptic Malignant Syndrome

reference	polymorphism	notes	sample	treatment	phenotype	result
Ueno 1996	CYP2D6: 1795T del, 1934G/A, Arg296Cys (Hha I)		9 NMS patients	miscellaneous NL ?	?	n.s.
Iwahashi 1997	CYP2D6: HhaI	identical to Iwahashi 1999	56 japanese schizophrenics, 8 of them with NMS	miscellaneous NL	?	n.s.
Kawanishi 2000	Cyp2D6: Pro34Ser		36 patients with NMS, 107 schizophrenic controls	miscellaneous NL ?	NMS diagnosis according to the criteria of Pope et al. 1986	n.s.
Suzuki 2001c	DRD2: TaqI A	153 schizophrenic patients, 15 with NMS	miscellaneous NL ?	NMS criteria by Pope et al. 1986	A1 allele associated with NMS	
Kishida 2003	DRD2: TaqI A	49 patients with NMS, 123 schizophrenic controls	miscellaneous NL	NMS criteria Pope et al. 1986		n.s.
Kishida 2004	DRD2: TaqI A, -141C Ins/Del, Ser311Cys	164 japanese schizophrenics, 32 with NMS	miscellaneous NL ?	criteria by Pope et al. 1986	-141C Del more frequent in NMS	

n.s. not significant

5.5. Various side effects

Hsu et al. [2000] found no association of the adrenergic receptor α1A in Clozapine treated patients. Spina et al. [1992] report Cyp2D6 poor metabolizer

to be more prone to side effects of typical neuroleptics like postural hypotension, diplopia and dry mouth.

Table 22. Side Effect: Various

reference	polymorphism	notes	sample	treatment	phenotype	result
Hsu 2000	Adrenergic Receptor α 1A RsaI		80 schizophrenic patients	Clozapine	urinary incontinence in the first 3 month of treatment	n.s.
Spina 1992	CYP2D6 phenotyped by debrisoquine hydroxylation test		53 schizophrenics	typical neuroleptics	side effects: postural hypotension, diplopia, dry mouth	PM state associated with side effects

n.s. not significant

6. DISCUSSION

Currently the association findings of pharmacogenetic studies in psychosis are not very convincing with the most robust findings, at least for response, probably in the 5-HT2A, 5-HT2C, DRD2 and DRD3 receptor genes. There are a lot of studies with conflicting results, no replications or no replication trials at all.

Many studies are suffering from considerable methodological shortcomings and efforts have been undertaken to propose a stringent methodology for pharmacogenetic studies [Rietschel et al. 1999, Malhotra et al. 2000, Masellis et al. 2000]. The recommendations range from sample size and statistical power considerations over diagnostic stringency and statistical rigorousness to proposals of medication plasma levels control.

An important reason for this apparent problematic situation of pharmacogenetics after all is inherent in the nature of the topic. Genetics of psychiatric diseases are complex with environmental factors to be considered and in genetics of treatment effects we are definitely dealing with complex traits. Multiple genetic polymorphisms each of them with minor impact are involved, multiple receptors are targeted by different drugs and pharmacokinetic effects have to be considered.

Complex designs of studies with multiple polymorphisms as by Arranz et al. [2000b] are an interesting approach, however with considerable methodological problems. Haplotype studies might increase statistical power [Malhotra et al. 2004], as well as the use of candidate genes with a strong a priori rationale [Masellis et al. 2000]. Multicenter studies with great sample sizes have repeatedly been asked for and endophenotypes as well as neuroimaging approaches might refine the technique.

Genes of expression, regulation and transport mechanisms, second messenger, third messenger and transcription factors are increasingly taken into account to include the post receptor downstream systems into the picture.

A promising future strategy in pharmacogenetics seems to be the application of microarray expression profiling for the identification of relevant candidate target genes of antipsychotic drug action. Thus gene expression induction by Clozapine treatment has been found for example for Chromogranin

A and Calcineurin A, a decrease in Synaptotagmin V in rat frontal cortex. Chronic administration of Clozapine resulted in a differential expression pattern for Chromogranin A, son of sevenless (SOS: a RAS activator) and Sec-1 in the cortex. Chronic haloperidol treatment has been found to alter gene expression of "inhibitor of DNA-binding 2" (ID-2) and Rab-12. Since Chromogranin A and Synaptotagmin V seem to be involved in presynaptic vesicle formation and secretion, this might represent an important mode of action of antipsychotic medication. Post mortem studies with brain tissue of schizophrenic patients have found altered expression of genes involved in presynaptic functions [see Miyamoto et al. 2005].

Thus we will hopefully see the riddle of pharmacogenetics of psychosis being resolved step by step in the nearer future to be provided with a biologically based rationale for the administration of targeted antipsychotic medication.

7. REFERENCES

Abt, K., Gülich, A., Krupp, P., Reinberg, W., 1992, HLA- associations in Leponex/Clozaril (Clozapine)-induced granulozytopenia and agranulocytosis. Statistical viewpoints. *Drug Safety.* **7**(suppl. 1):10-16.

Aitchison, K., Munro, J., Wright, P., Smith, S., Makoff, A.J., Sachse, C., Sham, P.C., Murray, R.M., Collier, D.A., Kerwin, R.W., 1999, Failure to respond to treatment with typical antipsychotics is not associated with CYP2D6 ultrarapid hydroxylation. Br. J. *Cli. Psychopharmacol*. **48**:388-394.

Amar, A., Segman, R., Shtrussberg, S., Sherman, L., Safirman, C., Lerer, B., Brautbar, C.,1998, An association between clozapine-induced agranulocytosis in schizophrenics and HLA-DQB*0201. Int. J. *Neuropsychopharmacol*. **1**:41-44.

American Psychiatric Association, 1994, Diagnostic and Statistical Manual of Mental Disorder, 4th edition. American Psychiatric Association, Washington D.C.

Andreasen, N.C., 1989, The scale for the assessment of negative symptoms: conceptual and theoretical foundations. Br. *J. Psychiatry* Suppl. **7**:49-58.

Arinami, T., Gao, M., Hamaguchi, H., Toru, M.,1997, A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. *Hum. Mol. Genet*. **6**:577-582.

Arranz, M., Collier, D., Sodhi, M., Ball, D., Roberts, G., Price, J., Sham, P., Kerwin, R., 1995a, Association between clozapine response and allelic variation in 5-HT2A receptor gene. *Lancet.* **346**:281-282.

Arranz, M., Dawson, E., Shaikh, S., Sham, P., Sharma, T., Aitchison, K., Crocq, A., Gill, M., Kerwin, R., Collier, D., 1995b, Cytochrome P4502D6 genotype does not determine response to clozapine. Br. *J. Clin. Pharmac*. **39**:417-420.

Arranz, M.J., Collier, D.A., Munro, J., Sham, P., Kirov, G., Sodhi, M., Roberts, G., Price, J., Kerwin, R.W., 1996, Analysis of a structural polymorphism in the 5-HT2A receptor and clinical response to clozapine. *Neurosci. Letters.* **217**:177-178.

Arranz, M., Munro, J., Sham, P., Kirov, G., Murray, R.M., Collier, D.A., Kerwin, R.W., 1998a, Meta-analysis of studies on genetic variation in 5-HT2A receptors and clozapine response. *Schiz. Res*. **32**:93-99.

Arranz, M., Munro, J., Owen, M.J., Spurlock, G., Sham, P.C., Zhao, J., Kirov, G., Collier, D.A., Kerwin, R.W., 1998b, Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine. *Mol. Psychiatry.* **3**:61-66.

Arranz, M.J., Li, T., Munro, J., et al., 1998c, Lack of association between a polymorphism in the promoter region of the dopamine-2 receptor gene and clozapine response. *Pharmacogenetics.* **8**:481-484.

Arranz, M.J., Bolonna, A.A., Munro, J., Curtis, C.J., Collier, D.A., Kerwin, R.W., 2000a, The serotonin transporter and clozapine response. *Mol. Psychiatr*. **5**:124-125.

Arranz, M.J., Munro, J., Birkett, J., Bolonna, A., Mancama, D., Sodhi, M., Lesch, K.P., Meyer,

J.F.W., Sham, P., Collier, D.A., Murray, R.M., Kerwin, R.W., 2000b, Pharmacogenetic prediction of clozapine response. *Lancet.* **355**:1615-1616.

Arranz, M.J., Mancama, D., Kerwin, R.W., 2001, Neurotransmitter receptor variants and their influence on antipsychotic treatment (Review). *Int. J. Mol. Med*. **7**(1)27-30.

Asghari, V., Sanyal, S., Buchwaldt, S., et al., 1995, Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J. Neurochem*. **65**:1157-1165.

Austin, J., Buckland, P., Cardno, A.G., Williams, N., Spurlock, G., Hoogendoorn, B., Zammit, S., Jones, G., Sanders, R., Jones, L., McCarthy, G., Jones, S., Bray, N.J., McGuffin, P., Owen, M.J., O'Donovan, M.C., 2000, The high affinity neurotensin receptor gene (NTSR1): comparative sequencing and association studies in schizophrenia. *Mol. Psychiatry.* **5**:552-557.

Basile, V.S., Masellis, M., McIntyre, R.S., Meltzer, H.Y., Lieberman, J.A., Kennedy, J.L., 2001, Genetic dissection of atypical antipsychotic-induced weight gain: novel preliminary data on the pharmacogenetic puzzle. *J. Clin. Psychiatry.* **62** (suppl. 23):45-66.

Basile, V.S., Masellis, M., De Luca, V., Meltzer, H.Y., Kennedy, J.L., 2002a, -759C/T genetic variation of 5HT2C receptor and clozapine-induced weight gain. *Lancet.* **360**:1790-1791.

Basile, V.S., Masellis, M., Potkin, S.G., Kennedy, J.L., 2002b, Pharmacogenetics in schizophrenia: the quest for individualized therapy. *Hum. Mol. Genet*. **11**:2517-2530.

Bayley, J.P., Ottenhoff, T.H., Verweij, C.L., 2004, Is there a future for TNF promoter polymorphisms? *Genes Immun*. **5**(5):315-329.

Bengtsson, F., 2004, Therapeutic drug monitoring of psychotropic drugs TDM "Nouveau". Ther. *Drug Monit*. **26**:145-151.

Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W.J., Mattick, J.S., Haussler, D., 2004, Ultraconserved elements in the human genome. *Science.* **304**:1321-1325.

Birkett, J.T., Arranz, M.J., Munro, J., Osbourn, S., Kerwin, R.W., Collier, D.A., 2000, Assoziation analysis of the 5-HT5A gene in depression, psychosis and antipsychotic response. *Neuroreport.* **11**:2017-20.

Bolonna, A.A., Arranz, M.J., Munro, J., Osborne, S., Petouni, M., Martinez, M., Kerwin, R.W., 2000, No influence of adrenergic receptor polymorphisms on schizophrenia and antipsychotic response. Neurosci. *Letters.* **280**:65-68.

Brenner, H.D., Dencker, S.J., Goldstein, M.J., Hubbard, J.W., Keegan, D.L., Kruger, G., et al., 1990, Defining treatment refractoriness in schizophrenia. *Schizophr. Bull*. **16**:551-561.

Brockmöller, J., Kirchheiner, J., Schmider, J., Walters, S., Sachse, C., Müller-Öhrlinghausen, B., Roots, I. , 2002, *Clin. Pharmacol. Ther*. **72**:438-452.

Buckland, P.R., Hoogendoorn, B., Guy, C.A., Smith, S.K., Coleman, S.L., O'Donovan, M.C., 2005, Low gene expression conferred by association of an allele of the 5-HT2C receptor gene with antipsychotic-induced weight gain. *Am. J. Psychiatry*. **162**(3): 613-615.

Chiuve, S.E., Giovannucci, E.L., Hankinson, S.E., Hunter, D.J., Stampfer, M.J., Willett, W.C., Rimm, E.B., 2005, Alcohol intake and methylenetetrahydrofolate reductase polymorphism modify the relation of folate intake to plasma homocysteine. *Am. J. Clin. Nutr*. **82**:55-162.

Claas, F.H.J., Abbott, P.A., Witvliet, M.D., D'Amaro, J., Barnes, P.M., Krupp, P., 1992, No direct clinical relevance of the human leucocyte antigen (HLA) system in Clozapine-induced agranulocytosis. *Drug Safety.* **7**(Suppl. 1):3-6.

Cohen, B.M., Ennulat, D.J., Centorrino, F., et al., 1999, Polymorphisms of the dopamine D4 recepto and response to antipsychotic drugs. *Psychopharmacol*. **141**:6-10.

Collier, D.A., 2003, Pharmacogenetics in psychosis. *Drug News Perspect.* **16**(3):159-165.

Cravchik, A., Sibley, D.R., Gejman, P.V., 1996, Functional analysis of the human D2 dopamine receptor missense variants. *J. Biol. Chem*. **271**:26013-26017.

Cawley, S., Bekiranov, S., Ng, H.H., et al., 2004, Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell.* **116**:499-509.

Dahl, M.L., 2002, Cytochrome P450 phenotyping/genotyping in patients receiving antipsychotics. *CLIN Pharmacokinet*. **41**:453-470.

Dahmen, N., Müller, M.J., Germeyer, S., et al., 2001, Genetic polymorphisms of the dopamine D2 and D3 receptor and neuroleptic drug effects in schizophrenic. *Schizophr. Res*. **49**:223-225.

Daly, A.K., Brockmöller, J., Broly, F., Eichelbaum, M., Evans, W.E., Gonzales, F.J., Huang, J.D., et al., 1996, Nomenclature for human Cyp2D6 alleles. *Pharmacogenetics.* **6**:193-201.

De Luca, V., Tharmalingam, S., King, N., Strauss, J., Bulgin, N., Kennedy, J.L., 2005, Association study of a novel functional polymorphism of the serotonin transporter gene in bipolar disorder and suicidal behaviour. *Psychopharmacology.* (*Berl*). Jun **29**:1-4 [Epub ahead of print]

Dettling, M., Cascorbi, I., Hellweg, R., Deicke, U., Weise, L., Mueller-Oerlinghausen, B., 1999, Genetic determinants of clozapine-induced-agranulozytosis: Potential risk of Olanzapine. *Pharmacopsychiatry.* **32**:110-112.

Dettling, M., Cascorbi, I., Roots, I., Mueller-Oerlinghausen, B., 2001a, Genetic determinants of clozapine-induced-agranulozytosis: Recent results of HLA Subtyping in a non-jewish caucasian sample. *Arch. Gen. Psychiatry.* **58**:93.

Dettling, M., Schaub, R.T., Mueller-Oerlinghausen, B., Roots, I., Cascorbi, I., 2001b, Further evidence of human leucocyte antigen-encoded susceptibility to clozapine-induced agranulocytosis independent of ancestry. *Pharmacogenetics.* **11**:135-141.

Duan, J., Wainwright, M.S., Comeron, J.M., et al., 2003, Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Hum. *Mol. Genet*. **12**:205-216.

Durany, N., Thome, J., Palomo, A., Foley, P., Riederer, P., Cruz-Sanchez, F.F., 1996, Homozygosity at the dopamine D3 receptor gene in schizophrenic patients. *Neurosci. Lett.* **220**:151-154.

Ebstein, R.P., Macciardi, F., Heresco-Levi, U., et al., 1997, Evidence of an association between the dopamine D3 receptor gene DRD3 and schizophrenia. *Hum. Hered*. **47**:6-16.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003, The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell.* **112**(2):257-269.

Ellingrod, V.L., Perry, P.J., Lund, B.C., Bever-Stille, K., Fleming, F., Holman, T.L., Miller, D., 2002, 5HT2A and 5HT2C receptor polymorphisms and predicting clinical response to olanzapine in schizophrenia. *J. Clin. Psychopharmacol*. **22**(6):622-624.

Ellingrod, V.L., Lund, B.C., Miller, D., Fleming, F., Perry, P., Holman, T.L., Bever-Stille, K., 2003, 5-HT2A receptor promoter polymorphism, -1438G/A and negative symptom response to olanzapine in schizophrenia. *Psychopharmacol. Bull*. **37**:109-112.

Endicott, J., Spitzer, R.L., Fleiss, J.L., Cohen, J., 1976, The global assessment scale. A procedure for measuring overall severity of psychiatric disturbance. *Arch. Gen. Psychiatry.* **33**:766-771.

Endicott, J., Spitzer, R.L., 1978, A diagnostic interview: The Schedule for Affective Disorders and Schizophrenia. *Arch. Gen. Psychiatry.* **35**:837-844.

Felber, W., Naumann, R., Schuler, U., et al., 2000, Are thee genetic determinants of Olanzapine-induced-agranulozytosis? *Pharmacopsychiatry.* **33**:197-198.

Fentress, H.M., Grinde, E., Mazurkiewicz, J.E., Backstrom, J.R., Herrick-Davis, K., Sanders-Bush, E., 2005, Pharmacological properties of the Cys23Ser single nucleotide polymorphism in human 5-HT(2C) receptor isoforms. *Pharmacogenomics. J.* May **24** [Epub ahead of print].

Gaitonde, E.J., Morris, A., Sivagnanasundaram, S., McKenna, P.J., Hunt, D.M., Mollon, J.D., 1996, Assessment of association of D3 dopamine receptor MscI polymorphism with schizophrenia: analysis of symptoms ratings, family history, age at onset, and movement disorders. Am. J. Med. Genet. (Neuropsychiat. Genet.) **67**:455-458.

Gutierrez, B., Arranz, M.J., Huezo Diaz, P., Dempster, D., Matthiasson, P., Travis, M., Munro, J., Osborne, S., Kerwin, R.W., 2002, Novel mutations in 5-HT3A and 5-HT3B receptor genes not associated with clozapine response. *Schiz. Res.* **58**(1):93-97.

Guy, W., (ed.), 1976, Clinical global impression. In: ECDEU assessment manual for psychopharmacology, revised. National Institute of Mental Health, Rockville, MD.

Hamelin, B.A., Dorson, P.G., Pabis, D., Still, D., Bouchard, R.H., Pourcher, E., Rail, J., Turgeon, J., Crismon, M.L., 1999, CYP2D6 mutations and therapeutic outcome in schizophrenic patients. *Pharmacotherapy*. **19**(9):1057-1063.

Hazelwood, L.A. and Sanders-Bush, E., 2004, His452Tyr polymorphism in the human 5-HT2A receptor destabilizes the signal conformation. *Mol. Pharmacol*. **66**:1293-1300.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K.P., 1996, Allelic variation of human serotonin transporter gene expression. *J. Neurochem*. **66**:2621-2624.

Hong, C.J., Yu, Y.W.Y., Lin, C.H., Song, H.L., Lai, H.C., Yang, K.H., Tsai, S.J., 2000, Assoziation study of apolipoprotein Ee4 with clinical phenotype and clozapine response in schizophrenia. *Neuropsychobiology.* **42**:172-174.

Hong, C.J., Yu, Y.W.Y., Lin, C.H., Cheng, C.Y., Tsai, S.J., 2001a, Assoziation analysis for NMDA receptor subunit 2B (GRIN2B) genetic variants and psychopathology and clozapine response in schizophrenia. Psychiatr. *Genet*. **11**:219-222.

Hong, C.J., Lin, C.H., Yu, Y.W., Yang, K.H., Tsai, S.J., 2001b, Genetic variants of the serotonin system and weight change during clozapine treatment. *Pharmacogenetics.* **11**:265-268.

Hong, C.J., Yu, Y.W.Y., Lin, C.H., Tsai, S.J., 2003, An association study of a brain derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. *Neuroscience Letters.* **349**:206-208.

Hranilovic, D., Stefulj, J., Schwab, S., Borrmann-Hassenbach, M., Albus, M., Jernej, B., Wildenauer, D., 2004, Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. *Biol. Psychiatry.* **55**(11): 1090-1094.

Huezo, P., Dempster, D., Gutierrez, B., Munro, J., Osborne, S., Arranz, M.J., Kerwin, R., 2000, Serotonin-6 (5-HT6) receptor variant (267-C/T) not associated with clozapine response. *Am. J. Med. Genet*. **96**:P265.

Huezo-Diaz, P., Arranz, M.J., Munro, J., Osborne, S., Makoff, A., Kerwin, R.W., Austin, J., O'Donovan, M., 2004, An association study of the neurotensin receptor gene with schizophrenia and clozapine response. *Schizophr. Res*. **66**(2-3):193-195.

Hurko, O., 2001, Genetics and genomics in neuropsychopharmacology: the impact on drug discovery and development. *Eur. Neuropsychopharmacol*. **11**:491-499.

Hwu, H.G., Hong, C.J., Lee, Y.L., Lee, P.C., Lee, S.F.C., 1998, Dopamine D4 receptor gene polymorphisms and neuroleptic response in schizophrenia. *Biol. Psychiatry.* **44**:483-487.

Illi, A., Mattila, K.M., Kampman, O., Anttila, S., Roivas, M., Lehtimäki, T., Leinonen, E., 2003, Catechol-O-methyltransferase and monoamine oxidase A genotypes and drug response to conventional neuroleptics in schizophrenia. *J. Clin. Psychopharmacol*. **23**:429-434.

Iwahashi, K., Nakamura, K., Suwaki, H., Tsuneoka, Y., Ichikawa, Y., 1997, CYP2D6 HhaI genotype and the neuroleptic malignant syndrome (NMS). *Clin. Chim. Acta*. **265**:143-144.

Iwahashi, K., Yoshihara, E., Nakamura, K., Ameno, K., Watanabe, M., Tsuneoka, Y., Ichikawa, Y., Igarashi, K., 1999, CYP2D6 HhaI genotype and the neuroleptic malignant syndrome. *Neuropsychobiol*. **39**:33-37.

Jönsson, E., Lannfelt, L., Sokoloff, P., Schwartz, J.C., Sedvall, G., 1993, Lack of association between schizophrenia and alleles in the dopamine D3 receptor gene. *Acta Psychiatr. Scand.* **87**:345-349.

Jönsson, E., Nöthen, M.M., Bunzel, R., Propping, P., Sedvall, G.,1996, Letter. *The Lancet.* **347**:1831.

Jönsson, E.G., Nöthen, M.M., Grünhage, F., et al., 1999, Polymorphisms in the dopamine D2 receptor gene and their relationship to striatal dopamine receptor density in healthy volunteers. *Mol. Psychiatry.* **4**:290-296.

Jönsson, E.G., Flyckt, L., Burgert, E., et al., 2003, Dopamine D3 receptor gene Ser9Gly variant and schizophrenia: association study and meta-analysis. *Psychiatr. Genet*. **13**:1-12.

Joober, R., Benkelfat, C., Brisebois, K., Toulouse, A., Turecki, G., Lal, S., Bloom, D., Labelle, A., Lalonde, P., Fortin, D., Alda, M., Palmour, R., Rouleau, G.A., 1999, T102C polymorphism in the 5HT2A gene and schizophrenia:relation to phenotype and drug response variability. *J. Psychiatr. Neurosci*. **24**:141-146.

Joober, R., Toulouse, A., Benkelfat, C., et al., 2000a, DRD3 and DAT1 genes in schizophrenia: an association study. *J. Psychiatr. Res*. **34**:285-291.

Joober, R., Benkelfat, C., Lal, S., Bloom, D., Labelle, A., Lalonde, P., Turecki, G., Rozen, R., Rouleau, G.A., 2000b, Association between the methylenetetrahydrofolate reductase 677C-T missense mutation and schizophrenia. *Mol. Psychiatry.* **5**:323-326.

Kaiser, R., Könneker, M., Henneken, M., et al., 2000, Dopamine receptor 48-bp repeat polymorphism: no association with response to antipsychotic treatment, but association with catatonic schizophrenia. *Mol. Psychiatry.* **5**:418-424.

Kane, J., Honigfeld, G., Singer, J., Meltzer, H., 1988, Clozapine for treatment resistant schizophrenic. A double blind comparrison withchlorpromazine. *Arch. Gen. Psychiatry.* **45**:789-796.

Kapur, S., Seeman, P., 2000, Antipsychotic agents differ in how fast they come off the dopamine D2 receptors. Implications for atypical antipsychotic action. *J. Psychiatry Neurosci.* **25**:161-166.

Kawanishi, C., Furuno, T., Onishi, H., Sugiyama, N., Suzuki, K., Matsumura, T., Ishigami, T., Kosaka, K., 2000, Lack of association in Japanese patients between neuroleptic malignant syndrome and a debrisoquine 4-hydroxylase genotype with low enzyme activity. *Psychiatr. Genet*. **10**:145-147.

Kay, S.R., Opler, L.A., Lindenmaier, J.P., 1987, The Positive and Negative Syndrome Scale (PANNS) for schizophrenia. *Schizophr. Bull.* **13**:261-276.

Kennedy, J.L., Billet, E.A., Macciardi, F.M., et al., 1995, Association study of dopamine D3 receptor gene and schizophrenia. *Am. J. Med. Genet*. (*Neuropsychiat. Genet.)* **60**: 558-562.

Kerwin, R.W., Pilowsky, L., Munro, J., Shaikh, S., Gill, M., Collier, D., 1994, Functional neuroimaging and pharmacogenetic studies of clozapine`s action at dopamine receptors. *J. Clin. Psychiatry.* **55** (Suppl. B):57-62.

Kishida, I., Kawanishi, C., Furuno, T., Matsumura, T., Hasegawa, H., Sugiyama, N., Suzuki, K., Yamada, Y., Kosaka, K., 2003, Lack of association in Japanese patients between neuroleptic malignant syndrome and the TaqI A polymorphism of the dopamine D2 receptor gene. *Psychiatr. Genet*. **13**:55-57.

Kishida, I., Kawanishi, C., Furuno, T., Kato, D., Ishigami, T., Kosaka, K., 2004, Association in Japanese patients between neuroleptic malignant syndrome and functional polymorphisms of the dopamine D2 receptor gene. *Mol. Psychiatry.* **9**:293-298.

Kohn, Y., Ebstein, R.P., Heresco-Levy, U., et al., 1997, Dopamine D4 receptor gene polymorphisms: relation to ethnicity, no association with schizophrenia and response to clozapine in israeli subjects. *Eur*. *Neuropsychopharmacol*. **7**:39-43.

Kondo, T., Mihara, K., Suzuki, A., Yasui-Furukori, N., Kaneko, S., 2003, Combination of dopamine D2 receptor gene Polymorphism as a possible predictor of treatment-resistance to dopamine antagonists in schizophrenic patients. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* **27**:921-926.

Krebs, M.O., Sautel, F., Bourdel, M.C., et al., 1998, Dopamine D3 receptor gene variants and substance abuse in schizophrenia. *Mol. Psychiatr*. **3**:337-341.

Krebs, M.O., Guillin, O., Bourdel, M.C., Schwartz, J.C., Olie, J.P., Poirier, M.F., Sokoloff, P., 2000, Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. *Mol. Psychiatr*. **5**:558-562.

Lahdema, L., Ahokas, A., Andersson, L.C., Huttunen, M., Sarna, S., Koskimies, S., 1998, Association between HLA A1 allele and schizophrenia gene(s) in patients refractory to conventional neuroleptics but responsive to clozapine medication. *Tissue Antigens.* **51**:200-203.

Lahdema, L., Ahokas, A., Andersson, L.C., Suvisaari, J., Hovata, I., Huttunen, M., Koskimies, S., 2001, Human leucozyte antigen-A1 predicts a good therapeutic response to clozapine with a low risk of agranulocytosis in patients with schizophrenia. *J. Clin. Psychopharmacol*. **21**:4-7.

Lane, H.Y., Hu, O.Y.P., Jann, M.W., Deng, H.C., Lin, H.N., Chang, W.H., 1997, Dextromethorphan phenotyping and haloperidol disposition in schizophrenic patients. *Psychiatry. Res.* **69**:105-111.

Lane, H.Y., Chang, Y.C., Chiu, C.C., Chen, M.L., Hsieh, M.H., Chang, W.H., 2002, Association of risperidon treatment response with a polymorphism in the 5 HT2A receptor gene. *Am. J. Psychiatry.* **159**:1593-1595.

Laruelle, M., Gelernter, J., Innis, R.B., 1998, D2 receptors binding potential is not affected by Taq1 polymorphism at the D2 receptor gene. *Mol. Psychiatry.* **3**:261-265.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996, Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science.* **274**(5292):1527-1531.

Lieberman, J.A., Yunis, J., Egea, E., Canosa, R., Kane, J.M., Yunis, E.J., 1990, HLA B38, DR4 and Dqw3 and clozapine induced agranulozytosis in jewish patients with schizophrenia. *Arch. Gen. Psychiatr*. **47**:945-948.

Lin, C.H., Tsai, S.J., Yu, W.Y., Song, H.L., Tu, P.C., Sim, C.B., Hsu, C.P., Yang, K.H., Hong, C.J., 1999, No evidence for association of serotonin 2A receptor variant (102T/C) with schizophrenia or clozapine response in a chinese population. *NeuroReport.* **10**:57-60.

Lundstrom, K., Turpin, M.P., 1996, Proposed schizophrenia-related gene polymorphism: Expression of the Ser9Gly mutant human dopamine D3 receptor with the Semliki Forest Virus System. Biochem. *Biophys. Res. Com*. **225**: 1068-1072.

Malhotra, A.K., Goldman, D., Ozaki, N., Breier, A., Buchanan, R., Pickar, D., 1996a, Lack of association between polymorphisms in the 5 HT2A receptor gene and the antipsychotic response to clozapine. *Am. J. Psychiatry.* **153**:1092-1094.

Malhotra, A.K., Goldman, D., Ozaki, N., Rooney, W., Clifton, A., Buchanan, R.W., Breier, A., Pickar, D., 1996b, Clozapine response and the 5HT2C Cys23Ser polymorphism. *Neuroreport.* **7**:2100-2102.

Malhotra, A.K., Goldman, D., Buchanan, R.W., et al., 1998, The dopamine D3 receptor (DRD3) Ser9Gly polymorphism and schizophrenia: a haplotype relative risk study and association with clozapine response. *Mol. Psychiatr*. **3**:72-75.

Malhotra, A.K., Buchanan, R.W., Kim, S., et al., 1999, Allelic variation in the promoter region of the dopamine D2 receptor gene and clozapine response. *Schiz. Res*. **36**:92-93.

Malhotra, A.K., 2003, The relevance of pharmacogenetics to schizophrenia. *Curr. Opin. Psychiatry.* **16**:171-174.

Malhotra, A.K., Murphy, G.M., Kennedy, J.L., 2004, Pharmacogenetics of psychotropic drug response. *Am. J. Psychiatry.* **161**:780-796.

Mancama, D., Arranz, M.J., Munro, J., Makoff, A., Kerwin, R., 2000, The histamine 1 and 2 receptor genes – candidates for schizophrenia and clozapine drug response. *Gene Screen.* **1**:29-34.

Mancama, D., Arranz, M.J., Munro, J., Osborne, S., Makoff, A., Collier, D., Kerwin, R., 2002, Investigation of promotor variants of the histamine 1 and 2 receptors in schizophrenia and clozapine response. *Neurosci*. *Letters.* **333**:207-211.

Mant, R., Williams, J., Asherson, P., Parfitt, E., McGuffin, P., Owen, M.J., 1994, Relationship between homozygosity at the dopamine D3 Receptor gene and schizophrenia. *Am. J. Med. Genet*. (*Neuropsychiat. Genet*.) **54**:21-26.

Masellis, M., Paterson, A.D., Badri, F., Lieberman, J.A., Meltzer, H.Y., Cavazzoni, P., Kennedy, J.L., 1995, Genetic variation of 5 HT2A receptor and response to clozapine. *Lancet.* **346**:1108.

Masellis, M., Basile, V., Meltzer, H.Y., Liebermann, J.A., Sevy S, Macciardi FM, Cola P, Howard A, Badri F, Nöthen MM, Kalow W, Kennedy JL (1998) Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenic patients. *Neuropsychopharmacol*. **19**:123-132.

Masellis, M., Basile, V.S., Özdemir, V., Meltzer, H.Y., Macciardi, F., Kennedy, J.L., 2000, Pharmacogenetics of antipsychotic treatment: lessons learned from clozapine. *Biol. Psychiatry.* **47**:252-266.

Masellis, M., Basile, V.S., Meltzer, H.Y., Liebermann, J.A., Sevy, S., Goldman, D.A., Hamblin, M.W., Macciardi, F.M., Kennedy, J.L., 2001, Lack of association between the T/C 267 serotonin 5 HT6 receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia. *Schizophrenia. Res*. **47**:49-58.

Meged, S., Stein, D., Sitrota, P., Melamed, Y., Elizur, A., Shmuelian, I., Gazit, E., 1999, Human leucocyte antigen typing, response to neuroleptics, and clozapine induced aganulocytosis in jewish israeli schizophrenic patients. *Int. Clin. Psychopharmacol*. **14**:305-312.

Miyamoto, S., Duncan, G.E., Marx, C.E., Lieberman, J.A., 2005, Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol. Psychiatry.* **10**:79-104.

Nimgaonkar, V.L., Wessely, S., Tune, L.E., Murray, R.M., 1988, Response to drugs in schizophrenia: the influence of family history, obstetric complications and ventricular enlargement. *Psychol. Med*. **18**:583-592.

Nimgaonkar, V.L., Zhang, X.R., Caldwell, J.G., Ranguli, R., Chakravarti, A., 1993, Association study of schizophrenia with dopamine D3 receptor gene polymorphisms: probable effects of family history of schizophrenia? *Am. J. Med. Genet. (Neuropsychiat. Genet.)* **48**: 214-217.

Nimgaonkar, V.L., Zhang, X.R., Brar, J.S., DeLeo, M., Ganguli, R., 1996a, 5 HT2 receptor gene locus: association with schizophrenia or treatment response not detected. *Psychiatr. Genet*. **6**:23-27.

Nimgaonkar, V.L., Sanders, A.R., Ganguli, R., et al., 1996b, Association study of schizophrenia and the dopamine D3 receptor gene locus in two independant samples. *Am. J. Med. Genet. (Neuropsychiat*. *Genet.)* **67**:505-514.

Nöthen, M.M., Rietschel, M., Erdmann, J., Oberländer, H., Möller, H.J., Naber, D., Propping, P., 1995, Genetic variation of the 5 HT2A receptor and response to clozapine. *Lancet.* **346**:908-909.

Nurnberger, J.I., Blehar, M.C., Kaufmann, C.A., York-Cooler, C., Simpson, S.G., Harkavy-Friedma, J., Severe, J.B., Malaspina, D., Reich, T., Collaborators from the NIMH Genetics Initiative (1994) Diagnostic Interview for Genetc Studies. Rationale, unique features and training. *Arch. Gen. Psychiatry.* **51**:799-812.

Ohara, K., Nakamura, Y., Xie, D.W., et al., 1996, Polymorphisms of dopamine D2-like (D2, D3, and D4) receptors in schizophrenia. *Biol. Psychiatry* **40**:1209-1217.

Ohara, K., Nagai, M., Ohara, K., 1997, Apolipoprotein E ε4 and clinical phenotype in schizophrenia. *The Lancet.* **350**:1857.

Ohara, K., Nagai, M., Tani, K., Nakamura, Y., Ino, A., Ohara, K., 1998, Functional polymorphism of -141C Ins/Del in the dopamine D2 receptor gene promotor and schizophrenia. *Psych. Res*. **81**:117-123.

Overall, J.E. and Gorham, D.R., 1962, The Brief Psychiatric Rating Scale. *Psychol. Rep*. **10**: 799-812.

Özdemir, V., Masellis, M., Basile, V.S., et al., 1999, Variability in response to clozapine: potential role of cytochrome P450 1A2 and the dopamine D4 receptor gene. *CNS. Specr*. **4** (6): 30-56.

Özdemir, V., Kalow, W., Okey, Ab., et al., 2001, Treatment resistance to Clozapine in Assoziation with Ultrarapid CYP1A2 activity and the C-A polymorphism in intron 1 of the CYP1A2 gene: effect of grapefruit juice and low-dose fluvoxamine. *J. Clin. Psychopharmacol*. **21**:603-607.

Perneger, T.V., 1998, Whats wrong with the Bonferroni adjustments. *Br. Med. J.* **316**:1236-1238.

Prior, T.I., Chue, P.S., Tibbo, P., Baker, G.B., 1999, Drug metabolism and atypical antipsychotics. *Eur. Neuropsychopharmacol*. **9**:301-309.

Pohjalainen, T., Rinne, J.O., Nagren, K. et al., 1998, The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Mol. Psychiatry.* **3**:356-360.

Pohjalainen, T., Nagren, K., Syvalahti, E.K., Hietala, J., 1999, The dopamine D2 receptor 5'-flanking variant, -141C Ins/Del, is not associated with reduced dopamine D2 receptor density in vivo. *Pharmacogenet*. **9**:505-509.

Pope, G.H., Keck, P.E., McElroy, S.L., 1986, Frequency and presentation of neuroleptic malignant syndrome in a large psychiatric hospital. *Am. J. Psychiatry.* **143**:1227-1233.

Potkin, S.G., Basile, V.S., Jin, Y., Masellis, M., Badri, F., Keator, D., Wu, J.C., Alva, G., Carreon, D.T., Bunney, W.E., Fallon, J.H., Kennedy, J., 2003, D1 receptor alleles predict PET metabolic correlates of clinical response to clozapine. *Mol. Psychiatry.* **8**:109-113.

Rao, P.A., Pickar, D., Gejman, P.V., Ram, A., Gershon, E.S., Gelernter, J., 1994, Allelic variation in the D4 dopamine receptor (DRD4) gene does not predict response to clozapine. *Arch. Gen. Psychiatry.* **51**:912-917.

Reynolds, G.P., Zhang, Z.J., Zhang, X.B., 2002, Association of antipsychotic drug induced weight gain with a 5-HT2C receptor gene polymorphism. *Lancet.* **359**:2086-2087.

Reynolds, G.P., Zhang, Z.J., Zhang, X.B., 2002, Polymorphism of the promotor region of the serotonin 5-HT2C receptor gene and Clozapine-induced weight gain. *Am. J. Psychiatry.* **160**:677-679.

Rietschel, M., Naber, D., Oberländer, H., et al., 1996, Efficacy and side effects of clozapine: testing for association with allelic variation in the dopamine D4 receptor gene. *Neuropsychopharmacol*. **15**(5):491-496.

Rietschel, M., Naber, D., Fimmers, R., Möller, H.J., Propping, P., Nöthen, M.M., 1997, Efficacy and side effects of clozapine not associated with variation in the 5 HT2C receptor. *Neuroreport.* **8**:1999 2003.

Rietschel, M., Kennedy, J.L., Macciardi, F., Meltzer, H.Y., 1999, Application of pharmacogenetics to psychotic disorders: the first consensus conference. *Schizophrenia. Res*. **37**:191-196

Ring, H.Z., and Kroetz, D.L., 2002, Candidate gene approach for pharmacogenetic studies. *Pharmacogenomics.* **3**:47-56.

Rosenberg, N.A., Pritchard, J.K., Weber, J.L., et al., 2002, Genetic structure of human populations. *Science.* **298**:2381-2385.

Sabol, S.Z., Hu, S., Hamer, D., 1998, A functional polymorphism in the monoamine oxidase A gene promoter. *Hum. Genet*. **103**:273-279.

Sachse, C., Brockmöller, J., Bauer, S., Roots, I., 1997, Cytochrome P450 2D6 variants in a caucasian populatio: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet*. **60**: 284-295.

Sachse, C., Brockmöller, J., Bauer, S., et al., 1999, Functional significance of a C->A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. *Br. J. Clin. Pharmacol*. **47**:445-449.

Scarselli, M., Novi, F., Schallmach, E., et al., 2001, D2/D3 dopamine receptor heterodimers exhibit unique functional properties. *J. Biol. Chem*. **32**:30308-30314.

Schäfer, M., Rujescu, D., Giegling, I., et al., 2001, Association of short-term response to haloperidol treatment with a polymorphism in the dopamine D2 receptor gene. *Am. J. Psychiatry.* **158**:802-804.

Scharfetter, J., Chaudhry, H.R., Hornik, K., et al., 1999, Dopamine D3 receptor gene polymorphism and response to clozapine in schizophrenic pakistani patients. *Eur. Neuropsychopharmacol*. **10**:17-20.

Schumacher, J., Schulze, T.G., Wienker, T.F., Rietschel, M., Nöthen, M.M., 2000, Pharmacogenetics of Clozapine response. *Lancet.* **356**:506-507.

Scordo, M.G., and Spina, E., 2002, Cytochrome P450 polymorphisms and response to antipsychotic therapy. *Pharmacogenomics.* **3**(2):201-218.

Shaikh, S., Collier, D., Kerwin, R.W., et al., 1993, Dopamine D4 receptor subtypes and response to clozapine. *Lancet.* **341**:116.

Shaikh, S., Collier, D., Arranz, M., Ball, D., Gill, M., Kerwin, R., 1994, DRD2 Ser311/Cys311 polymorphism in schizophrenia. *Lance.t* **343**:1045-1046.

Shaikh, S., Collier, D.A., Sham, P., et al., 1995, Analysis of clozapine response and polymorphisms of the dopamine D4 receptor gene (DRD4) in schizophrenic patients. *Am. J. Med. Genet. (Neuropsychiat. Genet.)* **60**:541-545.

Shaikh, S., Collier, D.A., Sham, P.C., et al., 1996, Allelic association between a Ser-9-Gly polymorphism in the dopamine D3 receptor gene and schizophrenia. *Hum. Genet*. **97**:714-719.

Sivagnanasundaram, S., Morris, A.G., Gaitonde, E.J., McKenna, P., Mollon, J.D., Hunt, D.M., 2000, A cluster of single nucleotide polymorphisms in the 5'-leader of the human dopamine D3 receptor gene (DRD3) and its relationship to schizophrenia. *Neurosci. Letters.* **279**:13-16.

Sodhi, M.S., Arranz, M., Curtis, D., Ball, D.M., Sham, P., Roberts, G.W., Price, J., Collier, D.A., Kerwin, R.W., 1995, Association between clozapine response and allelic variation in the 5 HT2C receptor gene. *Neuro Report.* **7**:169-172.

Spina, E., Ancione, M., DiRosa, A.E., Meduri, M., Caputi, A.P., 1992, Polymorphic debrisoquine oxidation and acute neuroleptic-induced adverse effects. *Eur. J. Clin. Pharmacol*. **42**:347-348.

Spitzer, R.L., Williams, J.B.W., Gibbon, M., First, M.B., 1990, User's Guide for the Structured Clinical Interview for DSM-III-R. American Psychiatric Press, Inc.

Staddon, S., Arranz, M.J., Mancama, D., Mata, I., Kerwin, R.W., 2002, Clinical application of pharmacogenetics in psychiatry. *Psychopharmacology.* **162**:18-23.

Suzuki, A., Mihara, K., Kondo, T., et al., 2000, The relationship between dopamine D2 receptor polymorphism at the Taq1 A locus and therapeutic response to nemonapride, a selective dopamine antagonist, in schizophrenic patients. *Pharmacogenetics.* **10**(4):335-341.

Suzuki, A., Kondo, T., Mihara, K., et al., 2001a, The –141C Ins/Del Polymorphism in the dopamine D2 receptor gene promoter region is associated with anxiolytic and antidepressive sffects during treatment with dopamine antagonists in schizophrenic patients. *Pharmacogenet*. **11**:545-550.

Suzuki, A., Kondo, T., Mihara, K., et al., 2001b, Association between Taq1 A dopamine D2 receptor polymorphism and therapeutic response to bromperidol: a preliminary report. *Eur. Arch. Psychiatry. Clin. Neurosci*. **251**:57-59.

Suzuki, A., Kondo, T., Otani, K., Mihara, K., Yasui, Furukori, N., Sano, A., Koshiro, K., Kaneko, S., 2001c, Association of the TaqIA polymorphism of the Dopamine D2 Receptor gene with predisposition to neuroleptic malignant syndrome. *Am. J. Psychiatry.* **158**:1714-1716.

Szekeres, G., Keri, S., Juhasz, A. et al., 2004, Role of dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphism in cognitive dysfunctions and therapeutic response to atypical antipsychotics in patients with schizophrenia. *Am. J. Med. Genet (Neuropsychiatr. Genet.)* **124B**:1-5.

Theisen, F.M., Hinney, A., Bromel, T., Heinzel-Gutenbrunner, M., Martin, M., Krieg, J.C., Remschmidt, H., Hebebrand, J., 2004, Lack of association between the -759C/T polymorphism of the 5-HT2C receptor gene and clozapine-induced weight gain among German schizophrenic individuals. *Psychiatr. Genet*. **14**(3):139-142.

Theodoropoulous, S., Pappa, H., Lykouras, L., Papapeorgiou, G., Papasteriades, C., Sakalis, G., 1997, Human leukocyte antigen system in Clozapine induced agranulocytosis. *Neuropsychobiol*. **36**:5-7.

Thompson, J., Thomas, N., Singleton, A., et al., 1997, D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics.* **7**:479-484.

Tsai, S.J., Hong, C.J., Yu, W.Y., Lin, C.H., Song, H.L., Lai, H.C., Yang, K.H., 2000, Association study of a functional serotonin transporter gene polymorphism with schizophrenia, psychopathology and clozapine response. *Schiz. Res*. **44**:177-181.

Tsai, S.J., Wang, Y.C., Yu, Y.W.Y., Lin, C.H., Yang, K.H., Hong, C.J., 2001, Association analysis of polymorphism in the promoter region of the α2a-adrenoceptor gene with schizophrenia and clozapine response. *Schiz. Res*. **49**:53-58.

Tsai, S.J., Hong, C.J., Yu, W.Y., Lin, C.H., 2002, 759C/T genetic variation of 5HT2C receptor and clozapine induced weight gain. *Lancet.* **360**:1790.

Tsuang, M.T., Stone, W.S., Faraone, S.V., 2000, Towards the prevention of schizophrenia. *Biol. Psychiat*. **48**:349-356.

Turbay, D., Lieberman, J., Alper, C.A., Delgado, J.C., Corzo, D., Yunis, J.J., Yunis, E.J., 1997, Tumor necrosis factor constellation polymorphism and clozapine-induced agranulocytosis in two different ethnic groups. *Blood.* **89**:4167-4174.

Ueno, S., Otani, K., Kaneko, S., Koshiro, K., Kondoh, K., Kotani, Y., Sano, A., 1996, Cytochrome P 450 2D6 gene polymorphism is not associated with neuroleptic malignant syndrome. *Biol. Psychiatry.* **40**:72-74.

Valevski, A., Klein, T., Gazit, E., Meged, S., Stein, D., Elizur, A., Narinsky, E.R., Kutzuk, D., Weizman, A., 1998, HLA B38 and clozapine induced agranulocytosis in Israeli Jewish schizophrenic patients. *Eur. J. Immunogenetics.* **25**:11-13.

Veenstra, VanderWeele, J., Anderson, G.M., Cook, E.H., 2000, Pharmacogenetics and the serotonin system: initial studies and future directions. *Eur. J. Pharmacol*. **410**:165-181.

World Health Organization, 1992, The ICD 10 Classification of Mental and Behavioural Disorders. Clinical Descriptions and Diagnostic Guidelines. WHO, Genf.

Wright, P., Nimgaonkar, V.L., Donaldson, P.T., Murray, R.M., 2001, Schizophrenia and HLA: a review. *Schizophr. Res*. **47**:1-12.

Yamanouchi, Y., Iwata, N., Suzuki, T., Kitajima, T., Ikeda, M., Ozaki, N., 2003, Effect of DRD2, 5 HT2A, and COMT genes on antipsychotic response to risperidone. *Pharmacogenomics J.* **3**:356-361.

Yang, L., Li, T., Wiese, C., et al., 1993, No association between schizophrenia and homozygosity at the D3 dopamine receptor gene. *Am. J. Med. Genet. (Neuropsychiat. Genet.)* **48**:83-86.

Yu, W.Y., Tsai, S.J., Lin, C.H., Hsu, C.P., Yang, K.H., Hong, C.J., 1999, Serotonin 6 receptor variant (C267T) and clinical response to clozapine. *NeuroReport.* **10**:1231-1233.

Yunis, J.J., Lieberman, J., Yunis, E.J., 1992, Major histocompatibility complex associations with clozapine induced agranulozytosis. The USA Experience. *Drug Safety* **7**(Suppl. 1):7-9.

Yunis, J.J., Corzo, D., Salazar, M., Lieberman, J.A., Howard, A., Yunis, E.J., 1995, HLA association in clozapine induced agranulocytosis. *Blood.* **86**:1177-1183.

Zalsman, G., Frisch, A., Lev-Ran, S., et al., 2003 DRD4 exon III polymorphism and response to risperidone in Israeli adolescents with schizophrenia: a pilot pharmacogenetic study. *Eur. Neurophsychopharmacol*. **13**:183-185.

6. ALZHEIMER'S DISEASE AND OTHER DEMENTIAS

Lucie Maréchal, Isabelle Le Ber, Didier Hannequin, Dominique Campion and Alexis Brice

Dementias represent a major health problem because the number of patients is increasing in most countries, due to aging of the populations. Alzheimer's disease was the first to be individualized and is by far the most frequent. More recent, clinical and neuropathological studies have distinguished a second major form of dementia, frontotemporal dementia, although less frequent. Interestingly, both Alzheimer's disease and frontotemporal dementia have features in common, such as neuronal loss in a number of brain structures and the intra or extracellular accumulation of misfolded proteins which constitute markers for the diseases. Sub-groups of Alzheimer's disease and frontotemporal dementia are monogenic. Several of the responsible genes have been identified, making possible to approach the physipathology of the disease and to generate in-vivo and in-vitro models.

1. INTRODUCTION

Alzheimer disease (AD) is the most common cause of dementia. In the past decade, many advances have been made in the understanding of AD etiology. Advances in neuropathology have helped to accurately describe the lesions responsible for this disease. More recently, the identification of mutations involved in familial early onset AD (EOAD), has permitted to characterize the physiopathological cascade. In the future, it could be possible to develop new therapeutic strategies targeted against the causative alterations.

Alexis BRICE, MD, INSERM U289 and Département de Génétique, Cytogénétique et Embryologie, Hôpital Pitié-Salpêtrière, AP-HP, 47 boulevard de l'Hôpital, 75013 PARIS, France.
Lucie Maréchal, INSERM U614, Faculté de Médecine-Pharmacie, 22, boulevard Gambetta, 76000 Rouen, Département de Neurologie, Hôpital Charles Nicolle, 1 rue de Germont, 76000 Rouen, France.
Isabelle Le Ber, INSERM U289, Hôpital Pitié-Salpêtrière, AP-HP, 47 boulevard de l'Hôpital, 75013 PARIS, France.
Didier Hannequin, INSERM U614, Faculté de Médecine-Pharmacie, 22, boulevard Gambetta, 76000 Rouen, Département de Neurologie, Hôpital Charles Nicolle, 1 rue de Germont, 76000 Rouen France.
Dominique Campion, INSERM U614, Faculté de Médecine-Pharmacie, 22, boulevard Gambetta, 76000 Rouen, France.

2. CLINIC

Clinical AD is diagnosed according to the NINCDS-ADRDA criteria (McKhann 1984): *National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.* Whereas clinical findings may provide possible diagnosis, final confirmation is only obtained based on pathological results. Diagnosis of AD is often preceded by a predementia state during several years, described as mild cognitive impairment (MCI). The diagnosis of MCI is made if the patient met the following criteria: memory complaint, normal activities of daily living, normal generalcognitive function, abnormal memory for age, and not demented (Petersen 1999). Individuals with MCI develop AD at the rate of 10% to 12% per year. The initial characteristics of AD are mainly disorders of episodic memory reported by the family, but can also appear as changes in behavior, reduction in attention, word finding difficulties or time and space disorientation. In some cases, the disease is revealed by a confusion syndrome. The patient is often anosognosic at the dementia state of AD and the diagnosis is therefore carried out with the help of a family member.

Reduction of cognitive functions are assessed either by different global scales, as the Mattis Dementia Rating Scale (Schmidt) or more specific tools evaluating memory, as the Grober and Buschke test (Grober), temporal and space orientation, image identification, verbal fluency and executive functions, praxis and gnosia (knowledge of famous faces, drawings of objects) and judgment. Cognitive functions initially affected are memory (encoding, recall, storage), praxis and visuospatial functions. There are frequently word-finding difficulties and paraphasias. Repercussions of cognitive disorders are a major factor which must be assessed with a member of the family using the Instrumental Activities of Daily Living (IADL). Behavior is assessed with scales such as the Neuropsychiatric Inventory (Cummings 1994) reporting various modifications such as apathy, depression and delirious ideas of paranoia or jealousy. Additional cognitive disorders appear at a later stage and behavioral disorders then worsen, with aggressive behavior, irritability and a defiant attitude. Confusion can complicate the disease, caused by infectious, metabolic or iatrogenic factors. Behavioral assessment is a necessary step in order to select the appropriate therapeutic agent. The well known Mini-Mental Score (MMS) is necessary to describe the severity stage of the disease; its scoring is useful to determine the use of drugs such as cholinesterase inhibitors or NMDA-antagonist.

Neurological examination is frequently normal at the onset of the disease. At an advanced stage, patients are confined to bed and stiffness, myoclonic jerks or epileptic seizures may occur.

3. COMPLEMENTARY EXAMINATIONS

Cerebral CT-scan is necessary to eliminate other causes of dementia (vascular, tumoral or different), and may reveal both global and regional atrophy (initial temporal areas) but initially, the CT-scan can appear completely normal.

There is currently no blood nor sufficiently specific cephalo-spinal liquid biological marker for AD that could be used to achieve an accurate diagnosis.

4. NEUROPATHOLOGY

The two neuropathological hallmarks of AD are the presence of senile (neuritic) plaques and neurofibrillary pathology (Tolnay 2003). Senile plaques are extra cellular lesions constituted by amyloid deposits, surrounded by neurofibrillary tangles. The amyloid deposits, present in the core of the senile plaques and in the vascular walls, contain a 40 to 42 amino-acids (AA) long peptide, named Aβ, which is a fragment of the amyloid precursor protein (APP). Antibodies directed against the Aβ peptide also label diffuse deposits that are devoid of the tinctorial affinities and biochemical properties of amyloid substances. While Aβ in the β-sheet conformation is the main component of senile plaques, several other proteins are found in senile plaques such as the apolipoprotein E which could act as a chaperone protein, inducing or facilitating the formation of amyloid.

Neurofibrillary pathology includes neurofibrillary tangles and neuropil threads. Neurofibrillary lesions are characterized by the same ultrastructure, i.e. the accumulation of paired helical filaments and the same immunological characteristics: they are labeled by antibodies directed against the Tau protein. The Tau protein constitutes the principal component of neurofibrillary pathology and is bound to ubiquitin, which means that it is intended to be degraded by the proteasome.

5. DOMINANTLY INHERITED FAMILIAL ALZHEIMER DISEASE

Early-onset autosomal dominant Alzheimer's disease (EOAD) is a heterogeneous disorder that can be caused by mutations in at least three different genes. These monogenic forms of AD account for approximately 0.25% of the entire pathology (Campion 1999). Presenilin 1 (*PS1*) mutations account for the majority of monogenic forms (70%), mutations of amyloid precursor protein (*APP*) are rare (15%) while Presenilin 2 (*PS2*) are exceptional.

5.1. APP gene

The first *APP* mutation was identified in 1991 (Goate 1991). This mutation causes an amino acid (AA) substitution (Val717Ile) located near the carboxy terminus of the Aβ peptide on its precursor. The *APP* gene is located in q21 region of chromosome 21 and has several isoforms generated by alternative splicing. All of these encode multidomain proteins with a single membrane-spanning region. This gene is triplicated in trisomy 21 (Down Syndrome) a condition in which senile plaques appear very early, from the age of 20.

Missense *APP* mutations are concentrated in three sites which are APP physiological cleavage sites (see Fig. 1). Val717Ile mutation is most frequent

and was found in 23 pedigrees. Other substitutions at the same codon are Val717Phe mutation (Murrell 1991), Val717Gly mutation (Chartier-Harlin 1994) and Val717Leu mutation (Murrell 2000). Other mutations are clustered around this site including the Ile716Val mutation (Eckman 1997), the Val715Met mutation (Ancolio 1999), the Thr714Ile mutation (De Jonghe 2000), the Thr714Ala mutation (Pasalar 2002) and the Leu723Pro mutation (Kwok 2000). In two large families linked by genealogy and containing multiple cases of Alzheimer disease (Mullan 1992), found a double mutation in exon 16, the Swedish mutation. Two nucleotide transversions, G to T and A to C, were observed in affected individuals at codons 670 and 671, respectively. These changes resulted in the substitution of a lysine to an asparagine and a methionine to a leucine (Lys670Asp, Met671Leu).

Finally, five mutations located in the middle of the Aβ sequence at the vicinity of the α-secretase cleavage site have been described: Ala692Gly, Flemish mutation (Hendriks 1992), Glu693Gln, Dutch mutation (Levy 1990), Glu693Gly, Arctic mutation (Kamino 1992), Glu693Gly, Italian mutation and Asp694Asn, Iowa mutation (Grabowski 2001). Some patients bearing these mutations have classical AD whereas cerebral hemorrhages occur in others. Currently, 17 pathogenic changes of *APP* have been reported.

Mutations around codon 717 in one hand and the Swedish mutation on the other hand, correspond to the sites where APP is cleaved by γ-secretases and by β-secretases, respectively releasing the Aβ peptide from its precursor. The consequence of the Swedish mutations is a quantitative increase in the production at the same time of the short form (Aβ-40, 40 AA) and long (Aβ-42, 42 AA) of the Aβ peptide. The other changes modify the Aβ42/Aβ40 ratio, thus favoring the production of the longer form of the peptide, which is more prone to aggregation. Indeed, the last two AA play an important role in the conformation of the peptide in β-pleated sheet, which are characteristic of senile plaque deposits (Jarrett 1993). Aβ peptide in fibrillary form is toxic for neurons (Lorenzo 1994), although the exact mechanisms which act as mediators of this toxicity still remain to be defined i.e. disruption of calcium homeostasis, oxidative stress, and induction of apoptosis.

5.2. Presenilin 1 (*PS1*) gene and Presenilin 2 (*PS2*)

In 1995, two other genes were discovered, which were implicated in monogenic forms of EOAD. These genes, known as *PSl* and *PS2*, were located respectively on chromosomes 14 and 1 and present major sequence homologies (Sherrington 1995; Levy-Lahad 1995). *PS1* mutations are extremely diverse, as

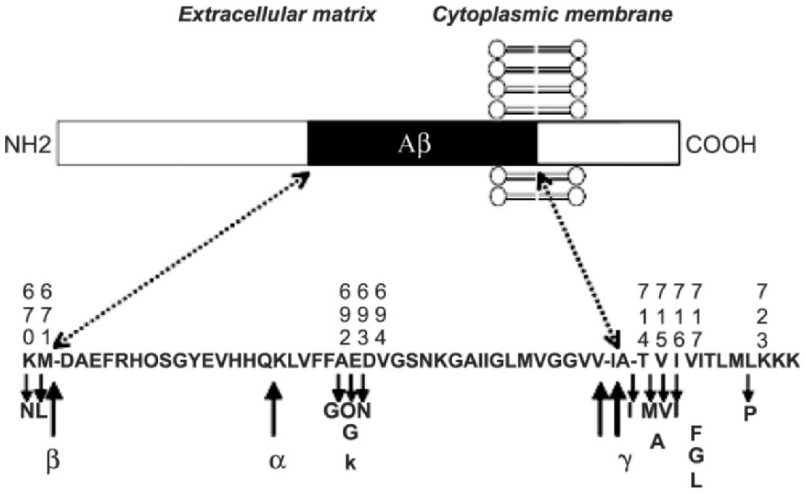


Figure 1. Transmembrane structure of APP with cleavage sites of α-, β- and γ-secretases. Sites of mutations are indicated by arrows and substituted AA is indi cated below.

opposed to *APP* (see Fig. 2). The same mutation is rarely found in both non-related families. Currently, 134 *PS1* missense mutations have been reported. It therefore appears that the structure of this protein is extremely sensitive and the slightest variation of AA composition can provoke AD.

In several families, a mutation on the splicing site produced a deletion of exon 9, conserving the reading frame and generating a missense mutation at the splice junction (*PS1* δ9 S290C). In a Finnish family, the loss of exon 9 was due to a major deletion which equally including adjacent introns (Crook 1998). In 7 families, who were thought to have a common ancestor, an intron 4 mutation produced three different transcripts, two coding for truncated *PS1* and the third for a protein with an AA insertion: T113-114Ins (De Jonghe 1999). Finally, another insertion of one AA after codon 352 in exon 10 has been described (Rogaeva 2001).

PS2 mutations are exceptional and only 9 missense mutations have been reported in the literature (Rogaeva 1995).

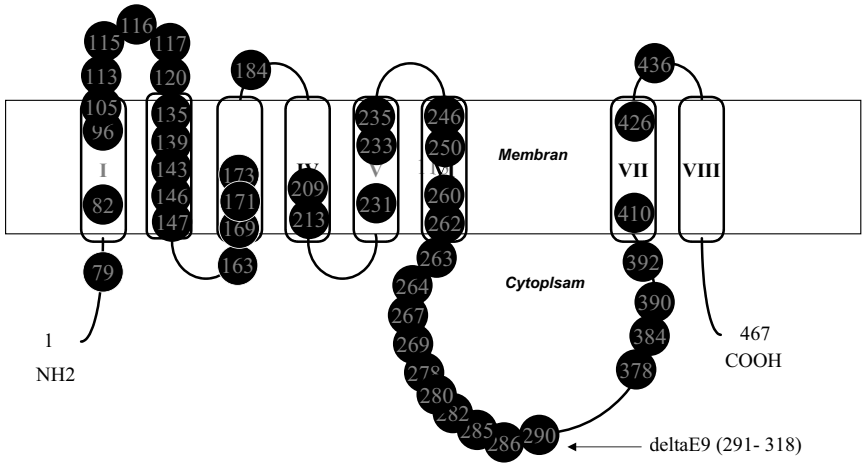


Figure 2. Presenilin 1 structure and mutations diversity.

5.3. Clinical characteristic of dominantly inherited alzheimer disease

5.3.1 Age of onset

As regards *APP* mutations, the age of onset of the illness varies between 37 years and 64 years for the same Val717Ile mutation. This onset age diversity has prompted to search for factors, primarily genetic, which may modulate disease expression. It has been suggested that *APOE* gene polymorphism could modify age of onset. In fact, a difference has been found for the same mutation and *APOE* genotype, which suggest that either genetic or environmental factors should be further investigated.

As regards *PS1*, comparison of numerous mutations confirms major diversity of onset age among mutations. Age of onset is always below age 60. Early occurrences are possible: from 24 to 33 years for Pro117Leu mutation (Wisniewski 1998); from 24 to 29 years for Leu173Trp mutation (Campion 1999). One hypothesis is that these mutations involve particular modifications of *PS1* structure, which may be particularly deleterious. The role played by biochemical properties of substituted AA is illustrated by comparing both mutations at codon 143: the average age of onset is 35 years in cases of non-conservative substitution (Ile143Thr), whereas onset age is approximately 55 years in cases with semi-conservative substitution (Ile143Phe).

However, a great intra-familial diversity has been reported in certain families with a large number of cases. Therefore, the concept of diversity according the specific type of mutation remains questionable. For example, age of onset varied from 34 to 62 years in a large family with Leu392Val mutation. Recently, it has been shown that APOE genotype may play a role to modulate age of onset in a cohort of 52 affected subjects bearing the same Glu280Ala *PS1* mutation (Pastor 2003)[31].

5.3.2 Neurological signs associated to AD

Most of the *PS1* mutations are associated with classical AD; however, some families develop AD and spastic paraparesis. The relevant mutations are a deletion of 2 AA in exon 4 (δI83/M84) (Houlden 2000), an insertion of 2 AA in exon 5 (InsFI) (Rogaeva 2001) and the complete deletion of exon 9 (δ9) (Kwok 1997; Crook 1998; Sato 1998). This phenotype was also observed with certain specific point mutations such as Phe237Ile, Val261Phe, Pro264Leu and Arg278Thr, but it is not constant. Spastic paraparesis may occur before cognitive impairment. In family Finn 2, memory impairment was preceded by walking difficulty due to spasticity of the lower extremities among 10 patients out of 14 (Verkkoniemi 2000). These forms are characterized on the hispathological ground by "cotton wool" plaques lacking congophilic dense core or marked plaque related neuritic pathology (Crook 1998).

In two families harboring either the Leu113Pro mutation (Raux 2000) or the insertion of one codon in position 352 (Rogaeva 2001), patients had personality changes and behavioral disorders, whereas spatial orientation and praxis were unchanged late in the course of the illness. This presentation was consistent with the diagnosis of fronto-temporal dementia. Recently a novel *PS1* missense mutation associated with Pick's disease but not senile plaques has been described (Dermaut 2004).

6. BIOLOGICAL CONSEQUENCES

6.1. Amyloid cascade hypothesis: *APP* mutations

There are two pathways of APP maturation. The first one consists of APP cleavage by the α-secretase enzyme in the middle of Aβ peptide. This pathway which produces a soluble APPα fragment is non amyloidogenic because it avoids the Aβ peptide production.

The second maturation pathway which consists in the sequential cleavage of APP by β- and γ-secretases is amyloidogenic. It produces the amyloid-peptide, a soluble APP-β fragment and also releases an intracellular fragment (AICD) which is translocated in the nucleus where it has a role of transcriptional activator (Cao 2004). Aβ peptide has an unknown exact function and as long as this function is not known, it will be difficult to consider therapeutic blocking the Aβ peptide production which could be noxious.

APP mutations were localized on the 3 *APP* cleavage sites, by α-, β- and γ-secretases. All these mutations have the same biological consequence: overproduction of Aβ peptide, by blocking α-secretase in one case, or by increasing β- and γ-secretase activities in other cases. To specify the mutation characterization, animal models were assessed, which overproduced Aβ peptide.

6.2. *APP* transgenic mice

Transgenic mice expressing normal or mutant human *APP* were evaluated (Calhoun 1998). Mice have an *APP* homolog gene, containing the Aβ peptide sequence but this sequence is slightly different and no senile plaque was observed in mice, probably because aggregation capacity was less significant.

Brains of transgenic mice expressing human mutant *APP* exhibit typical pathologic features of AD, including numerous extracellular Aβ deposits, neuritic plaques, synaptic loss, astrocytosis, and abnormal phosphorylated Tau protein. However, it should be stressed that neurofibrillary tangles were never observed. Neurofibrillary tangles can be formed in transgenic mice; they have indeed been observed in transgenic mice with *Tau* mutations, whereas this gene is never mutated in AD (Lewis 2001).

APP transgenic mice are thus suffering of cognitive deterioration and neuronal loss without neurofibrillary tangles whereas in human, cognitive deterioration is correlated with neurofibrillary lesions. Observation of these transgenic mice raises another problem: cognitive deterioration appears before senile plaques. Together, these observations show that cognitive deterioration related to Aβ toxicity can occur very early, before neurofibrillary tangles and senile plaques, suggesting that these lesions would be a late event in AD evolution and emphasize the pathogenic role of soluble, incompletely aggregated forms of Aβ, so called “protofibrills.”

6.3. Presenilin mutations

Immunochemical analyses indicated that *PS1* and *PS2* localize to similar intracellular compartments, such as the endoplasmic reticulum and Golgi complex, where *APP* undergoes biochemical maturation.

Consequences of *Presenilin* mutations are the same than that of *APP* mutations i.e. an overproduction of Aβ peptide and particularly of the Aβ42 form. Cell lines transfected with mutant *PS1* produce a more significant increase in Aβ42 than cells transfected with wild type *PS1* (Murayama 1999). Transgenic mice overexpressing mutant *PS1*, but not wild type *PS1*, show a selective increase in brain Aβ42 (Duff 1996). Thus, mutations of three different genes have the same biological consequence and this observation strongly support the conclusion that progressive cerebral deposition of amyloid β protein is a seminal event in familial AD pathogenesis.

Asides from APP, Presenilin interact with several proteins, including the transmembrane protein Notch. Signaling through the receptor Notch, which is involved in crucial cell fate decisions during development, requires ligand-induced cleavage of Notch. This cleavage occurs within the predicted

transmembrane domain, releasing the Notch intracellular domain (NICD), and is reminiscent of the γ-secretase-mediated cleavage of APP. Indeed, deficiency of presenilin-1 inhibits processing of APP by γ-secretase in mammalian cells, and genetic interactions between Notch and PS1 homologs in C. elegans indicate that the presenilins may modulate the Notch signaling pathway. De Strooper et al. (1999) reported that in mammalian cells PS1 deficiency also reduces the proteolytic release of NICD from a truncated Notch construct, thus identifying the specific biochemical step of the Notch signaling pathway that is affected by PS1.

Moreover, several γ-secretase inhibitors block this same step in Notch processing, indicating that related protease activities are responsible for cleavage within the predicted transmembrane domains of Notch and APP. Presenilins are now known to be members of a large multimolecular complex (also including Nicastrin, APH1 and PEN2) which is responsible for the γ- secreatase activity (Mattson 2003). Targeting of γ-secretase activity for the treatment of Alzheimer disease may risk toxicity caused by reduced Notch signaling. Ye et al. (1999) described loss-of-function mutations in the Drosophila *PS1* gene that caused lethal Notch-like phenotypes such as maternal neurogenic effects during embryogenesis, loss of lateral inhibition within proneural cell clusters, and absence of wing margin formation. They showed that PS1 is required for the normal proteolytic production of carboxy-terminal Notch fragments that are needed for receptor maturation and signaling, and that genetically it acts upstream of both the membrane-bound form and the activated nuclear form of Notch. Thus, to interfere with the Notch signaling could be very deleterious but there is now an inhibitor of γ-secretase able to alterate only APP signaling (Netzer 2003).

7. GENETIC RISK FACTOR: APOE

In the central nervous system Apolipoprotein E (APOE) is primarily synthesized by astrocytes. APOE assures the transport role of different lipoproteins and binds to neurons via LDL receptors (LDL, VLDL, LRP, and gp330) (Poirier 1994),. APOE is present in three isoforms APOE2, APOE3 and APOE4, with respective frequencies of 7%, 78% and 15% in a European or American caucasian population (Strittmatter 1995). These isoforms are encoded by three alleles ε2, ε3, ε4 of the *APOE* gene localized in the q13.2 region of chromosome 19. While the APOE3 isoform has a cystein to codon 112 and an arginine to codon 158, the APOE4 contains an arginine in 112 and APOE2 a cysteine in 158. An increase of ε4 allele frequency was initially reported in groups of old subjects with familial cases of AD. This association was further confirmed in groups with late and early sporadic AD (Chartier-Harlin). Numerous studies have now reported this association between AD and the ε4 allele. In a meta-analysis study including 6262 subjects and 5107 AD patients of caucasian origin, respective percentages of different genotypes were 61% versus 36% of ε3/ε3, 21% vs 41% of ε3/ε4, 1% vs 15% of ε4/ε4, and 17% vs 8% of

ε2/ε*[50]. The genotype relative risk is calculated by taking ε3/ε3 as a genotype reference (odds ratio (O.R.) = 1). This risk is multiplied by 2.7 (2.2-3.2) for ε3/ε4 genotype, by 12.5 (8.8 - 17.7) for ε4/ε4 genotype, and 0.6 for ε2/ε3[50] (Farrer 1997) Therefore, a genetic dosage effect exists with a greater risk occurring in homozygotes ε4/ε4 as compared to ε3/ε4. The ε2/ε3 genotype has a protective effect as the risk is lower compared to genotype ε3/ε3. The imputable risk of allele ε4 remains present whatever the age range but it is most significant between 60 and 69 years [O.R.= 4.1(2.3-7.5)] than before 59 years [O.R.= 1.9 (.96-3.7)] and over 80 years [O.R.=1.7 (.9-3.4)] (Bickeboller 1997)**.** In cases of genotype ε3/ε4, the risk of AD is higher for women; the adjustment for age range eliminates all bias due to their greater longevity (Farrer 1997)[50].

This gender effect therefore suggests the intervention of another factor. The importance of the risk associated with the allele ε4 and the frequency of this allele in the general population raises the question of the involvement of the APOE genotype in familial, non autosomal dominant, aggregation. The cumulated risk of developing AD at the age of 90 years was estimated in the first degree relatives of a proband, according to the propositus genotype. These risks were respectively 30% (±11), 46% (±13) and 61% (±16) in relatives of a ε3/ε3, ε3/ε4, or ε4/ε4 propositus (Martinez 1998). These estimates were comparable with those reported by Farrer et al. (1995). These figures should be compared to predictive percentages of carriers who have at least one ε4 allele in family member, which are on average 14 %, 58 % et 90% depending on ε3/ε3, ε3/ε4, and ε4/ε4 genotype of propositus. The first conclusion is that the allele ε4 is a major risk factor, which may explain significant family aggregation in relatives of a propositus bearing at least one ε4 allele. The presence of several cases in the same family may therefore result from the effects of this sole genetic risk factor, particularly in relatives old and ε4/ε4 proband. The second conclusion is that AD aggregation (30%) observed in the propositus family with a genotype ε3/ε3, may be due in part to another risk factor as allele ε4, which only would explain approximately 50% of observed cases. Finally, allele ε4 is only a risk factor as certain allele ε4 carriers will not develop the disease even beyond 90 years of age. Similarly, approximately 30% of family related women and 60% family related men, carriers of one or two ε4 alleles would not experience AD if they reach that age. Nevertheless, the previously mentioned data may be under estimated as ε4 allele is a risk factor for myocardial infarction. A large number of deaths due to cardiovascular disease have been observed in relatives, primarily men, of AD patients who are ε4 carriers (Li 1996).

The direct role played by APOE on peptide Aβ deposits has been demonstrated by using transgenic mice, which not only overexpress mutated *APP* but are knock out for the mouse *APOE* gene. The number of amyloid deposit is very low in *APOE* knock out mice, intermediary in hemizygotes and increased in those mice which express mutated *APP* in a normal APOE background (Bales 1997). In transgenic mice expressing the human *APOE* gene, an APOE isoform dependent amyloid deposition and neuritic degeneration has been reported.

In addition, APOE may also intervene in cholesterol metabolism and reduction of oxydative stress induced by the Aβ peptide on neurons. APOE 4 has been shown to have a reduced anti-oxydative effect as compared to the other two isoforms[56].

8. GENES AND THERAPY

Cholinesterase inhibitors are established for the treatment of mild-to-moderate Alzheimer's disease. Memantine is the first drug to demonstrate a clinical benefit in the treatment of patients with moderately-severe to severe AD. Acetylcholinesterase inhibitors had comparable efficacy as well as similar significant side effects. Some patients are very receptive to anticholinesterasic drugs whereas other patients are only slightly or not at all. Predictive factors of positive response to anticholinesterasic drugs have been poorly reported. Nevertheless, Poirier et al. (1995) have reported the influence of APOE4 on Tacrine effects. Allele ε4 was considered to be predictive of less satisfactory response to treatment as compared to allele ε3 or ε2. Cacabelos (2002)[58] reported a similar effect with galantamine. However, this effect remains debatable since not reported by others (Farlow 1999; Aerssens 2001) but the understanding of functional genomics in AD will foster productive pharmacogenomic studies in the search for effective medications and preventive strategies in AD.

8.1. New therapeutic strategies

Enormous effort is devoted to develop drugs that slow neurodegeneration in Alzheimer's disease, although insights into AD genetics and molecular pathogenesis only arose in the last 15 years. The existence of pathogenic mutations in *APP* and the *presenilin* genes provides strong support for the hypothesis that Aβ-production and deposition contribute to the etiology of AD. A variety of approaches are being tried to interrupt the disease process, including reducing the production of the Abeta peptide, inhibiting its aggregation, and promoting its removal, for example via immunotherapy. γ-Secretase activity is involved in the generation of Aβ and therefore likely contributes to the pathology of AD. Blocking this activity would have been a major therapeutic target to slow down or arrest Aβ-related AD progression. Drugs that modulate the production of Aβ by inhibiting γ-secretase could provide an effective therapy for AD, but like most disease targets, the γ-secretase appear to have more than a single function. The discovery of drugs that could selectively inhibit β-APP cleavage is an important objective. Several inhibitors seem to be able to prevent Aβ production without triggering unwanted cleavages of other proteins (Netzer 2003). The efficacy of these inhibitors in reducing Aβ without affecting Notch cleavage may prove useful as a basis for developing novel therapies for Alzheimer's disease. Little is known about exchange of the β-peptide between the brain and blood. Increased understanding

of this process in experimental animal models and humans, and how it changes with aging, will likely open new therapeutic approaches.

Finally, discovery of abnormally phosphorylated tau protein in neurofibrillary tangles in AD brain has led to strategies for identifying selective inhibitors of tau kinases and central nervous system/brain-permeable drugs that help maintain microtubule integrity.

All the future knowledge of physiopathological neurodegeneration will help to develop neuroprotective strategies.

FRONTOTEMPORAL DEMENTIAS

During the past decade, neurologists and psychiatrists have become increasingly aware that a significant proportion the degenerative dementias are of the "non-Alzheimer" type. The frontotemporal dementias, characterized by progressive personality changes and language impairment related to a frontotemporal atrophy, account for approximately 5-20% of these diseases (Jackson 1996; Sleegers 2004). The first pathological description of a particular form of frontotemporal dementia (FTD) was made by Arnold Pick in the early 20th century, but the first clearly indexed cases of patients with FTD were reported in 1987 by Gustafson in Lund-Sweden (Gustafson 1987), and in 1988 by Neary et al. in Manchester-United Kingdom (Neary 1988). The clinical and pathological nosology was further clarified in 1994 and the term frontotemporal dementia adopted during a consensus conference bringing both the teams of Lund and Manchester (The Lunds and Manchester groups 1994). The frontotemporal dementias in fact included a wide range of distinct entities, which are now divided into subgroups, according to their genetic and pathological characteristics (D.M.A. 2000). The term *Pick disease*, sometimes used as a clinical term for patients presenting progressive frontal syndrome with frontotemporal lobar atrophy, should now be restricted to a minority of pathologically proven cases with specific Pick bodies.

8.1.1. Epidemiology

The prevalence of FTD is age-dependant and varies from 3.6 per 100 000 at age 50-59, and 9.4 at age 60-69 (Rosso 2003; Bird 2003). A positive family history is noted in 33 to 56% of patients (Stevens 1998; Morris 2001; Hodges 2003). Approximately 25% of the probands presenting a family history compatible with an AD mode of inheritance have a mutation in the tau gene (Dumanchin 1998; Hutton 1998; Poorkaj 1998).

8.1.2. Diagnosis

FTD are characterised by prominent personality changes (apathy, agitation, aggression, disinhibition, depression, inappropriate affect), impaired reasoning and insight, lack of thematic understanding and difficulty planning, in the absence of ideomotor apraxia or agnosia. Most of cases begin between the age

of 45 and 65, with a mean age at onset at 58.0 years (Rosso 2003). The phenotypes and age at onset may vary, however, in patients with a microtubule associated protein tau (*MAPT*) gene mutation, also referred to as FTDP-17, according to the type of mutation (Van Swieten 2004)[76]. Indeed, a subset of patients with the P301L and R406W develop the disease after 60 (Van Swieten 1999), whereas those with the P301S mutation have earlier age at onset, around age 35 (Bugiani 1999; Yasuda 2000; Lossos 2003; Werber 2003). The clinical diagnosis of FTD, based on the criteria established by the Lund and Manchester group, later revised by Neary, includes: (i) progressive behavioural disorder with insidious onset; (ii) affective symptoms; (iii) preserved orientation and praxis; (iv) selective frontotemporal atrophy on brain imaging or frontotemporal hypoperfusion on single photon emission computed tomography (SPECT) (The Lund and Manchester groups 1994; Neary 1998).

Clinical presentation depends on whether the frontal or the temporal cortex is frist affected (Perry 2000; Hodges 2001). The frontal - or behavioural-variant is characterized by progressive changes in personality and social cognition, with disinhibition, loss of empathy, changes in eating patterns, stereotyped behaviours, apathy. The temporal variant – or semantic dementia- is characterized by the deficits in language and semantic knowledge. Physical manifestations are limited to frontal signs as grasping, sucking and rooting reflexes, and the occasional late development of parkinsonism. Motor neuron disease (MND) is associated with frontotemporal dementia in approximately 15% of patients. Mean survival after onset is approximately 6.0 to 10.4 years (Hodges 2003; Pasquier 2004).

Brain morphology visualized by MRI or CT can be normal at onset. As the disease progresses, bilateral atrophy of the frontal lobes, sometimes asymmetrical, and of the anterior region of the temporal lobes becomes visible. Atrophy of the hippocampal area can also be associated with frontotemporal atrophy (Frisoni 1996). SPECT studies show hypoperfusion in the frontal lobe and anterior region of the temporal lobes, often before atrophy can be visualized by CT or MRI (Miller 1991).

8.1.3. Neuropathology

The definite diagnosis of frontotemporal dementia is made by neuropathological examination. Circumscribed focal atrophy may be seen in the frontal and/or temporal lobes, and may be accompanied by ventricular dilatation. Although microscopic pathological changes are variable, neuronal loss, gliosis and diffuse spongiosis in superficial layers of the cortex are lesions observed in

all forms of FTD. The substantia nigra, hippocampus, and others subcortical structures show neuronal loss. Immunochemistry has greatly facilitated the diagnosis of these conditions, with the identification of disease specific abnormalities, including Pick bodies, tau inclusions or motor neuron disease-type ubiquitin positive inclusions. A classification of frontotemporal dementias has been proposed based on to the pathological hallmarks (Morris 2001; Munoz 2003). However, the lesions are heterogeneous not only among the different entities, but also among families with the same mutation, and even in the same brain.

DFT with tauopathy is characterized by frontotemporal neuronal loss, gliosis, and the presence of tau-positive inclusions in neurons and glial cells. They account for approximately 30-40% of the DFT (D.M.A. 2000), among which 50% have FTDP-17 with tau mutations. The neuropathological characteristics of patients with tau mutations are variable, although all cases reported to date had filamentous pathology made of hyperphosphorylated tau protein in neurons and glial cells (Götz 2001; Rosso 2002). The morphology of tau filaments and the tau isoform involved is determined by whether the mutation affects or not the splicing of exon 10, but most of the mutations cause aggregation predominantly of insoluble four repeats (4R) tau proteins (Bué 1999). Pick disease, another form of FTD, is characterized neuropathologically by the presence of ballooned neurons (Pick cells) containing granulofilamentous material in cortical layers of the most severely affected areas. Pick cells are immunostained with antibodies against alpha-B crystalline and phosphorylated neurofilaments, but only inconsistently with tau and ubiquitin antibodies. In addition, intraneuronal argyrophilic tau-positive round inclusions, called Pick's bodies, that constitute the pathological hallmark of the disease, are found essentially in the hippocampus, dentate fascia, amygdala and ventral temporal lobe. They are composed of tau- and ubiquitin-positive straight and twisted filaments, that do not contain alpha-synuclein reactivity (Dickson 2001). The insoluble tau proteins contains only the 3R isoform in most cases (Bué 1999; Taniguchi 2004), or more rarely a mixture of 3R and 4R tau (Taniguchi 2004).

The major form of FTD without pathologically confirmed tauopathy includes dementia with motor neuron disease (MND)-type type inclusions, progressive subcortical gliosis and dementia lacking distinctive histology. Dementia with MND-type inclusions is characterized by ubiquitinated neurites in neocortex and neuronal inclusions in neocortex and dentate gyrus, containing intermediate filaments. The inclusions are ubiquitin-positive but tau-negative (Wightman 1992). Dementia lacking distinctive histologic features (DLDH) is characterised by neuronal loss and gliosis only; there are no tau- or ubiquitin-positive neuronal inclusions and no senile plaques (Knopman 1990). Zhukareva

et al. (2001) reported a particular form of DLDH characterized by the absence of native tau protein (Zhukareva 2001). Progressive subcortical gliosis is characterized by frontotemporal atrophy and fibrillary astrogliosis in superficial and deep cerebral cortical layers, and in the white matter immediately subcortical. Immunoreactive tau is either absent (Lanska 1994), or may be present in both neurons and glial cells in rare families with tau mutations (Goedert 1999).

Dementia lacking distinctive histologic features is the most common neuropathological form of FTD in a recent Japanese neuropathological study of 55 autopsied cases. The relative frequencies are 42% for DLDH, 18% for FTD with ubiquitin inclusions, 15% for Pick disease and 11% for FTDP-17 with identified tau mutation and inclusions (Taniguchi 2004).

8.1.4. Genetic of familial FTD

The MAPT gene and tau protein

Many cases of FTD are sporadic, but some pedigrees strongly support the existence of an autosomal dominant entity with high penetrance. Mutations in the MAPT gene (microtubule associated protein tau) have been identified in approximately 25% of the probands with family histories compatible with an autosomal dominant mode of inheritance (Heutink 1997; Dumanchin 1998; Hutton 1998; Poorkaj 1998). The reported frequency of MAPT gene mutations in familial forms of FTD varies among different populations and studies, ranging from 10% to 50% (Houlden 1999; Morris 2001; Rizzu 1999). The MAPT gene on chromosome 17 (17q21-22) encodes the tau protein, which is widely expressed in adult human tissues including central nervous system. In neurons, tau is mainly present in axons, where it binds to microtubule and promotes their assembly and stabilizes the cytoskeleton. Tau is a major component of neurofibrillary tangles, a pathological characteristic of Alzheimer disease. In addition, the accumulation of filamentous deposits of hyperphosphorylated tau in various brain areas is a hallmark of several neurodegenerative diseases including frontotemporal dementia linked to chromosome 17 (FTDP-17), and other tauopathies such as Pick disease, progressive supranuclear palsy and corticobasal degeneration (Bué 1999).

Six major tau isoforms, resulting from alternative splicing of exons 2, 3 or 10, are expressed in the adult human brain. Exons 2 and 3 encode for a N-terminal insertion of 29 or 58 amino acids respectively, which may play a role in the spacing of microtubules. Alternative splicing of exon 2, or 2+3, results in the absence (0N) or the presence of one (1N) or two (2N) 29 amino-acid domains. Exons 9 to 13 encode the C-terminal region of the protein, containing 3 or 4 imperfectly repeated domains involved in interaction and binding to microtubules, one of which being encoded by exon 10 (E10). Alternative splicing of E10 results in two distinct isoforms containing either 3 (3R, exon 10) or 4 (4R, exon 10+) repeated domains. Splicing of E10 is regulated by at least 8 cis-acting regulatory elements affecting the efficiency of normally weak 5' and 3' splice sites (D'Souza 2000 and 2002). The 5'splice sites regulatory sequences enhance (exon splicing enhancer, ESE) or inhibit (exon splicing silencer, ESS)

the use of the E10 5' splice site (Lee 2001). The binding affinities of the 3R and 4R isoforms for tubulin differ. The 4R isoform binds tubulin with a 3-fold higher affinity and assembles microtubules more efficiently than the 3R isoform (Goeder 1990; Goode 2000). In contrast to adult human brain, in which all six isoforms are found, with slight preponderance of 3R over 4R forms (Goedert 1990), fetal human brain expresses only the 3R isoform. This may explain the greater stability of the cytoskeleton in adult compared to fetal neurons in development.

In addition to E10 alternative splicing, tau is also affected by posttranslational modifications, including glycosylation, glycation and ubiquitination. The major post-translational modification, however, is phosphorylation of the tau protein at at least 25 different sites. Phosphorylation of tau affects its potential to form aggregates, in a positive or a negative manner, depending on the site of phosphorylation (Yen 1999). Hyperphosphorylation of tau at physiological or additional sites, alter its ability to bind to microtubules, increasing the pool of soluble tau that may promotes tau filament assembly.

8.1.4a. MAPT mutations

Thirty-five mutations, including 21 missense mutations, 8 intronic mutations found in the 5' splice donor site of exon 10, 3 silent mutations and two 3-basepair deletion in positions 280 and 296, have been identified so far in more than 100 families. The mutations are listed in Table 1. Most are clustered in exons 9 to 13 that encode the microtubule-binding domain, or in flanking regions, except for two mutations in exon 1. The P301L and E10+16 are the most frequent mutations (Rosso 2002). Ancestral founder events have been identified for the E10+16 mutation in British, Australian and North American families (Pickering-Brown 2004) and for the N279K in Japanese families (Tsuboi 2002). Phenotypic heterogeneity is observed in families with *MAPT* gene mutations. Some mutations give rise to variable phenotypes and/or pathologic hallmarks in classical FTD, Pick disease (K257T, G272V, S305N, S320F, Q336R, G389R, K369I) (Spillantini 1998; Ghetti 2000; Murrell 1999; Pickering-Brown 2000 and 2004; Rizzini 2000; Neumann 2001; Rosso 2002; Kobayashi 2004), pallido-ponto-nigral degeneration (N279K, V337M) (Tsuboi 2002; Clark 1998), progressive subcortical gliosis (E10+16) (Petersen 1995; Goedert 1999), progressive supranuclear palsy (R5L, N279K, S305S, ΔN296, E10+16) (Poorkaj 2002; Delisle 1999; Solvieri 2003; Stanford 2000; Wszolek 2001; Pastor 2001; Morris 2003), corticobasal degeneration (N296N, P301S) (Spillantini 2000; Bugiani 1999), dementia with epilepsy (P301S) (Rosso 2003), and tauopathy with respiratory failure (S352L) (Nicholl 2003).

Table 1. Mutations of the MAPT gene and their consequences on the expression and function of the tau protein.

Mutations	Exon	Tau isoforms	Pathologic alteration
R5L	1	-	↓ MT assembly, ↑ FF
R5H	1	-	↓ MT assembly, ↑ FF
K257T	9	-	↓ MT assembly, ↑ FF
I260V	9	-	↓ MT assembly, ↑ FF
L266V	9	-	↓ MT assembly, ↑ FF
G272V	9	Normal ratio	↓ MT assembly, ↑ FF
N279K	10	Increase 4R	E10 splicing
ΔK280	10	Increase 3R	E10 splicing, ↓ MT assembly, ↑ FF
L284L	10	Increase 4R	E10 splicing
ΔN296	10	Increase 4R	E10 splicing, ↓ MT assembly, ↑ FF
N296H	10	Increase 4R	E10 splicing, ↓ MT assembly, ↑ FF
N296N	10	Increase 4R	E10 splicing
P301L	10	-	↓ MT assembly, ↑ FF
P301S	10	-	↓ MT assembly, ↑ FF
S305N	10	Increase 4R	E10 splicing
S305S	10	Increase 4R	E10 splicing
IVS10+3	Intron 10	Increase 4R	E10 splicing
IVS10+11	Intron 10	Increase 4R	E10 splicing
IVS10+12	Intron 10	Increase 4R	E10 splicing
IVS10+13	Intron 10	Increase 4R	E10 splicing
IVS10+14	Intron 10	Increase 4R	E10 splicing
IVS10+16	Intron 10	Increase 4R	E10 splicing
IVS10+19	Intron 10	Increase 3R	E10 splicing
IVS10+29	Intron 10	Increase 3R	E10 splicing
L315R	11	Normal ratio	↓ MT assembly, No effect on FF
S320F	11	-	↓ MT assembly,
Q336R	12	-	↑ MT assembly, ↑ FF
V337M	12	Normal ratio	↓ MT assembly[1], ↑ FF
E342V	12	Increased 4R	Not available
S352L	12	-	↓ MT assembly, ↑ FF
K369I	12	-	↓ MT assembly, ↑ FF
G389R	13	Normal ratio	↓ MT assembly
R406W	13	Normal ratio	↓ MT assembly

MT: microtubule, FF: filament formation

8.1.4b. Effects of MAPT mutations on tau fonction

The electrophoretic tau profiles and functional consequences of tau mutations vary according to the type and location of the mutations in the protein (Hong 1998). Missense mutations in constitutively expressed exons affect all six major isoforms and result in neurofibrillary tangles similar to those present in Alzheimer disease (Goedert 1992). These mutations are associated with predominantly neuronal tau pathology. Conversely, mutations that affect the alternatively spliced E10, or its 5' splice regulatory region alter the ratio of the tau isoforms constituting tangles, resulting in filamentous inclusions resembling those seen in other tauopathies such as progressive supranuclear palsy, corticobasal degeneration and Pick disease. These mutations are associated with both glial and neuronal tau pathology.

Most of the missense mutations identified so far (K257T, I260V, L266V, G272V, N279K, L284L, N296N, N296H, P301L, P301S, S305N, S305S, V337M, E342V, S352L, K369I, G389R, R406W) and two deletions (ΔK280, ΔN296) are localized in the microtubule-binding domain. All intronic mutations and most of the mutations located in exon 10 affect alternative splicing of the exon 10 by altering cis-acting regulatory sequences, changing the ration of 4R to 3R isoform (Hasegawa 1998). Most of these mutations increase E10 containing mRNA, leading to a preponderance of the 4R isoform. The 5' splicing site is predicted to form a stem-loop RNA structure, that might regulate the E10 splicing by partially blocking access by the splicing machinery to the splice site. Intronic mutations might destabilize this critical stem-loop, facilitating access to and the binding of splicing factors to the splice site, increasing inclusion of exon 10. Three mutations (E10+19, E10+29, ΔK280), however, increase the 3R isoform by disrupting an intron silencer modulator (E10+19) or a splicing enhancer near the 3' splice site (ΔK280) with consequent exclusion of E10.

Additionally, most missense mutations lead to a partial loss of function by altering the ability of tau protein to bind to tubulin and to promote microtubule assembly, and increasing its ability to aggregate (Hasegawa 1998). Most of the missense mutations are located in the microtubule binding domain and are hypothesized to alter tau-microtubule interactions. *In vitro* studies using recombinant 4R tau have confirmed that the P301L, P301S, V337M, R406W and ΔK280 mutations significantly reduce the affinity of tau for microtubules[133]. G272V, ΔK280, P301L, V337M and R406W mutations reduce the ability of 3R and 4R to polymerize tubulin. Reduced microtubule binding increase the pool of unbound tau which is consequently available for pathological aggregation in neurons, consistent with a toxic gain of function. *In vitro* aggregation studies with recombinant wild-type and mutant 4R tau (P301L, V337M, R406W) incubated with arachidonic acid or heparin, demonstrated that the missense mutations alter the ability of tau to interact with itself, accelerate tau polymerization and increase the tendency of tau to polymerize into insoluble filaments (Barghorn 2000; Götz 2001).

Animal models have been generated with overexpressed normal tau protein or with a number of different human tau mutations (P301L, P301S, G272V,

V337M, R406W) to study its biology, neurofibrillary tangles formation and their relationship with neuronal loss (Trojanowski 1999; Götz 2001). None exactly replicate, however, the neuropathological pattern seen in human tauopathies. Transgenic mice with the missense P301L mutation, expressing 4R tau isoforms have motor and behavioural phenotype and an age- and gene dose-dependant accumulation of neurofibrillary tangles (Lewis 2000; Götz 2001). The distribution of abnormal tau deposits in neurons, but also in oligodendrocytes and astrocytes, mimicks human tauopathies with E10 and intron 10 mutations in human (Lin 2003). *MAPT* knock-out mice are clinically normal, but embryonic hippocampal cultures from these animals show significant delays in neuronal maturation (Dawson 2001).

8.1.4c. Other monogenic forms of FTD

The genetic basis of familial FTD has not yet been elucidated in the 3/4 of cases not caused by mutations in the *MAPT* gene. Several families with FTD, or FTD with amyotrophic lateral sclerosis are linked to the *MAPT* locus on chromosome 17 but no mutations have been found in the coding sequence of gene, suggesting variations in non-coding regions or in another that maps close to *MAPT* (Wilhelmsen 2004). In a large family, a second locus has been identified in a 13cM region on chromosome 3, but the responsible gene has not yet been identified (Brown 1995; Gydesen 2002).

8.1.4d. Genetic risk factors

Non-monogenic FTD has been less well studied. Several groups have examined the potential association of FTD with the apolipoprotein E gene, but the results have been contradictory. Initially, Gustafson et al. and Stevens et al. reported a higher frequency of E4 alleles and E4E4 genotypes s in patients with FTD than in controls (Gustafson 1997; Stevens 1997). Other studies have indicated that the E2 allele might represent a risk factor for FTD. A recent study confirmed that the E2E2 genotype was overrepresentated in a large group of 94 FTD patients and 392 controls, suggesting that patients homozygous for allele E2 are at increased risk for developing FTD (Verpillat 2002). The extended tau H1 haplotype, that covers the entire human tau gene, and the H1H1 genotype were significantly overrepresented in patients with FTD compared with controls with an odds ratio at 1.95, confirming the primary role of tau in FTD (Verpillat 2002). The genotype QQ for the Q7R polymorphism in the saitohin gene, a novel determinant of the H1 haplotype, was also associated with FTD.

8.1.5. Therapy

No effective therapy for FTD is available at present. Several neurotransmitter systems are altered in the frontal cortex of patients with FTD, and a conservative approach to restore deficient neurotransmission with pharmacologic agents has been proposed. The principal neurotransmitters involved in frontal lobe function are serotonin, catecholamines and acetylcholine. Since cholinergic neurons are not affected in FTD patients, anticholinesterases are not indicated. Decreased serotonin receptor binding has been reported, however, in frontal and temporal cortex of FTD patients, as well as decreased serotonin levels in CSF. The effect of serotoninergic re-uptake inhibitors has, therefore, been evaluated. Fluoxetine, sertraline, and paroxetin

are reported to reduce signs such as disinhibition, depression, hyperorality and compulsive behaviours (Swartz 1997). Decreased CSF dopamine levels and reduced binding of dopaminergic receptor ligands in the superior frontal cortex have also been described in FTD patients (Frisoni 1994). Evaluations of dopamine agonists have shown that bromocriptine might improves some frontal functions, such as performances of executive functions and perseveration (Imamura 1998). The effect of dopaminergic therapy still need further study (Litvan 2001).

Another potential approach, is target the consequences of tau dysfunction or aggregation (Trojanowski 1999). Phosphorylation of tau appears to be critical for its toxicity. Tau phosphorylation, which requires the intervention of several successive kinases in a temporally ordered sequence, has partially identified. It has been shown that the drosophila PAR-1 kinase initiate tau toxicity by phosphorylating tau at S262 and S356. The phosphorylation of S262 and S356 is a prerequisite for the action of downstream kinases (GSK-3, Cdk5) that phosphorylate other sites and generate disease associated phospho-epitopes (Nishimura 2004). Disrupting the phosphorylation process by inactivating the PAR-1 kinase might be expected to reduce tau toxicity. PAR-1 and the other tau kinases may therefore be interesting targets for future therapeutic intervention in tauopathies.

9. ACKNOWLEDGMENTS

The authors wish to thank Richard Medeiros, Rouen University Hospital Medical Editor, for his valuable help in editing the manuscript.
This work was partly supported by the GIS-Maladies Rares grant (INSERM A03081DS/APS03002DSA), (A.B).

10. REFERENCES

Aerssens, J., Raeymaekers, P., Lilienfeld, S., Geerts, H., Konings, F., Parys, W., 2001, APOE genotype: no influence on galantamine treatment efficacy nor on rate of decline in Alzheimer's disease, *Dement Geriatr Cogn Disord.* 12(2): 69-77.

Ancolio, K., Dumanchin, C., Barelli, H., Warter, J.M., Brice, A., Campion, D., Frebourg, T., Checler, F., 1999, Unusual phenotypic alteration of beta amyloid precursor protein (beta-APP) maturation by a new val715-to-met beta-APP-770 mutation responsible for probable early-onset Alzheimer's disease, *Proc Nat Acad Sci* **96**: 4119-4124.

Bales, KR., Verina, T., Dodel, RC., Du, Y., Altstiel, L., Bender, M., Hyslop, P., Johnstone, EM., Little, S.P., Cummins, D.J., Piccardo, P., Ghetti, B., Paul, S.M., 1997, Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition, *Nat Genet.* **17**(3): 263-4.

Barghorn, S., Zheng-Fischhöfer, Q., Ackmann, M., Biernat, J., von Bergen, M., Mandelkow E.M., et al., 2000, Structure, microtubule interactions, and peired helical filament aggregation by tau mutatnts of frontotemporal dementia, *Biochemistry*. **39**:11714-11721.

Bickeboller, H., Campion, D., Brice, A., Amouyel, P., Hannequin, D., Didierjean, O., Penet, C., Martin, C., Perez-Tur, J., Michon, A., Dubois, B., Ledoze, F., Thomas-Anterion, C., Pasquier, F., Puel, M., Demonet, J.F., Moreaud, O., Babron, M.C., Meulien, D., Guez, D., Chartier-Harlin, M.C., Frebourg, T., Agid, Y., Martinez, M., Clerget-Darpoux, F., 1997,

Apolipoprotein E and Alzheimer disease: genotype-specific risks by age and sex, *Am J Hum Genet*. **60**(2): 439-46.

Bird, T., Knopman, D., Van Swieten, J., Rosso, S., Feldman, H., Tanabe, H., Graff-Raford, N., Geschwind, D., Verpillat, P., Hutton, M., 2003, Epidemiology and genetics of frontotemporal dementia/Pick's disease, *Ann Neurol*. 54 Suppl 5: S29-31.

Brown, J., Ashworth, A., Gydesen, S., Sorensen, A., Hardy, J., Collinge, J., 1995, Familial non-specific dementias maps to chromosome 3, *Hum Mol Genet*. **4**: 1625-8.

Bué, L., Delacourte, A., 1999, Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease, *Brain Pathol.* **9**: 681-693.

Bugiani, O. Murrell, J.R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., Morbin, M., Primavera, A., et al., 1999, Frontotemporal dementia and corticobasal degeneration in a family with P301S mutation in tau, *J Neuropathol Exp Neurol.* **58**: 667-677.

Cacabelos, R., 2002, Pharmacogenomics for the treatment of dementia, *Ann Med.* **34**(5): 357-79.

Calhoun, M.E., Wiederhold, K.H., Abramowski, D., Phinney, A.L., Probst, A., Sturchler-Pierrat, C., Staufenbiel, M., Sommer, B., Jucker, M., 1998, Neuron loss in APP transgenic mice, *Nature.* **395**(6704): 755-6.

Campion, D., Dumanchin, C., Hannequin, D., Dubois, B., Belliard, S., Puel, M., Thomas-Anterion, C., Michon, A., Martin, C., Charbonnier, F., Raux, G., Camuzat, A., Penet, C., Mesnage, V., Martinez, M., Clerget-Darpoux, F., Brice, A., Frebourg, T., 1999, Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum, *Am J Hum Genet*. **65(3)**: 664-70.

Cao, X., Sudhof, T.C., 2004, Dissection of amyloid-beta precursor protein-dependent transcriptional transactivation, *J Biol Chem*. **279**(23): 24601-11.

Chartier-Harlin, M.C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J., Mullan, M., 1991, Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene, *Nature.* **353**: 844-846.

Chartier-Harlin, M.C., Parfitt, M., Legrain, S., Perez-Tur, J., Brousseau, T., Evans, A., Berr, C., Vidal, O., Roques, P., Gourlet, V., et al., 1994, Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region, *Hum Mol Genet.* **3**(4): 569-74.

Clark, L.N., Poorkaj, P., Wszolek, Z., Geshwind, D.H., Nasreddine, Z.S., Miller, B., Li, D., et al., 1998, Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17, *Proc Natl Acad Sci USA.* **95**: 1310-13107.

Crook, R., Verkkoniemi, A., Perez-Tur, J., Mehta Baker, N., Houlden, M., Farrer, H., Hutton, M., Lincoln, M., Hardy, S., Gwinn, J., Somer, K., Paetau, M., Kalimo, A., Ylikoski, H., Poyhonen, R., Kucera, M., S., Haltia, M., 1998, A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1, *Nature Med.* **4**: 452-455.

Cummings, J.L., Mega, M.S., Gray, K., Rosemberg-Thompson, S., Gornbein, T., 1994, The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia, *Neurology.* **44**: 2308-2314.

Dawson, H.N., Ferreira, A., Eyster, M.V., Ghoshal, N., Binder, L.I., Vitek, M.P., 2001, Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice, *J cell Sci*. **114**: 1179-1187.

De Jonghe, C., Cruts, M., Rogaeva, E.A., Tysoe, C., Singleton, A., Vanderstichele, H., Meschino, W., Dermaut, B., Vanderhoeven, I., Backhovens, H., Vanmechelen, E., Morris, C.M., Hardy, J., Rubinsztein, D.C., St George-Hyslop, P.H., Van Broeckhoven, C., 1999, Aberrant splicing in the presenilin-1 intron 4 mutation causes presenile Alzheimer's disease by increased A-beta-42 secretion, *Hum Mol Genet.* **8**: 1529-1540.

De Jonghe, C., Kumar-Singh, S., Cruts, M., Kleinert, R., Vanderstichele, H., Vanmechelen, E.J.M., Kroisel, P., Van Broeckhoven, C., 2000, Unusual Aβ amyloid deposition in Alzheimer's disease due tu an APP T714I mutation at the γ42-secretase site, *Neurobiology of Aging.* 21 **Supp1**: S200.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J.S., Schroeter, E.H., Schrijvers, V., Wolfe, M.S., Ray, W.J., Goate, A., Kopan, R., 1999, A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain, *Nature.* **398**(6727): 518-22.

Delisle, M.B., Murell, J.R., Richardson, R., Trofatter, J.A., Rascol, O., Soulages, X., Mohr, M., et al., 1999, A mutation at codon 279 (N279K) in exon 10 of the tau gene causes a tauopathy with dementia and supranuclear palsy, *Acta Neuropath*. **98**: 62-77.

Dermaut, B., Kumar-Singh, S., Engelborghs, S., Theuns, J., Rademakers, R., Saerens, J., Pickut, B.A., Peeters, K., van den Broeck, M., Vennekens, K., Claes, S., Cruts, M., Cras, P., Martin, J.J., Van Broeckhoven, C., Deyn, De P.P., 2004, A novel presenilin 1 mutation associated with Pick's disease but not beta-amyloid plaques, *Ann Neurol*. **55**(5): 617-26.

Dickson, D.W., 2001, Neuropathology of Pick's disease, *Neurology*. **56**: S16-S20.

D'Souza, I., Schellenberg, G.D., 2000, Determinants of 4-repeat tau expression. Coordination between enhancing and inhibitory splicing sequences for exon 10 exclusion, *J Biol Chem*. **275**: 17700-17709.

D'Souza, I., Schellenberg, G.D., 2002, Tau Exon 10 expression involves a bipartite intron 10 regulatory sequence and weak 5' and 3' splice sites, *J Biol Chem*. **277**: 26587-26599.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.M., Perez-tur, J., Hutton, M., Buee, L., Harigaya, Y., Yager, D., Morgan, D., Gordon, M.N., Holcomb, L., Refolo, L., Zenk, B., Hardy, J., Younkin, S., 1996, Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1, *Nature.* **383**(6602): 710-3.

Dumanchin, C., Camuzat, A., Campion, D., Verpillat, P., Hannequin, D., Dubois, B., Saugier-Veber, P., Martin, C., Penet, C., Charbonnier, F., Agid, Y., Frébourg, T., Brice, A., 1998, Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism, *Hum Mol Genet*. **7**: 1825-1829.

Eckman, C.B., Mehta, N.D., Crook, R., Perez-tur, J., Prihar, G., Pfeiffer, E., Graff-Radford, N., Hinder, P., Yager, D., Zenk, B., Refolo, L.M., Prada, C.M., Younkin, S.G., Hutton, M., Hardy, J., 1997, A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A-beta-42(43). *Hum Mol. Genet*. **6**: 2087-2089.

Farlow, M.R., Cyrus, P.A., Nadel, A., Lahiri, D.K., Brashear, A., Gulanski, B., 1999, Metrifonate treatment of AD: influence of APOE genotype, *Neurology*. **53**(9): 2010-6.

Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., Pericak-Vance, M.A., Risch, N., van Duijn, C.M., 1997, Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium, *JAMA*. **278**(16): 1349-56.

Farrer, L.A., Cupples, L.A., van Duijn, C.M., Kurz, A., Zimmer, R., Muller, U., Green, R.C., Clarke, V., Shoffner, J., Wallace, D.C., et al., 1995, Apolipoprotein E genotype in patients with Alzheimer's disease: implications for the risk of dementia among relatives, *Ann Neurol.* **38**(5): 797-808.

Frisoni, G.B., Beltramello, A., Geroldi, C., Weiss, C., Bianchetti, A., Trabucchi, M., 1996, Brain atrophy in frontotemporal dementia, *J Neurol Neurosurg Psychiatry*. **61**: 157-65.

Frisoni, G.B., Pizzolato, G., Bianchetti, A., Chierichetti, F., Ferlin, G., Battistin, L., Trabucchi, M., 1994, Single photon emission computed tomography with [99Tc]-HM-PAO and [123I]-IBZM in Alzheimer's disease and dementia of frontal type: preliminary results, *Acta Neurol Scand*. **89**: 199-203.

Ghetti, B., Murrell, J.R., Zolo, P., Spillantini, M.G., Goedert, M., 2000, Progress in hereditary tauopathies: a mutation in the Tau gene (G389R) causes a Pick disease-like syndrome, *Ann N Y Acad Sci*. **920**: 52-62.

Goate, A., Rossor, M., Roques, P., Hardy, M., Mullan, 1991, Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta- J., amyloid precursor protein gene. *Nature.* **353**: 844-846.

Goedert, M., Jakes, R., 1990, Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerisation, *EMBO*. **9**: 4225-4230.

Goedert, M., Jakes, R., Crowther, J., 1999, Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments, *FEBS Lett*. **450**: 306-311.

Goedert, M., Spillantini, M.G., Cairns, N.J., Crowther, R.A., 1992, Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms, *Neuron*. **8**: 159-168.

Goedert, M., Spillantini, M.G., Crowther, J., Chen, S.C., Parchi, P., Tabaton, M., Lanska, et al., 1999, Tau gene mutation in progressive sucortical gliosis, *Nat Med*. **5**: 454-457.

Goode, B.L., Chau, M., Denis, P.E., Feinstein, S.C., 2000, Structural and functional differences between 3-R repeat and 4-repeat tau isoforms. Implications for normal tau function and the onset of neurodegenerative disease, *J Biol Chem*. **275**: 38182-38189.

Götz, J., 2001, Tau and transgenic animal models, *Brain Res Brain Res Rev*. **35**: 266-286.

Grabowski, T.J., Cho, H.S., Vonsattel, J.P. Rebeck, G., S.M., G.W., 2001, Greenberg, Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy, *Ann Neurol.* **49**: 697-705.

Grober, E., Buschke, H., 1987, Genuine memory deficits in dementia, *Developmental Neuropsychology.* **3**: 13-36.

Gustafson, L. 1987, Frontal lobe degeneration of non-Alzheimer type. II. Clinical picture and differential diagnosis, *Arch Gerontol Geriatr*. **6**: 209-223.

Gustafson, L., Abrahamson, M., Grubb, A., Nilsson, K., Fex, G., 1997, Apolipoprotein-E genotyping in Alzheimer's disease and frontotemporal dementia, *Dement Geriatr Cogn Disord.* **8**: 240-3.

Gydesen, S., Brown, J.M., Brun, A., Chakrabarti, L., Gade, A., Johannsen, P., Rossor, M., et al., 2002**,** Chromosome 3 linked frontotemporal dementia (FTD-3), *Neurology*. **59**: 1585-1594.

Hasegawa, M., Smith, M.J., Goedert, M., 1998, Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembl, *FEBS Lett*. **437**: 207-210.

Hendriks, L., van Duijn, C.M., Cras, P., Cruts, M., Van Hul, W., van Harskamp, F., Warren, A., McInnis, M. Antonarakis, G., Martin, S.E., Hofman, J.J., Van Broeckhoven, A., C., 1992, Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene, *Nature Genet.* **1**: 218-221.

Heutink, P., Stevens, M., Rizzu, P., Bakker, E., Kros, J.M., Tibben, A., Niermeijer, M.F., van Duijn, C.M., Oostra, B.A., van Swieten, J.C., 1997, Hereditary frontotemporal dementia is linked to chromosome 17q21-22: a genetic and clinicopathological study of three Dutch families, *Ann Neurol*. **41**: 150-159.

Hodges, J.R., 2001, Frontotemporal dementia (Pick's disease): clinical features and assessment, *Neurology*. **56**: S6-S10.

Hodges, J.R., Davies, R., Xuereb, J., Kril, J., Halliday, G., 2003, Survival in frontotemporal dementia, *Neurology*. **61**: 349-354.

Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B.I., Geschwind, D.H., et al., 1998, Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17, *Science.* **282**: 1914-1917.

Houlden, H., Baker, M., Adamson, J., Grover, A., Waring, S., Dickson, D., Lynch, T., et al., 1999, Frequency of tau mutations in three series of non-Alzheimer's degenerative dementia, *Ann Neurol*. **42**: 243-248.

Houlden, H., Baker, M., McGowan, E., Lewis, P., Hutton, M., Crook, R., Wood, N.W., Kumar-Singh, S., Geddes, J., Swash, M., Scaravilli, F., Holton, J.L., Lashley, T., Tomita, T., Hashimoto, T., Verkkoniemi, A., Kalimo, H., Somer, M., Paetau, A., Martin, J.J., Van Broeckhoven, C., Golde, T., Hardy, J., Haltia, M., Revesz, T., 2000, Variant Alzheimer's disease with spastic paraparesis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-beta concentrations, *Ann Neurol.* **48**: 806-808.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, et al., 1998, Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17, *Nature*. **393**: 702-705.

Imamura, T., Takanashi, M., Hattori, N., Fujimori, M., Yamashita, H., Ishii, K., Yamadori, A., 1998, Bromocriptine treatment for perseveration in demented patients, *Alzheimer Dis Assoc Disord*. **12**: 109-13.

Jackson, M., Lowe, J., 1996, The new neuropathology of degenerative frontotemporal dementias, *Acta Neuropathol*. **91**: 127-134.

Jarrett, J.T., Berger, E.P., Lansbury, P.T., 1993, The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease, *Biochemistry.* **32**: 4693-4697.

Kamino, K., Orr, H.T., Payami, H., Wijsman, E.M., Alonso, M.E., Pulst, S.M., Anderson, L., O'dahl, S., Nemens, E., White, J.A., Sadovnick, A.D., Ball, M.J., Kaye, J., Warren, A., McInnis, M., Antonarakis, S.E., Korenberg, J.R., Sharma, V., Kukull, W., Larson, E., Heston, L.L., Martin, G.M., Bird, T.D., Schellenberg, G.D., 1992, Linkage and mutational analysis of

familial Alzheimer disease kindreds for the APP gene region, *Am J Hum Genet.* **51**: 998-1014.

Knopman, D.S., Mastri, A.R., Frey, W.H., Sung, J.H., Rustan, T., 1990, Dementia lacking distinctive histologic features: a commun non-alzheimer degenerative dementia, *Neurology*. **40**: 251-256.

Kobayashi, K., Hayashi, M., Kidani, T., Ujike, H., Iijima, M., Ishihara, T., Nakano, H., et al., 2004, Pick's disease pathology of a missens mutation of S305N of frontotemporal dementia and parkinsonism linked to chromosome 17: another phenotype of S305N, *Dement Geriatr Cogn Disord*. **17**: 293-297.

Kwok, J.B., Li, Q.X., Hallupp, M., Whyte, S., Ames, D., Beyreuther, K., Masters, C.L., Schofield, P.R., 2000, Novel Leu723Pro amyloid precursor protein mutation increases amyloid beta42(43) peptide levels and induces apoptosis, *Ann Neurol*. **47**(2): 249-53.

Kwok, J.B., Taddei, K., Hallupp, M., Fisher, C., Brooks, W.S., Broe, G.A., Hardy, J., Fulham, M.J., Nicholson, G.A., Stell, R., George Hyslop, St P.H., Fraser, P.E., Kakulas, B., Clarnette, R., Relkin, N., Gandy, S.E., Schofield, P.R., Martins, R.N., 1997, Two novel (M233T and R278T) presenilin-1 mutations in early-onset Alzheimer's disease pedigrees and preliminary evidence for association of presenilin-1 mutations with a novel phenotype, *Neuroreport*. **8**(6): 1537-42.

Lanska, D.J., Currier, R.D., Cohen, M., Gambetti, P., Smith, E.E., Bebin, J., Jackson, J.F., Whitehouse, P.J., Markesbery, W.R., 1994, Familial progressive subcortical gliosis, *Neurology*. **44**: 1633-1643.

Lee, V.M., Goedert, M., Trojanowski, J.Q., 2001, Neurodegenerative tauopathies, *Annu Rev Neurosci*. 24: 1121-1159.

Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., van Duinen, S.G., Bots, G.T., Luyendijk, W., Frangione, B., 1990, Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type, *Science.* **248**(4959): 1124-6.

Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C., Jondro, P.D., Schmidt, S.D., Wang, K., Crowley, A.C., Fu, Y.H., Guenette, S.Y., Galas, D., Nemens, E., Wijsman, E.M., Bird, T.D., Schellenberg, G.D., Tanzi, R.E., 1995, Candidate gene for the chromosome 1 familial Alzheimer's disease locus, *Science.* **269**: 973-977.

Lewis, J., Dickson, D.W., Lin, W.L., Chisholm, L., Corral, A., Jones, G., Yen, S.H., Sahara, N., Skipper, L., Yager, D., Eckman, C., Hardy, J., Hutton, M., McGowan, E., 2001, Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP, *Science* **293**(5534): 1487-91.

Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Van Slegtenhorst, M., Gwinn-Hardy, K., et al., 2000, Neurofibrilary tangles, amyotrophy and progressive motor disturbances in mice expressing mutant (P301L) tau mutation, *Nat Genet*. **25**: 402-405.

Li, G., Silverman, J.M., Altstiel, L.D., Haroutunian, V., Perl, D.P., Purohit, D., Birstein, S., Lantz, M., Mohs, R.C., Davis, K.L., 1996, Apolipoprotein E-epsilon 4 allele and familial risk in Alzheimer's disease, *Genet Epidemiol*. **13**(3): 285-98.

Lin, W.L., Lewis, J., Yen, S.H., Hutton, M., Dickson, D.W., 2003, Filamentous tau in oligodendrocytes and astrocytes of transgenic mice expressing the human tau isoform with the P301L mutation, *Am J Pathol*. **162**: 213-218.

Litvan, I., 2001, Therapy and management of frontal lobe dementia patients, *Neurology*. **56**: S41-S45.

Lorenzo, A., Yankner, B.A., 1994, Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red, *Proc Natl Acad Sci*. **91**(25): 12243-7.

Lossos, A., Reches, A., Gal, A., Newman, J.P., Soffer, D., Gomori, J.M., Boher, M., Ekstein, D., Biran, I., Meiner, Z., Abramsky, O., Rosenmann, H., 2003, Frontotemmporal dementia and parkinsonism with the P301S tau gene mutation in a Jewish family, *J Neurol*. **250**: 733-740.

Mann, D.M.A., MacDonagh, A.M., Snowden, J., Neary, D., Pickering-Brown, S.M., 2000, Molecular classification of the dementias, *Lancet*. **355**: 626.

Martinez, M., Campion, D., Brice, A., Hannequin, D., Dubois, B., Didierjean, O., Michon, A., Thomas-Anterion, C., Puel, M., Frebourg, T., Agid, Y., Clerget-Darpoux, F., 1998, Apolipoprotein E epsilon4 allele and familial aggregation of Alzheimer disease, *Arch Neurol.* **55**(6): 810-6.

Mattson, M.P., 2003, Neurobiology: Ballads of a protein quartet, *Nature.* **422**(6930): 385, 387.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984, Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease, *Neurology.* **34**(7): 939-44.

Miller, B.L., Cummings, J.L., Villanueva-Meyer, J., Boone, K., Mehringer, C.M., Lesser, I.M., Mena, I., 1991, Frontal lobe degeneration: clinical, neuropsychological, and SPECT characteristics, *Neurology*. **41**: 1374-82.

Miyata, M., Smith, J.D., 1996, Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides, *Nat Genet*. **14**(1): 55-61.

Morris, H.R., Khan, M.N., Janssen, J.C., Brown, J.M., Perez-Tur, J., Baker, M., Ozansoy, M., Hardy, J., Hutton, M., Wood, N.W., Lees, A.J., Revesz, T., Lantos, P., Rossor, M., 2001, The genetic and pathological classification of familial frontotemporal dementia, *Arch Neurol.* **58**: 1813-1816.

Morris, H.R., Osaki, Y., Holton, J., Lees, A.J., Wood, N.W., Revesz, T., Quinn, N., 2003, Tau exon 10 +16 mutation FTDP-17 presenting clinically as sporadic young onset PSP, *Neurology*. 61: 102-104.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., Lannfelt, L., 1992, A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid, *Nature Genet*. **1**: 345-347.

Munoz, D.G., Dickson, D.W., Bergeron, C., Mackenzie, I.R.A., Delacourte, A., Zhukareva, V., 2003, The neuropathology and biochemistry of frontotemporal dementia, *Ann Neurol.* **54**: S24-S28.

Murayama, O., Tomita, T., Nihonmatsu, N., Murayama, M., Sun, X., Honda, T., Iwatsubo, T., Takashima, A., 1999, Enhancement of amyloid beta 42 secretion by 28 different presenilin 1 mutations of familial Alzheimer's disease, *Neurosci Lett.* **265**(1): 61-3.

Murrell, J., Farlow, M., Ghetti, B., Benson, M.D., 1991, A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease, *Science.* **254**: 97-99.

Murrell, J.R., Hake, A.M., Quaid, K.A., Farlow, M.R., Ghetti, B., 2000, Early-onset Alzheimer disease caused by a new mutation (V717L) in the amyloid precursor protein gene, *Arch Neurol*. **57**(6): 885-7.

Murrell, J.R., Spillantini, M.G., Zolo, P., Guazzelli, M., Smith, M.J., Hasegawa, M., Redi, F., et al., 1999, Tau gene mutation G389R causes a tauopathy with abundant Pick body-like inclusions and axonal deposits, *J Neuropathol Exp Neurol*. **58**: 1207-1226.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., et al., 1998, Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria, *Neurology*. **51**: 1546-1554.

Neary, D., Snowden, J.S., Northen, B., Goulding, P., 1988, Dementia of frontal lobe type, *J Neurol Neurosurg Psychiatry*. **51**: 353-361.

Netzer, W.J., Dou, F., Cai, D., Veach, D., Jean, S., Li, Y., Bornmann, W.G., Clarkson, B., Xu, H., Greengard, P., 2003, Gleevec inhibits beta-amyloid production but not Notch cleavage, *Proc Natl Acad Sci USA.* **100**(21): 12444-9.

Neumann, M., Schulz-Schaeffer, W., Crowther, R.A., Smith, M.J., Spillantini, M.G., Goedert, M., Kretzschmar, H.A., 2001, Pick's disease associated with the novel tau gene mutation K369I, *Ann Neurol*. **50**: 503-513.

Nicholl, D.J., Greenstone, M.A., Clarke, C.E., Rizzu, P., Crooks, D., Crowe, A., Trojanowski, J.Q., Lee, V.M.Y., Heutink, P., 2003, An English kindred with a novel recessive tauopathy and repiratory failure, *Ann Neurol*. **54**: 682-686.

Nishimura, I., Yang, Y., Lu, B., 2004, PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in Drosophila, *Cell*. **116**: 671-682.

Pasalar, P., Najmabadi, H., Noorian, A.R., Moghimi, B., Jannati, A., Soltanzadeh, A., Krefft, T., Crook, R., Hardy, J., 2002, An Iranian family with Alzheimer's disease caused by a novel APP mutation (thr714-to-ala), *Neurology.* **58**: 1574-1575.

Pasquier, F., Richard, F., Lebert, F., 2004, Natural history of frontotemporal dementia: comparison with Alzheimer's disease, *Dement Geriatr Cogn Disord.* **17**: 253-257.

Pastor, P., Pastor, E., Carnero, C., Vela, R., Garcia, T., Amer, G., Tolosa, E., Oliva, R., 2001, Familial atypical progressive supranuclear palsy associated with homozygosity for the delN296 mutation in the tau gene, *Ann Neurol*. **48**: 859-867.

Pastor, P., Roe, C.M., Villegas, A., Bedoya, G., Chakraverty, S., Garcia, G., Tirado, V., Norton, J., Rios, S., Martinez, M., Kosik, K.S., Lopera, F., Goate, A.M., 2003, Apolipoprotein

Epsilon4 modifies Alzheimer's disease onset in an E280A PS1 kindred, *Ann Neurol*. **54(2)**: 163-9.

Perry, R.J., Hodges, J.R., 2000, Differentiating frontal and temporal variant frontotemporal dementia from Alzheimer's disease, *Neurology*. **54**: 2277-84.

Petersen, R.B., Tabaton, M., Chen, S.G., Monari, L., Richardson, S.L., Lynches, T., Manetto, V., 1995, Familial progressive subcortical gliosis: presence of prions and linkage to chromosome 17, *Neurology.* 45: 1062-1067.

Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G., Kokmen, E., 1999, Mild cognitive impairment: clinical characterization and outcome, *Arch. Neurol*. **56**(3): 303-8.

Pickering-Brown, S., Baker, M., Bird, T., Trojanowski, J., Lee, V., Morris, H., Rossor, et al., 2004, Evidence of a founder effect in families with frontotemporal dementia that harbor the tau +16 splice mutation, *Am J Med Genet*. **125**: 79-82.

Pickering-Brown, S., Baker, M., Yen, S.H., Liu, W.K., Hasegawa, M., Cairns, N., Lantos, P.L., et al., 2000, Pick's disease is associated with mutations in the tau gene, *Ann Neurol*. **48**: 859-867.

Pickering-Brown, S.M., Baker, M., Nonaka, T., Ikeda, K., Sharma, S., Mackenzie, J., Simpson, S.A., Moore, J.W., Snowden, J.S., de Silva, R., Revesz, T., Hasegawa, M., Hutton, M., Mann, D.M.A., 2004, Frontotemporal dementia with Pick-type histology associated with Q336R mutation in the tau gene, *Brain*. **127**: 1415-1426.

Poirier, J., 1994, Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease, *Trends Neurosci.* **17**(12): 525-30.

Poirier, J., Delisle, M.C., Quirion, R., Aubert, I., Farlow, M., Lahiri, D., Hui, S., Bertrand, P., Nalbantoglu, J., Gilfix, B.M., et al., 1995, Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease, *Proc Natl Acad Sci USA.* **92**(26): 12260-4.

Poorkaj, P., Bird, T.D., Wijsman, E., Nemens, E., Garruto, R.M., Anderson, L., Andreadis, A., Wiederholt, W.C., Raskind, M., Schellenberg, G.D., 1998, Tau is a candidate gene for chromosome 17 frontotemporal dementia, *Ann Neurol*. **43**: 815-825.

Poorkaj, P., Muma, N.A., Zhukareva, V., Cochran, E.J., Shannon, K.M., Hurtig, H., Koller, W.C., et al., 2002, An R5L tau mutation in asubject with a progressive supranuclear palsy phenotype, *Ann Neurol*. **52**: 511-516.

Raux, G., Gantier, R., Thomas-Anterion, C., Boulliat, J., Verpillat, P., Hannequin, D., Brice, A., Frebourg, T., Campion, D., 2000, Dementia with prominent frontotemporal features associated with L113P presenilin 1 mutation, *Neurology.* **55**: 1577-1578.

Rizzini, C., Goedert, M., Hodges, J.R., et al., 2000, Tau gene mutation K257T causes a tauopathy similar to Pick's disease, *J neuropathol Exp Neurol*. **59**: 990-1001.

Rizzu, P., Van Swieten, J.C., Joosse, M., Hasegawa, M., Stevens, M., Tibben, A., Niermeijer, et al., 1999, High prevalence of mutations in the microtubule-associated protein tau in a population study of frontotemporal dementia in the Netherlands, *Am J Hum Genet*. **64**: 414-421.

Rogaeva, E.I., Frommelt, P., Sadovnick, A.D., Meschino, W., Rockwood, K., Boss, M.A., Mayeux, R., St. George-Hyslop, P., 2001, Screening for PS1 mutations in a referral-based series of AD cases: 21 novel mutations, *Neurology.* **57**: 621-625.

Rogaeva, E.I., Sherrington, R., Rogaeva, E.A., Levesque, G., Ikeda, M., Liang, Y., Chi, H., Lin, C., Holman, K., Tsuda, T., Mar, L., Sorbi, S., Nacmias, B., Piacentini, S., Amaducci, L., Chumakov, I., Cohen, D., Lannfelt, L., Fraser, P.E., Rommens, J.M., St George-Hyslop, P.H., 1995, Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene, *Nature.* **376**: 775-778.

Rosso, S.M., Donker, K.L., Baks, T., Joosse, M., de Koning, I., Pijnenburg, Y., de Jong, D., Dooijes, D., Kamphorst, W., Ravid, R., Niermeijer, M., Verheij, F., Kremer, H.P., Scheltens, P., van Duijn, C.M., Heutink, P., van Swieten, J.C., 2003, Frontotemporal dementia in the Netherlands: patients characteristics and prevalence estimates from a population-based study, *Brain*. **126**: 2016-2022.

Rosso, S.M., van Herpen, E., Deelen, W., Kamphorst, W., Severijnen, L.A., Willemsen, R., Ravid, R., et al., 2002, A novel tau mutation, S320F, causes a tauopathy with inclusions similar to those in Pick's disease, *Ann Neurol*. **51**: 373-376.

Rosso, S.M., van Swieten, J.C., 2002, New developments in frontotemporal dementia and parkinsonim linked to chromosome 17, *Curr Opin Neurol.* **15**: 423-428.

Sato, S., Kamino, K., Miki, T., Doi, A., Ii, K., St George-Hyslop, P.H., Ogihara, T., Sakaki, Y., 1998, Splicing mutation of presenilin-1 gene for early-onset familial Alzheimer's disease, *Hum Mutat*. **Suppl 1**: S91-4.

Schmidt, R., Freidl, W., Fazekas, F., Reinhart, B., Grieshofer, P., Koch, M., Eber, B., Schumacher, M., Polmin, K., Lechner, H., 1994, The Mattis Dementia Rating Scale: normative data from 1,001 healthy volunteers, *Neurology*. **44**(5): 964-6.

Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J.F., Bruni, A.C., Montesi, M.P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R.J., Wasco, W., Da Silva, H.A.R., Haines, J.L., Pericak-Vance, M.A., Tanzi, R.E., Roses, A.D., Fraser, P.E., Rommens, J.M., St George-Hyslop, P.H., 1995, Cloning of a gene bearing mis-sense mutations in early-onset familial Alzheimer's disease, *Nature*. **375**: 754-760.

Sleegers, K., Roks, G., Theuns, J., Aulchenko, Y.S., Rademakers, R., Cruts, M., van Gool, W.A., Van Broeckhoven, C., Heutink, P., Oostra, B.A., van Swieten, J.C., van Duijn, C.M., 2004, Familial clustering and genetic risk for dementia in a genetically isolated Dutch population, *Brain*. **127**: 1641-1649.

Solvieri, P., Rossi, G., Monza, D., Tagliavini, F., Piacentini, S., Albanese, A., Bugiani, O., Girotti, F., 2003, A case of dementia parkinsonism ressembling progressive supranuclear palsy due to mutation in the tau protein gene, *Arch Neurol*. **60**: 1454-1456.

Sperfeld, A.D., Collatz, M.B., Baier, H., Palmbach, M., Storch, A., Schwarz, J., Tatsch, K., Reske, S., Joosse, M., Heutink, P., Ludolph, A.C., 1999, FTDP-17: an early-onset phenotype with parkinsonism and epileptic seizures caused by a novel mutation, *Ann Neurol*. **46**: 708-715.

Spillantini, M.G., Murrell, J.R., Goedert, M., Farlow, M.R., Klug, A., Ghetti, B., 1998, Mutation in the tau gene in familial multiple system tauopathy with presenile dementia, *Proc Natl Acad Sci USA*. **95**: 7737-7741.

Spillantini, M.G., Yoshida, H., Rizzini, C., Lantos, P.L., Khan, N., Rossor, M.N., Goedert, M., Brown, J., 2000, A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies, *Ann Neurol*. **48**: 939-943 .

Stanford, P.M., Halliday, G.M., Brooks, W.S., Kwok, J.B.J., Storey, C.E., Creasey, H., Morris, J.G.L., et al., 2000, Progressive supranuclear palsy pathology caused by a novel silent mutation in exon 10 of the tau gene, *Brain*. **123**: 880-893.

Stevens, M., van Duijn, C.M., de Knijff, P., van Broeckhoven, C., Heutink, P., Oostra, B.A., Niermeijer, M.F., van Swieten, J.C., 1997, Apolipoprotein E gene and sporadic frontal lobe dementia, *Neurology*. **48**: 1526-9.

Stevens, M., van Duijn, C.M., Kamphorst, W., de Knijff, P., Heutink, P., van Gool, W.A., Scheltens, P., Ravid, R., Oostra, B.A., Niermeijer, M.F., van Swieten, J.C., 1998, Familial aggregation in frontotemporal dementia, *Neurology*. **50**: 1541-1545.

Strittmatter, WJ., Roses, AD., 1995, Apolipoprotein E and Alzheimer disease, *Proc Natl Acad Sci USA*. **92**(11): 4725-7.

Swartz, J.R., Miller, B.L., Lesser, I.M., Darby, A.L., 1997, Frontotemporal dementia: treatment response to serotonin selective reuptake inhibitors, *J Clin Psychiatry*. **58**: 212-216.

Taniguchi, S., McDonagh, A.M., Pickering-Brown, S.M., Umeda, Y., Iwatsubo, T., Hasegawa, M., Mann, D.M., 2004, The neuropathology of frontotemporal lobar degeneration with respect to the cytological and biochemical charachteristics of tau protein, *Neuropathol Appl Neurobiol*. **30**: 1-18.

The Lund and Manchester groups, 1994, Clinical and neuropathological criteria for frontotemporal dementia, *J Neurol Neurosurch Psychiatry*. **57**: 416-418.

Tolnay, M., Probst, A., 2003, The neuropathological spectrum of neurodegenerative tauopathies, *IUBMB Life*. **55(6)**: 299-305.

Trojanowski, J.Q., Lee, V.M.Y., 1999, Transgenic models of tauopathies and synucleinopathies, *Brain Pathol*. **9**: 733-739.

Tsuboi, Y., Baker, M., Hutton, M.L., Uitti, R.J., Rascol, O., Delisle M.B., Soulages, X., et al., 2002, Clinical and genetic studies of families with ths N279K mutation (FTDP-17), *Neurology*. **59**: 1791-1793.

Van Swieten, J.C., Rosso, S.M., van Herpen, E., Kamphorst, W., Ravid, R., Heutink, P., 2004, Phenotypic variation in frontotemproal dementia and parkinsonism linked to chromosome 17, *Dement Geriatr Cogn Disord*. **17**: 261-264.

Van Swieten, J.C., Stevens, M., Rosso, S.M., Rizzu, P., Josse, M., de Koning, I., Kamphorst, W., Ravid, R., Spillantini, M.G., Niermeijer, M.F., Heutink, P., 1999,Phenotypic variation in hereditary frontotemporal dementia with tau mutations, *Ann Neurol.* **46**: 617-626.

Verkkoniemi, A., Somer, M., Rinne, J.O., Myllykangas, L., Crook, R., Hardy, J., Viitanen, M., Kalimo, H., Haltia, M., 2000, Variant Alzheimer's disease with spastic paraparesis: clinical characterization, *Neurology.* **54**: 1103-1109.

Verpillat, P., Camuzat, A., Hannequin, D., Thomas-Anterion, C., Puel, M., Belliard, S., Dubois, B., et al., 2002, Apolipoprotein E gene in frontotemporal dementia: An association study and meta-analysis, *Eur J Hum Genet*. **10**: 399-405.

Verpillat, P., Camuzat, A., Hannequin, D., Thomas-Anterion, C., Puel, M., Belliard, S., Dubois, B., et al., 2002, Association between the extended tau haplotype and frontotemporal dementia, *Arch Neurol*. **59**: 935-939.

Verpillat, P., Ricard, S., Hannequin, C., Dubois, B., Bou, J., Camuzat, A., Pradier, L., Frebourg, T., Brice, A., Clerget-Darpoux, F., Deleuze, J.F., Campion, D., 2002, French Study Group on Alzheimer's Disease and Frontotemporal Dementia, Is the saitohin gene involved in neurodegenerative diseases? *Ann Neurol*. **52**(6): 829-32.

Werber, E., Klein, C., Grünfeld, J., Rabey, J.M., 2003, Phenotypic presentation of frontotemporal dementia with parkinsonism-chromosome 17 type P301S mutation in a patient of Jewish-Algerian origin, *Mov Disorders* **18**: 595-598.

Wightman, G., Anderson, V.E., Martin, J., Swash, M., Anderton, B.H., Neary, D., et al., 1992, Hippocampal and neocortical ubiquitin-immunoreactive inclusions in amyotrophic lateral sclerosis with dementia, *Neurosci Lett.* **139**: 269-74.

Wilhelmsen, K.C., Forman, M.S., Rosen, H.J., Alving, L.I., Goldman, J., Feiger, J., Lee, J.V., et al., 2004, 2004,17q-linked frontotemporal dementia-amyotrophic lateral sclerosis without tau mutations with tau and alpha-synuclein inclusions, *Arch Neurol*. **61**: 398-406.

Wisniewski, T., Dowjat, W.K., Buxbaum, J.D., Khorkova, O., Efthimiopoulos, S., Kulczycki, J., Lojkowska, W., Wegiel, J., Wisniewski, H.M., Frangione, B., 1998, A novel Polish presenilin-1 mutation (P117L) is associated with familial Alzheimer's disease and leads to death as early as the age of 28 years, *Neuroreport.* **9**(2): 217-21.

Wszolek, Z.K., Tsuboi, Y., Uitti, R.J., Reed, L., Hutton, M.L., Dickson, D.W., 2001, Progressive supranuclear palsy as a disease phenotype caused by the S305S tau gene mutation, *Brain*. **124**: 1666-1670.

Yasuda, M., Yokoyama, K., Nakayasu, T., Nishimura, Y., Matsui, M., Yokoyama, T., Miyoshi, K., Tanaka, C., 2000, A japanese patient with frontotemporal dementia and parkinsonism by a tau P301S mutation, *Neurology*. **55**: 1224-1227.

Ye, Y., Lukinova, N., Fortini, M.E., 1999, Neurogenic phenotypes and altered Notch processing in Drosophila Presenilin mutants, *Nature.* **398**(6727): 525-9.

Yen, S.H., Hutton, M., DeTure, M., Ko, L.W., Nacharaju, P., 1999, Fibrillogenesis of tau: insights from tau missense mutations in FTDP-17, *Brain pathology*. **9**: 695-705.

Zhukareva, V., Vogelsberg-Ragaglia, V., Van Deerlin, V.M., Bruce, J., Shuck, T., Grossman, M., Clark, C.M., Arnold, S.E., Masliah, E., Galasko, D., Trojanowski, J.Q., Lee, V.M., 2001, Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia, *Ann Neurol*. **49**: 165-175.

7. PHARMACOGENETICS OF ALCOHOL-DEPENDENCE

Philip Gorwood, Gunter Schumann, Jens Treutlein and Jean Adès*

1. PHENOTYPE DEFINITION

The term "alcoholism", which covers a set of complex relationships with alcohol, is no more used in the international literature because of its heterogeneity. An expert group from the WHO, since 1976 (Edward et al., 1976), decided that it was important to distinguish the alcoholic behaviour itself, the syndrome of alcohol-dependence, and the multiple consequences of chronic alcohol intoxication, concerning somatic, psychic and social domains. This perspective was a core element in the building of "substance abuse" and "substance dependence" criteria, as they are defined in DSM-III, ICD-10, DSM-IV and DSM-IV-TR. Distinguishing two diagnostic categories is thus generally admitted:

-alcohol abuse (DSM IV TR) relates to an inadequate use of alcohol, leading to functional impairment or a clinically significant suffering. The core criteria are limited to social, interpersonal, physical or judicial consequences of repeated consumptions of alcohol beverages.

-alcohol dependence is characterised by physical (tolerance and withdrawal processes), psychological and/or behavioural symptoms, close to the Jellinek's "loss of control" concept.

This distinction is used for all addictive disorders and should help to describe more homogenous diagnostic categories. Epidemiological, biological and genetic research may thus benefit from such differences in analysing the complex entity of alcoholic behaviours. The complexity of pathological relationships with alcohol is, in fact, imperfectly described in international classifications.

Philip Gorwood and Jean Adès, INSERM Unit 675 (IFR02) Faculty Xavier Bichat. 16 rue Henri Huchard, 75018 Paris-France and CHU Louis Mourier (AP-HP, Paris VII), 178 rue des Renouillers, 92700 Colombes, France.
Gunter Schumann and Jens Treutlein, Molecular Genetics Laboratory, Central Institute of Mental Health. University of Heidelberg, J5, 68159 Mannheim, Germany.

Alcohol abuse, as it is defined in the DSM-IV-TR, is not comparable with the ICD-10 (WHO criteria) diagnostic of "harmful use of alcohol". In ICD-10 criteria, negative somatic and psychic (such as depression and anxiety) consequences of alcohol consumption are the only variables taken into account, independently of social and interpersonal related difficulties. Patients with a DSM or an ICD criteria of alcohol abuse could thus be different. Having the diagnosis with one criteria is not systematically associated with the presence of the diagnosis with the other criteria. Such discrepancy has a strong impact, with potentially low reproducibility of researches on alcohol abu, based on the DSM versus the ICD criteria.

Alcohol dependence also appears as an heterogeneous concept, the DSM classification proposing to isolate alcohol dependence with or without physical symptoms. It distinguishes a purely compulsive form of psychological dependence, from a form with physical dependence and an increased risk of medical consequences and relapses.

Comorbity of psychiatric disorders and alcohol abuse or dependence is frequently observed. Once again, two types of categories are distinguished. Independent psychiatric disorders, such as mood, anxiety, psychotic or personality disorders, should be present before the onset of alcohol problems, or should be detected out of intoxication periods, in abstinent patients. Mark Schuckit proposed, in case of double-diagnostic, to distinguish primary alcoholism -that aroused before the onset of the comorbid psychiatric disorder- with alcoholism secondary to a mental or a personality disorder. This distinction is important for assessing the prognosis and making treatment choice (Schukit, 1989). Accordingly, the DSM-IV-TR identifies alcohol-induced psychotic disorder, mood disorders and anxiety disorders. Psychic symptoms, in these disorders, develop in less than one month after alcohol intoxication and withdrawal.

Alcohol abuse and dependence are two distinct modalities of pathological relationships with alcohol. A large and recent epidemiological analysis, the "National Epidemiologic Survey on Alcohol and Related Condition", based on a sample of 42.392 subjects residing in the United States, showed that one third of patients with alcohol dependence did not have an associated alcohol abuse diagnostic. This specific subgroup of patients, with alcohol dependence but without alcohol abuse, is mainly observed in women and in certain ethnic minorities of the United-States (Hasin & Grant, 2004). These data confirm the heterogeneity of the alcohol-dependence concept, reinforcing the importance to distinguish alcohol-dependence with or without physical dependence, and with or without alcohol-abuse. Epidemiological and genetic researches, as new treatment developments, require that homogenous phenotype of alcohol related problems are being proposed. Current diagnostic categories imperfectly feet this necessity.

The psychopharmacogenetics of alcohol dependence or abuse has thus to cope with a complex phenotype, based on intuitive diagnostic criteria (satisfying the importance of a diagnostic validity, as different clinicians may have comparable conclusions) rather than built up from the core mechanisms that are involved (because a large part of them are still unknown). In this view, and until

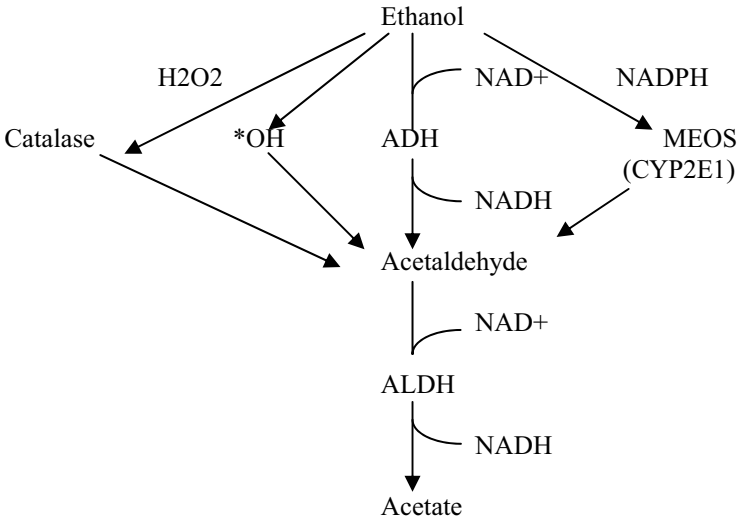
more precise biological pathways are being clearly defined, it is expected that such a precise approach as pharmacogenetics (i.e., defining which alcohol dependent patient is going to benefit from a specific treatment according to its genome) is still (but for how long?) an ambitious aim.

2. ALCOHOL METABOLISM

The pharmacogenetics of alcohol-dependence has the particularity to be interconnected with the genetics of the vulnerability to alcohol-dependence, because the risk for alcohol-dependence is also (but incompletely) related to the way alcohol, i.e., the molecule ethanol (CH_3-CH_2-OH), is metabolized. The specific behavioral and physiologic effects of alcohol depend (Schuckit, 1995) on dose, distribution, and metabolism of alcohol, but also prior drinking experience and concurrent use of other drugs for the same reasons involving metabolism.

In humans, ethanol is rapidly absorbed by simple diffusion from the upper gastrointestinal tract. Diffusion is slow in the stomach and mostly (70% 80%) occurs in the intestines. The distribution of ethanol after ingestion, unbounded to protein, is observed a few minutes after intake (the half-life of distribution being between 7 and 8 minutes) with a prominent contact with highly vascularized organs such as brain, lung and liver. Ethanol also has the particularity of a large diffusion, and except for bones and fat, is unrestricted by placenta and brain barriers.

Part of (from 2% to 10%) the ingested ethanol is directly eliminated through air, urine and sweat, but ethanol is mainly metabolized by oxydation in acetaldehyde and then in acetate. The main metabolism of ethanol is occurring in the liver, with a first passage of less than 20% of the total dosage of drunken alcohol. Quantitatively, the most important component of alcohol metabolism occurs in the stomach by ADH isozymes (ADH6 and ADH7) and in hepatocytes (ADH1, ADH2 and ADH3). Alcohol is oxidized to a toxic intermediate, acetaldehyde, which is then rapidly oxidized to acetate by cytosol (ALDH1) or mitochondrial (ALDH2) aldehyde dehydrogenase enzymes. The microsomal ethanol oxidizing system (MEOS) located in the smooth endoplasmic reticulum and the catalase pathway located in peroxisomes have a less important role in alcohol metabolism (figure 1).



Legend: ADH= alcohol dehydrogenase. ALDH= acetaldehyde dehydrogenase. MEOS= microsomal ethanol oxidizing system. CYP2E1= cytochrome P450 isoform.

Figure 1. Metabolism of ethanol

The interplay between the kinetics of absorption, distribution and elimination is important in determining the pharmacodynamic responses to alcohol. There is a large degree of variability in alcohol absorption, distribution and metabolism, as a result of both genetic and environmental factors. The between-individual variation in alcohol metabolic rates is, in part due to allelic variants of the genes encoding the alcohol metabolizing enzymes.

2.1 Minor metabolism pathways

MEOS activity has been attributed to CYP2E1, an isoform of cytochrome P450. Its role in ethanol metabolism in non-habitual drinkers is probably small, at least when circulating ethanol concentrations are low. Catalase does not appear to play a major role in ethanol oxidation in the liver, but is the main enzyme to oxidise ethanol to acetaldehyde in the brain, at least under physiological conditions.

However, upon continued exposure to ethanol, the progression of liver injury involves ethanol metabolism via CYP2E1 and consequent oxidant stress. Ethanol-induced oxidative stress appears to play a major role in mechanisms by which ethanol causes liver injury.

CYP2E1 is of interest because of its ability to metabolize and activate many toxicological substrates, including ethanol, to more reactive, toxic products. Levels of CYP2E1 are elevated after acute and chronic alcohol treatment. CYP2E1 is also an effective generator of reactive oxygen species such as the superoxide anion radical and hydrogen peroxide, and in the presence of iron catalysts, produces powerful oxidants such as the hydroxyl radical (Kessova et al., 2003).

The large interindividual variability of the chlorzoxazone hydroxylase activity could be partly due to the genetic polymorphisms observed in the CYP2E1 gene. An RsaI (in the 5'-flanking region), a DraI (in intron 6) and a TaqI (in intron 7) polymorphisms were described, with frequencies of the rare allele above 1%. No relationship between these three polymorphisms and CYP2E1 activity was clearly established. Nevertheless, other results indicate that genetic polymorphisms in the 5'-flanking region of the human CYP2E1 gene affect its binding of trans-acting factor and change its transcriptional regulation (Hayashi et al., 1991).

Interestingly, many association studies detected that one genetic variant of the CYP2E1 was a significant risk factor in alcohol drinkers or abusers for alcohol-related diseases, such as a precursor of stomach cancer (incomplete intestinal metaplasia) for patients with the c1/c1 genotype (Chen et al., 2004), alcohol-induced chronic pancreatitis for the CYP2E1 intron 6 D allele (Verlaan et al., 2004), a higher risk of developing esophageal and lung cancer in subjects with the homozygous mutant-type-A4/A4- (Nomura et al., 2003), or developing breast cancer in ever-drinking women with the CYP2E1 c2 allele containing genotypes (Choi et al., 2003).

The role of the CYP1E2 gene is thus quiet convincing in alcohol related somatic disorders. The pharmacogenetic role of the CYP2E1 is nevertheless exposed to confounding factors that renders the assessment of its importance, for example in alcohol related liver disease, more difficult. For example the development of anti-cytochrome P4502E1 (CYP2E1) autoantibodies in alcohol abusers enhances the risk of liver diseases. It is thus interesting to note that patients with alcoholic fibrosis/cirrhosis who carry the G allele of the cytotoxic T-lymphocyte antigen-4, had a higher risk of developing anti-CYP2E1 autoreactivity (Vidali et al., 2003) than patients without this allele.

Less works were devoted to a direct analyse of the role of the CYP1E2 on alcohol consumption and/or abuse, with the exception of a significant heterogeneity with age observed in one study. The CYP2E1 RsaI polymorphism was indeed significantly more frequently observed in subjects who had no drinking experience although being above 68 years (odds ratio=2.4), while the association was reversed at ages below 47 years (OR=0.5) (Raimondi et al., 2004). This study will require replication, and the genes involved in the other metabolic pathways seem to have a much clearer impact on alcohol consumption.

2.2 Alcohol dehydrogenase enzyme

Alcohol dehydrogenase (ADH) is a dimeric protein responsible for the majority of ethanol oxidation, and constitutes a complex family in humans. Class I to V ADH isozymes exhibit tissue-specific distribution (table I).

Table 1. Classification of ADH enzymes

Class (tissue)	Gene	Allele	Sub-unit	Km*	Vmax#	Location
I	ADH1	ADH1	α	4.40	23	Liver
	ADH2	ADH2*1	β1	0.05	9	Liver, lung
		ADH2*2	β2	0.94	340	Liver, stomach
		ADH2*3	β3	34.00	320	
	ADH3	ADH3*1	γ1	1.00	88	
		ADH3*2	γ2	0.63	35	
II	ADH4	ADH4	π	34.00	20	Liver
III	ADH5	ADH5	χ	1,000.00		Ubiquitous
IV	ADH7	ADH7	σ,μ	37.00	1510	Oesophagus, stomach
V	ADH6	ADH6	?	?	?	Liver

*Km (Michaelis constant) represents the concentration of ethanol for which the enzyme is activated at 50% of its biggest potential. #Vmax represents the maximum speed of the enzyme activity, by enzyme mol and minute.

The allozymes exhibit distinct maximal activities due to single amino acid exchanges at different sites in the coenzyme-binding domain. Six *ADH* genes have been characterized (Pastino et al., 2000). Among them, only the *ADH2* and *ADH3* isoenzyme are polymorphic (table I). Among the *ADH2* locus, the $ADH2^1$ encodes for the β^1 subunit with low activity, rarely expressed in Japanese (Bosron et al., 1986) and $ADH2^2$ encodes for the β^2 subunit with high activity. The *ADH3* locus has two alleles: $ADH3^1$, which encodes for the γ1 subunit; and $ADH3^2$, which encodes for the γ^2 subunit (Hashimoto et al., 2000). Because of the large difference in kinetic properties of the β^1 and β^2 subunits, the *ADH2* gene polymorphisms play an important role in individual variations regarding ethanol elimination (Bosron et al., 1986). The ADH2 His47 allele increases the rate of acetaldehyde formation, so that the altered enzymatic function due to this polymorphism leads to an accumulation of acetaldehyde after alcohol intake.

2.3 Acetaldehyde dehydrogenase

Acetaldehyde is mainly oxidized to acetate by the activity of the enzyme acetaldehyde dehydrogenase (ALDH) (Bosron et al., 1986). The acetate produced as a result of acetaldehyde oxidation is rapidly metabolized to carbon dioxide and water. Two major isoforms of ALDH have been identified which play a major role in hepatic acetaldehyde metabolism. Of these, the mitochondrial form (ALDH2) is the more important. The ALDH2 gene has two polymorphic forms; the 'wild' type gene ($ALDH2^1$) encodes the active enzyme whereas the 'mutant' form ($ALDH2^2$) encodes an inactive enzyme (Hsu et al., 1985; Yoshida et al., 1985). The mutant allele is rarely observed in Caucasians but is found in some 40% of Orientals. Individuals carrying the mutant allele

have a markedly reduced capacity to metabolize acetaldehyde and the resultant increase in circulating acetaldehyde concentrations.

2.4 Genetics of the flushing reaction to alcohol

Acetaldehyde, which is normally virtually absent from the blood because of the high speed of alcohol metabolism, causes a specific reaction entitled "flushing reaction". This reaction is very similar to that produced if alcohol is consumed following drugs that block aldehyde dehydrogenase (for example Disulfiram, which is an antabuse treatment for alcohol-dependence). Facial flushing associates different symptoms such as palpitations, perspiration, nausea (occasionally with vomiting), and headache (Harada et al., 1981; Johnson et al., 1984). Physiological flushing when drinking small amount of alcohol is inherited as a dominant trait (Schwitters et al., 1982b). Studies of ethnic differences in alcohol-induced facial flushing indicate that the phenomenon is more common in Asians (50–80%) than Caucasians (3–12%) (Goedde and Agarwal, 1987; Schwitters et al., 1982a).

All enzyme specificities that reduce the speed of acetaldehyde catabolism (such as slow ALDH), or increase the speed of acetaldehyde production (such as fast ADH), may increase the risk of facial flushing. Indeed, the *ALDH2*[2] gene contributes to the manifestations of increased blood acetaldehyde after alcohol drinking (Harada et al., 1981; Takeshita et al., 1985). ALDH2 is responsible for most acetaldehyde metabolism in hepatocytes, and the inactive allele Lys487 acts dominantly. In the epidemiology of alcoholism, the ALDH2 Glu487Lys polymorphism plays the most important role. Glu487/Lys487 heterozygotes have little residual enzyme activity because one Lys487 subunit is sufficient to largely inactivate the ALDH2 tetramer. The flushing reaction is immediate in Glu487/Lys487 heterozygotes after consumption of even one drink of alcohol (Harada et al., 1982), and is most severe in Lys487/Lys487 homozygous individuals. The frequency of Lys487 is approximately 30% in Japanese and Chinese (Bosron and Li, 1986; Thomasson et al., 1994; Higuchi et al., 1995; Chen et al., 1996). Therefore, approximately half the Japanese and Chinese populations experience flushing after alcohol consumption.

The action of the ALDH2 Lys487 allele is additive with the ADH2 His47 allele (Chen et al., 1996), a catalytically more active ADH2 allele that is independently associated with higher acetaldehyde levels and flushing (Impraim et al., 1982; Hsu et al., 1988). The His47 allele is also found in non-Oriental populations. Monteiro et al., (1991) estimated that His47 accounts for 20 to 30% of the variance in alcohol intake variance between two groups of light drinking and heavy drinking Israeli Jews, and proposed that the relatively high frequency of the His47 allele in that population might contribute to lower levels of alcohol consumption among Jews. An ADH3 polymorphism Ile271Val also produces a difference in enzyme activity, and the superactive allele (Val271) is again more abundant in East Asia (Tanaka et al., 1992; Edenberg and Bosron, 1997).

Because the ALDH2 Lys487 and ADH2 His47 alleles, aversive for alcohol intake, are common in Asian countries (including China, Japan, and Korea) but rare in Caucasian and African populations (Enomoto et al., 1991; Yoshida et al.,

1991; Goedde et al., 1992; Bosron et al., 1993), they have been studied to explain why rates of alcohol dependence are lower in many Asian populations compared to Caucasian populations. Even at an individual level, there has been so far only one reported case of an alcoholic Lys487/Lys487 homozygote, and this individual had an unusual drinking pattern in which small amounts of alcohol were drunken regularly throughout the day (Chen et al., 1999b). The genetic polymorphisms of ADH and ALDH enzymes could thus modulate the risk of alcohol-dependence. Increasing negative consequences of drinking alcohol use, but does not abolishes, the risk of alcohol abuse. In Lys487/Glu487 heterozygotes, the risk of alcoholism is reduced 4- to 10-fold (Thomasson et al., 1994). The ADH3 gene could also be involved, but findings of association of ADH3 to alcoholism vulnerability appear to be attributable to linkage disequilibrium with ADH2, located in the same gene cluster on chromosome 4q, at a distance of only 15 kb (Chen et al., 1999a; Osier et al., 1999).

It is of interest that the protective effect of alcohol metabolic gene polymorphisms may have a different impact according to environmental backgrounds or thresholds (Goldman, 1993). Tu and Israel (1995) found that the ALDH2-specific odds ratio for alcoholism was only about 2:1 among individuals of Korean and Taiwanese ancestry when they were born in North America. Acculturation accounted for 7 to 11% of the variance in alcohol consumption, and the ALDH2 polymorphism predicted two-thirds of the vulnerability to alcohol consumption and excessive alcohol use. Furthermore, in Southeast Asian populations with similar ALDH2 Glu487Lys allele frequencies, there are large differences in the prevalence of alcohol dependence (i.e., 2.9% in Taiwan and 17.2% in Korea).

These results concern alcohol metabolism, without considering the effect of ethanol on the brain. If it is easy to admit that genes that increases the negative effects of drinking modulates the risk of alcohol-dependence, it is important to look at other candidate genes that may explain why few patients that use alcohol do get dependent, even in populations that do not have mutant forms of the ADH and ALDH enzymes, and why some patients with mutant forms of these enzymes can become alcohol-dependent. For this, the direct impact of alcohol on the brain, and more specifically on reward circuits is needed.

3. NEUROTRANSMITTER SYSTEMS INVOLVED IN ALCOHOL CENTRAL EFFECTS

Neurotransmitter systems involved in alcohol dependence include pathways triggered by the excitatory amino-acid glutamate, the inhibitory neurotransmitter GABA, the monoaminergic neurotransmitters dopamine, serotonine, noradrenaline as well as endogenous opioids, anandamide and other neuropeptides (Nevo and Hamon 1995).

3.1 Glutamate

Glutamate is the main excitatory neurotransmitter. While it can be detected throughout the brain, its main areas of expression are the cortex and the limbic system (Tsai and Coyle 1998). Post-synaptic glutamatergic signalling is mediated through ligand-activated ion channels, the ionotropic glutamate receptors or G-protein-coupled receptors, the metabotropic glutamate receptors (Collingridge und Lester 1989, Michaelis 1998).

Acute alcohol intake leads to inhibition of glutamatergic activity (Tsai und Coyle 1998, Spanagel and Bienkowski 2002), which is mediated by different biochemical mechanisms (Nie et al., 1994). Alcohol inhibits NMDA-receptor signalling by interacting with a glycine binding site with this receptor (Mihic et al., 1997, Mascia et al., 2000). Voltage-clamp experiments in hippocampal neurons revealed, that a concentration of 50 mM of ethanol is sufficient to reduce NMDA-activated currents by 60% (Lovinger et al., 1989). Additionally, glutamatergic signalling is reduced by an inhibitory input of GABA, which is activated upon acute alcohol intake (Spanagel and Bienkowski, 2002).

Chronic administration of ethanol, however, is thought to lead to a compensatory up-regulation of NMDA-receptors and resulting in tolerance to higher dosages of ethanol. In case of acute ethanol withdrawal and consecutive abrogation of inhibition, these up-regulated receptors are thought to mediate a hyperexcitatory state, which underlies the clinical symptoms of ethanol withdrawal (Tsai and Coyle 1998).

NMDA-receptor subunits are differentially expressed in brain regions and have distinct sensitivities to the inhibitory effects of ethanol. NMDA receptor subunits 2A and 2B were shown to be more sensitive to the inhibitory effects of ethanol than subunits 2C and 2D (Spanagel und Bienkowski 2002), suggesting that the receptor subunit composition is an important factor determining alcohol sensitivity in glutamatergic neurotransmission (Kumari und Ticku 2000). Nevertheless, various genetic association studies did not show an association of the NMDA receptor 2B subunit with alcohol dependence (Schumann et al., 2003a, Wernicke et al., 2003).

As acute ethanol intake inhibits NMDA-receptor function and results in a decreased Ca^{2+}-influx, a reduction of PKC activity was consequently observed (Kruger et al., 1993, Slater et al., 1993). Chronic ethanol administration, however, leads to increased activity due to increased expression of PKC isoenzymes δ and ε (Roivainen et al., 1994).

Chronic exposition to ethanol decreases the activity of the cAMP-signal transduction pathway, which is mediated through stimulatory G-proteins, adenylate cyclase, PKA and CREB (Pandey 1998, Davis and Wu 2001).

Regulation of calcium influx is not only subject to activation or inhibition of the NMDA-receptor with substances such as glutamate or ethanol, but is also exerted by regulatory proteins, which can influence alcohol drinking behaviour. One such protein is protein tyrosin kinase “fyn”, an intracellular src-like kinase which regulates NMDA-receptor activity by phosphorylating NMDA receptor 2A and 2B subunits (Cheung und Gurd 2001). Fyn-knock out mice show increased alcohol sensitivity and a genetic variation in the regulatory domain of

the PTK fyn gene was shown to be associated with alcohol dependence (Schumann et al., 2003b).

3.2. GABA

Gamma-amino-butyric acid (GABA) is the main inhibitory neurotransmitter. GABA synthesis from glutamate is catalysed by the enzyme GAD, the GAD1 and GAD2 isoforms of which have been implicated in alcohol withdrawal in genetic analyses of quantitative trait loci (QTL) (Buck 1997, Fehr et al., 2003). Similar to glutamate receptors, GABA receptors can be divided into ionotropic receptors, which are ligand-gated chloride channels ($GABA_A$, $GABA_C$) and metabotropic, G protein-coupled receptors ($GABA_B$), both eliciting an inhibitory post-synaptic potential (IPSP) (Davis and Wu 2001). The most frequent $GABA_A$ receptor complex in the human brain is a pentameric complex, consisting of α-, β-, and γ-peptides (Mihic and Harris 1997). Presently, 17 isoforms of the α-, β-, and γ-subunits have been identified in the mammalian brain (Davis und Wu 2001). The functional heterogeneity of the $GABA_A$ receptors is manifested in isoform-specific pharmacological properties as well as differential expression in distinct brain regions and developmental stages (McKernan und Whiting 1996).

The sedating effects of benzodiazepines as well as ethanol are mediated through ligation with specific binding sites, which result in activation of central $GABA_A$-receptors (Davis and Wu 2001, Heinz and Batra 2003). Chronic ethanol intake leads to compensatory down-regulation of $GABA_A$ receptors, resulting in increased alcohol tolerance. Upon acute alcohol withdrawal a reduced GABA-ergic activity will reduce inhibitory signals and contribute to clinical withdrawal symptoms (Mihic and Harris 1997; Heinz and Batra 2003).

The relevance of the $GABA_A$ receptor complex is supported by genetic studies showing an association of a functional variant of the α6-receptor subunit leading to a proline/serine amino acid exchange with alcohol tolerance and risk for alcohol dependence (Schuckit et al., 1999).

3.3. Dopamine

In the human brain five different dopamine receptor subtypes DRD1 – DRD5 were identified and classified in two families: The D1 receptor family is characterised by the activation of stimulatory G_s-proteins and consists of the DRD1 and DRD5. The D2 receptor family, which consists of DRD2, DRD3 and DRD4, is characterised by activation of inhibitory G_i-proteins as well as regulation of K^+-ion channels. (Forth 1996).

Dopamine secretion is inhibited by the effect of GABAergic neurons on dopaminergic neurons in the nucleus accumbens. Activation of opioid neurons in the ventral tegmental area induces secretion of beta-endorphin, which binds to μ-opioid receptors on the terminals of GABAergic neurons, thus, reducing the GABAergic inhibition of dopaminergic neurons (Mansvelder und McGehee 2002).

Activation of the dopaminergic system is a crucial property of all known addictive drugs. Dopaminergic neurons are localised in the brain stem and modulate many brain areas via ascending fibres. Dopaminergic activity is correlated with specific behavioural features, such as motivation and reward (Di Chiara 1997, Baumgarten und Gorzdanovic 1997, Wise 1988), which are triggered by dopaminergic stimulation of the nucleus accumbens in the ventral striatum (Robinson und Berridge 1993, Di Chiara 1995, Heinz 2000). Conditioning experiments have shown that receptors of both the D1 family (Shippenberg und Herz 1987, Weed und Wolverton 1995) and the D2 family (Le Foll et al., 2000) are involved in mediating positive reinforcement processes. While dopaminergic stimulation is thought to induce craving (Robinson und Berridge 1993; Verheul et al., 1999), activation of opioid receptors in the vebtral striatum could be responsible for the pleasant feeling associated with alcohol intake (Heinz und Batra 2003).

In alcohol dependent patients a reduced sensitivity of hypothalamic DRD2 receptors was observed (Balldin et al., 1992, Heinz et al., 1995a). Animal experiments in rats showed a decrease of density in ventral and dorsal striatal DRD2 receptors upon chronic alcohol administration (Rommelspacher et al., 1992). Furthermore, a reduction of dopamine transporter (DAT) activity in the striatum was observed (Heinz et al., 2000). Thus, chronic alcohol consumption leads to adaptive measures, which counteract excessive stimulation and contribute to the maintenance of homeostasis (Koob und Le Moal 1997).

There has been a very large number of studies on the genetic polymorphisms of receptors, transporter or regulating enzymes related to dopamine transmitter in alcohol-dependence (for review, see Goldman, 1995). The most frequently studied dopamine receptor gene analysed in alcohol dependence is the TaqI polymorphism of the dopamine receptor D2 (DRD2), representing at least 40 different studies and more than 4,500 patients. One hypothesis is that patients with the A1 allele of the DRD2 gene have a “reward deficiency syndrome”, leading to drink more alcohol to get an equivalent “high” effect than patients without this vulnerability allele, thus increasing their risk for alcohol dependence (Comings & Blum, 2000). A pharmacogenetic approach was used accordingly, probably for the first time in alcohol-dependence. Lawford et al., (1995) tested, in a double-blind study, the efficacy of Bromocriptine, a DRD2 agonist, versus placebo, administered to alcohol dependent patients with either the vulnerability A1 only the A2 allele of the DRD2 gene. As the greatest improvement in craving and anxiety occurred in the bromocriptine-treated A1 alcoholics, this study demonstrated that choosing the most adequate treatment on the basis of each patient’s specificities regarding different candidate genes is possible. As bromocriptine is usually not well tolerated, and as the study had different methodological limitations (sample too small, follow-up to short, no replication…), this study has more heuristic than pragmatic virtues.

3.4. Serotonin

Presently, 7 different families of Serotonin transporters have been observed (5-HT1–5-HT7). With the exception of 5-HT3, which is a ligand-activated ion channel, all are G-protein-coupled receptors. Serotonin is synthesized in neurons originating from the raphe nuclei of the brain, an area which influences attention, emotion and motivation (Copper et al., 1991, Lovinger 1997). Axons of these neurons extend to many cortical and subcortical regions, including the amygdala and the nucleus accumbens, where they secrete Serotonin (Baumgarten und Grozdanovic 1997; Lovinger 1997).

Chronic consumption of alcohol stimulates serotonin release and compensates for an existing serotonin deficit (Fils-Aime et al., 1996, Heinz und Batra 2003). A pharmacologic decrease of the central 5-HT concentration results in increased alcohol consumption (Nevo and Hamon 1995).

Genetic studies have demonstrated associations of 5-HT1B (Tyndale 2003; Lappalainen et al., 1998) and 5-HT2A (Tyndale 2003) with different phenotypes of alcohol dependence. Ethanol leads to increased ion-influx mediated by 5-HT3 receptors (Lovinger und Zhou 1994).

Synaptic concentration of serotonine is regulated by the serotonine reuptake transporter (5-HTT) (Lovinger 1997). Chronic alcohol consumption leads to an adaptive decreased activity of this transporter (Heinz et al., 2004). A functional variant in the promoter of 5-HTT consisting of a variable number of tandem repeats (VNTR) and leading to differential activity of the 5-HTT (Lesch et al., 1996) was associated with alcohol dependence in several studies (Türker et al., 1998, Schuckit et al., 1999, Sander et al., 1998). While selective serotonin reuptake inhibitors (SSRI's) influence alcohol intake in patients, the interindividual response is very large, leading to a decrease of alcohol consumption between 10 70% (Naranjo und Knoke 2001).

The recent development of a 5-HT3 antagonist, Ondansetron®, in alcohol-dependence, interestingly had a specific activity (regarding decrease in drinks per day, drinks per drinking day, and alcohol-related problems) in young onset alcohol dependent patients, excluding late onset patients. As Cloninger showed that young onset alcohol dependence (i.e., type II) has a more important heritability than type I alcohol dependence (Gilligan et al., 1987), it is possible that genetics, for example 5-HT_3 genetic polymorphisms, may help to disentangle this specific activity.

3.5. Noradrenaline

Biochemical studies show that alcohol withdrawal leads to increased release of noradrenaline. Furthermore, severity of withdrawal positively correlates with noradrenaline concentration (Linnoila et al., 1987), indicating activation of the sympathetic nervous system. The murine gene for the noradrenaline transporter (NET) is localised on chromosome 8, in a region where a QTL for alcohol sensitivity was identified. In genetic studies, however, no association with variants of this gene and phenotypes of alcohol dependence could be demonstrated so far (Samochowiec et al., 2002).

3.6. Opioids

Endogenous opioids include endorphins, enkephalins and dynorphines, which bind to μ-, δ- and κ-opioid receptors, respectively (Akil et al., 1998, Smith und Lee 2003). While enkephaline-producing neurons are localised in many different brain regions, beta endorphine- and dynorphin-producing neurons are mainly localised in the hypothalamus (Froehlich 1997).

Opioidergic signal transduction is mainly mediated by $G_{i/o}$-proteins, consisting of an α– and a βγ-subunit, which bind to different effectors, thereby regulating signal transduction through adenylate cyclase-, phosphoinositol- or MAP-kinase pathways (Fábián 2001). In target neurons, opioid receptor signalling has an inhibitory effect, which can manifest itself as pain relief, euphoria or behavioural alterations (Froehlich 1997).

Ethanol alters binding properties of opioid receptors, synthesis and secretion of endogenous opioids (Herz 1997). Alcohol consumption leads to the secretion of endorphins (Johnson and Ait-Daoud 2000), which bind to μ-opioid receptors resulting in an inhibition of GABAergic neurons, which express μ-opioid receptors. Reduced GABAergic output leads to disinhibition of dopaminergic neurons in the striatum and the ventral tegmentum, thus leading to an increased synaptic dopamine availability (Spanagel et al., 1992, Heinz und Batra 2003) and the feeling of pleasure related to alcohol consumption (Herz 1997).

Conversely, opioid receptor antagonists such as naltrexone can reduce alcohol intake and decrease the relapse rate in patients with alcohol dependence (Nille, 2000). Their success in relapse prevention, however, is limited. Characterisation of specific subgroups of addicts, which respond better to the given medications may aid in predicting the results this medication.

3.7. Cannabinoids

Identification and cloning of the cannabinoid receptor (Matsuda et al., 1990) led to increased molecular biological studies of the role of this system in addiction disorders. Cannabinoid receptor type 1 is a G protein-coupled receptor activating adenylate cyclases (Rhee et al., 1998) and PKA (Deadwyler et al., 1995). Alternative signal transduction pathways are mediated by Phosphoinositol – 3 Kinase (PI3Kinase) and MAP-kinase pathway (Sanchez et al., 1998).

Recent studies have demonstrated a neuromodulatory role for the endocannabinoid system in alcohol dependence. Chronic alcohol consumption leads to an increased secretion of CB1-receptor agonists and a consecutive downregulation of the CB1 receptor (Basavarajappa und Hungund 2002). Since activation of the CB1-recptor system increases search for alcohol in animal models, a participation of this system in excessive alcohol intake is likely (Basavarajappa & Hungund 2002). Furthermore, the blockage of the CB1 receptor with a specific antagonist (SR147778, Rimonabant®) blocks the consumption of alcohol in rodents. Indeed, Rimonabant® decreases rewarding effects of opiates, nicotine, cannabis and alcohol in animals (Cohen et al., 2002). Hence, it is of major interest in the treatment of addictions, as it has already been shown for the treatment of nicotine dependence (LeFoll et al., 2004).

Genetic studies of the CB1 gene (CNR1, 1359A/A polymorphism) in alcohol dependent patients showed contradictory results, with one positive association (Schmidt et al., 2002) with severe withdrawal symptoms such as delirium tremens, and one negative result with the same phenotype (Preuss et al., 2003). The complete sequence of the CB1/Cnr1 human gene is now available, and will certainly allow pharmacogenetic analyses of Rimonabant efficacy in tobacco dependence, and potentially in other addictions.

4. TREATMENT OF ALCOHOL-DEPENDENCE

The μ-opioid receptor antagonist Naltrexone and Acamprosate, which interacts primarily with the glutamatergic system, are the two most commonly used drugs for secondary relapse prevention in alcohol dependence in Europe. A number of double blind studies conducted during the last decade have shown that both, Naltrexone and Acamprosate prevent relapse in a relevant portion of patients – but not in all (Garbutt et al., 1999, Berglund et al., 2003). The results of recent meta-analyses suggest that the number of patients needed to treat in order to prevent one additional relapse of alcohol is about 7.5 for Acamprosate (Mann et al., 2004). This ratio points towards the need to identify predictors for response to pharmacological relapse prevention. Pharmacogenetic research may be useful in attaining this goal. Whereas for Acamprosate no pharmacogenetic analyses have been preformed, a recent study describes a dramatic improvement in treatment success for a specific genotype of a genetic variation of the μ-opioid receptor (Oslin et al., 2003.) Therefore, we will focus in this chapter on the biological effects of Naltrexone as they relate to secondary relapse prevention and to the identification of genetic predictors for treatment success.

One pharmacogenetic study analysed the impact of Naltrexone, an antagonist for the mu-opioid receptor, on the reduction of “rate of relapse” and “time to return to heavy drinking” in 82 alcohol-dependent patients who successfully completed detoxification from alcohol, compared to 59 controls. The potential impact of this pharmacogenetic approach is particularly rich according to the biological background of alcoholism, as detailed in the “neurotransmitter systems involved in alcohol central effects” section.

Indeed, many studies showed the potential interest of Naltrexone in alcohol-dependent patients. Volpicelli initially showed that Naltrexone reduced the risk of relapse (one alcohol-dependent patient out of four relapsed with Naltrexone compared to one patient out of two when treated by placebo), but also for the intensity of alcohol craving and days in which any alcohol was consumed (Volpicelli et al., 1992). As only 35 patients were treated by Naltrexone, this study could have been considered as a preliminary finding. Nevertheless, a nice replication was published in the same issue of the *Archives of General Psychiatry*. In a population of nearly 100 alcohol-dependent patients, Naltrexone was found superior to placebo in measures of drinking and alcohol-related problems, including abstention rates, number of drinking days, relapse, and severity of alcohol-related problems (O’Malley et al., 1992). The efficacy of

Naltrexone was assessed in other studies, the majority of them confirming the efficacy of this compound (Oslin et al., 1997; Chick et al., 2000; Anton et al., 2001; Monti et al., 2001; Morris et al., 2001), although not systematically (Kranzler et al., 2000; Krystal et al., 2001). Interestingly, some clues were proposed focusing on which clinical parameter Naltrexone could be effective. As alcohol dependence phenotype and addiction processes are particularly complex and heterogeneous, it is important to disentangle the mechanism by which a treatment may help patients to maintain their abstinence. A correct phenotype definition in pharmacogenetics is as important as looking at the appropriate polymorphism(s) of the involved gene(s).

Naltrexone blocked the euphoria produced by ethanol (Volpicelli et al., 1995; King et al., 1997). This clinical symptom of "feeling high" constitutes one of the most frequently quoted factor to explain relapse, and is presented as a way to cope against depressed mood (Strowig, 2000). As an opioid antagonist should block or reduce the effect of alcohol on opioid receptor activity, treated subjects are expected to be less reinforced by alcohol. Indeed, Voplicelli et al., (1995) found that from the reported alcohol effects during lapse from abstinence by detoxified alcoholics receiving Naltrexone, feeling "high" was the only parameter that significantly distinguished patients treated by Naltrexone compared to those treated by placebo. This was not the case for craving, but the limited size of the sample could not depict symptoms with too small effect.

The efficacy of Naltrexone in detoxified alcohol-dependent patients may alternatively be specifically involved in reducing the risk to shift from "slip" (i.e., punctual and occasional drinking) defined as less than 5 glasses on one drinking occasion, and less than 5 consecutive drinking days) to "relapse", as the primary effect of Naltrexone was seen in patients who drank any alcohol while attending outpatient treatment. (Volpicelli et al., 1992). In this view, the concepts of "binge drinking" or "losing control" may be more specifically involved regarding Naltrexone efficacy. A small dose of opiates increase alcohol drinking in rats (Hubbel et al., 1988), the opioidergic effect of the first drink may enhance alcohol craving and relapse. When opiate receptors are effectively blocked by Naltrexone, the first drink would not increase opioid activity, hence not eliciting further alcohol drinking.

An alternative mechanism could implicate the modification of stress coping capacity, as some alcohol-dependent patients sustain a relative deficiency in endogenous opioids after experiencing a stressful life event (Volpicelli, 1987; Volpicelli et al., 1990; Kreek, 1996). Fuerthermore, cortisol stimulation in early abstinent alcoholics showed a blunted response after psychosocial stress (Lovallo et al., 2000). A successful response to stress plays an important role in maintaining health and well-being. In recently detoxified alcoholics, the HPA system is indeed dysregulated with non-suppression of cortisol after dexamethasone administration. Corticotropin releasing factor (CRF) neurons, within the paraventricular nucleus of the hypothalamus, initiate activation of the hypothalamic-pituitary-adrenal (HPA) axis (Bell et al., 1998) and express mu-opioid receptors. CRF neurons are thus modulated by inhibitory tone imposed by β-endorphin neurons originating in the arcuate nucleus (Wand et al., 1998). Central nervous adaptation to the chronic action of alcohol can be observed in

the functional state of hypothalamic peptides regulating HPA system function (Madeira and Paula-Barbosa 1999), and subsequent changes in pituitary and adrenal regulation that are associated with chronic alcoholism and withdrawal can be observed in the peripheral circulation.

Whatever the exact mechanism(s) involved for the treatment efficacy of Naltrexone, a large inter-subjects variability is observed. Ethanol increase, in a dose-dependent manner, the plasma level of beta-endorphin-related peptides of subjects with a history of alcoholism, but not of subjects from families without a history of alcoholism (Gianoulakis et al., 1996). The role of family history as a predictor of treatment response has led to speculation that naltrexone may function differently in genetically predisposed individuals (Oslin et al., 2003), leading to the analysis of genetic polymorphisms of the receptor antagonised by Naltrexone, i.e., the Opioid Receptor μ 1 (OPRM1).

A classical "*problem*" in psychopharmacogenetics is that candidate genes involved in the vulnerability to the disorder (i.e., vulnerability genes) have to be distinguished from candidate genes involved in the therapeutic response to the treatment of the disorder (i.e., pharmacogenetic genes). It is possible and expected that a gene coding for a protein which is involved in one of the neurobiological pathways of the disorder (i.e., dopamine in schizophrenia, serotonin in depression, endorphin in addiction…) is also analysed in pharmacogenetics because it belongs to specific targets of the treatment which improves affected patients (i.e., antipsychotics in schizophrenia, antidepressants in mood disorders…). In this view, the OPRM1gene was initially analysed in addict patients compared to healthy controls in a large series of studies.

At least fourty-three variants were identified within the OPRM1 gene which was physically mapped to the chromosomal region 6q24-25 (Wang et al., 1993). Some mutations are potentially of specific interest. Some are located in the 5' untranslated region (T-1793A, -1699Tinsertion, A-1320G, G-172T, C-111T and C-38A), thus potentially affecting transcription rate. Three of the genetic polymorphisms (Ser4Arg; C17T [A6V] and A118G [N40D] [G24A]) may have functional consequences on the binding affinity of the receptor as they modify the amino acid sequence on the N-terminal region. Lastly, other genetic polymorphisms are located either in the third transmembrane domain (C440G [Ser147Cys] ? [N152D]) or in the third intracellular loop (G779A [R260H], [R265H], [S268P] and [D274]) (G877A [Ile Val]) (Bergen et al., 1997; Bond et al., 1998; Befort et al., 2001; Crowley et al., 2003; Wang et al., 2001). If domains in the third intra-cellular loop (R260H, R265H) have been shown to alter both G proteins coupling and calmodulin binding (Wang et al., 2001), the majority of studies were devoted to the impact of the A118G polymorphism. Indeed, the most prevalent single nucleotide substitution is at position 118 (the G118 allele being present in 10% to 15% in the Caucasian population), predicting an amino-acid change at a putative N-glycosylation site.

This A118G variant receptor binds beta-endorphin, an endogenous opioid that activates the μ opioid receptor, approximately three times more tightly than the most common allelic form of the receptor (A118), without showing altered binding affinities for other opioid peptides and alkaloids (Bond et al., 1998). Furthermore, β-endorphin is approximately three times more potent at the

A118G variant receptor than at the most common allelic form in agonist-induced activation of G protein-coupled potassium channels (Bond et al., 1998). Not all studies showed abnormalities of the OPRM1 receptor in patients with the 118G allele (compared to wild type allele), whether it concerns alteration of the glycosylation status of the receptor or binding affinities (Befort et al., 2001). Nevertheless, apart from sporadic finding about patients who had a different tolerance for morphine-6-glucuronide with versus without the G118 allele (Lötsch et al., 2002a), pupil constrictory after administration of morphine, or morphine-6-glucuronide (its active metabolite), was significantly correlated with the number of this allele (0,1 or 2), presence of the 118G allele reducing the potency of M6G in humans (Lötsch et al., 2002b). At a more clinical level, heroin-dependent patients carrying both G31A and C118G genotypes consumed relatively more drugs when compared to other addicts (Shi et al., 2002), although the latter genotype was not independently associated with higher consumption.

The role of the OPRM1 on cortisol response could also be modified in patients carrying the G118 allele. Those patients have higher cortisol concentrations at baseline and after naloxone infusion than subjects with the wild type allele (Hernandez-Avila et al., 2003). Furthermore, subjects expressing the A118G receptor variant had greater cortisol response to opiate receptor blockade (Wand et al., 2002).

If the A118G variants of the OPRM1 gene binds differentially β-endorphin, this polymorphism offer an interesting candidate gene in addictive disorders such as opiate dependence. Indeed, the genotype distribution was found comparable in opiate dependent patients compared to healthy controls in different samples, whether in was in 180 Caucasians (Schinka et al., 2002) or 100 (Tan et al., 2003) and 200 (Szeto et al., 2001) Chinese patients. Nevertheless, negative studies were also published with samples of comparable size (Berrettini et al., 1997) or even larger (Crawley et al., 2003; Franke et al., 2001. German; Gelernter et al., 1999. European and African).

The association that was sometimes detected seemed to be unrelated to a specific type of dependence (Schinka et al., 2002). In addition, the A118G polymorphism seems to have a role on cortisol level and/or response, which may be involved in the vulnerability to other type of dependence, such as alcoholism. Just as for opiate dependence, case-control studies have failed to demonstrate a consistent association between OPRM1 sequence variation and the presence of alcohol dependence (Bergen et al., 1997; Berrettini et al., 1997; Bond et al., 1998; Sander et al., 1998; Gelernter et al., 1999; Town et al., 1999; Gscheidel et al., 2000; Franke et al., 2001; Szeto et al., 2001; Schinka et al., 2002; Crowley et al., 2003), although sometimes showing a positive association (Kranzler et al., 1998; Hoehe et al., 2000; Rommelspacher et al., 2001).

A German group tried to give sense to these discrepant results in alcohol-dependence, looking at the role of the A118G genotype on an endophenotype of alcohol-dependence, i.e., variation of central dopaminergic sensitivity during alcohol withdrawal (Smolka et al., 1999; Rommelspacher et al., 2001). In fact, the dopaminergic reward system is activated by both ethanol and opioids, and genetically determined differences in the sensitivity of the endogenous opioid

system to alcohol among various individuals may be an important factor determining their risk for alcohol consumption. In two different samples, the GH response (stimulated by apomorphine) measured seven days after alcohol withdrawal was significantly increased in alcoholics with the Asn40Asp genotype compared with those carrying the Asn40Asn genotype (Smolka et al., 1999; Rommelspacher et al., 2001).

If the role of the A118G genetic polymorphism is difficult to demonstrate on the large phenotype of addictive disorders, focusing on a specific aspect, related to a precise neurobiological mechanism (i.e., an endophenotype), could be highlighting. Following the same idea, but even closer to the potential impact of this polymorphism, Oslin et al., (2003) analysed the clinical response of detoxified alcohol-dependent patients to Naltrexone, shifting from an endophenotype to a pharmacogenetic approach. This study probably represents the single psychopharmacogenetic analysis in addictive disorder that was performed until now. In this view it has many interesting aspects but also a series of pitfalls. Comparing patients treated by the active compound (Naltrexone) (1) with those treated by placebo, on the basis of a (2) follow-up analysis, taking into account rates of relapse but also (3) the time taken to return to heavy drinking, (4) assessing the percentage of patients treated by cognitive-behavioral therapy and (5) looking for the interaction between the A118G genotype and medication to explain the clinical variables of relapse constitute five important *strenghts* of this work. On the other hand, the sample is based on 120 alcohol-dependent patients only, and is derived from three studies which initially represented 466 patients, questioning the representativeness of the studied sample. Hence, the comparisons are mainly based on the 6 patients (out of 23) carrying the G allele who relapsed during the 12 weeks follow-up with the 25 others (out of 48) who did not relapse but who carried the AA genotype. Furthermore, because of this sample of limited size, it was not possible to focus on which core clinical features or group of patients this genotype might have a significant impact in predicting efficacy. For example, and in accordance with the three points developed before, it will be important to assess the clinical parameter for which Naltrexone could be effective knowing the genotype for the OPRM1 gene, such as possible euphoria during alcohol-consumption, occasional drinking during the follow-up, and importance of the stress that the patients faced during this same period. Knowing these three parameters will help to depict by which mechanisms the OPRM1 gene might be involved in the Naltrexeone efficacy. In another point of view, a pharmacokinetic approach should also be addressed, controlling for the inter-subjects variability of the correct dosage, even if the recent development of a depot Naltrexone may reduce the importance of this parameter (Kranzler et al., 2004). Clearly, taking into account these aspects when testing the role of a gene in order to detect which patient would benefit more clearly of such a product would mean a much larger sample of patients than the first published one.

5. REFERENCES

Adinoff B, Martin P.R., Bone G.H.A., Eckardt M.J., Roehrich L., George D.T. et al., 1990, Hypothalamic-pituitary-adrenal axis functioning and cerebrospinal fluid corticotropin releasing hormone and corticotropin levels in alcoholics after recent and long-term abstinence, *Arch Gen Psychiatry.* **47**, pp. 325-330.

Akil H, Owens C, Gutstein H, et al., 1998, Endogenous opioids: overview and current issues, *Drug and Alcohol Dependence*. **51**:127-140.

Anton R.F., Moak D.H., Latham P.K., Waid L.R., Malcolm R.J., Dias J.K. et al., 2001, Posttreatment results of combining naltrexone with cognitive-behavior therapy for the treatment of alcoholism, *J Clin Psychopharmacol*. **21**:72-77.

Balldin J.I., Berggren U.C., Lindstedt G., 1992, Neuroendocrine evidence for reduced dopamine receptor sensitivity in alcoholism, *Amc Clin Exp Res*. **16**:71-74.

Basavarajappa and Hungund, 2002.

Baumgarten H.G., Grozdanovic Z., 1997, Anatomy of central serotoninergic projection systems. In: *Baumgarten HG, Göthert (Hrsg) Handbook of experimental pharmacology*, Vol 129. Serotoninergic neurons and 5-HT receptors in the ZNS. Springer, Berlin, Heidelberg; 41-89.

Befort K., Filliol D., Decaillot F.M., Gaveriaux-Ruff C., Hoehe M.R., Kieffer B.L., 2001, A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs recptor signaling, *J Biol Chem.* Feb **2**: 276(5):3130-7.

Bell S.M., Reynolds J.G., Thiele T.E., Gan J, Figlewicz D.P., Woods S.C., 1998, Effects of third intracerebroventricular injections of corticotropin-releasing factor (CRF) on ethanol drinking and food intake. *Psychopharmacology*, Berl, **139**:128-135.

Bergen A., Kokoszka J., Peterson R., Long J., Virkkunen M., Linnoila M. et al., 1997, Mu-opioid receptor gene variants: lack of association with alcohol dependence, *Mol Psychiatry*. **2**: 490-494.

Berman J.D., Cook D.M., Buchman M. and Keith L.D., 1990, Diminished adrenocorticotropin response to insulin-induced hypoglycemia in nondepressed, actively drinking male alcoholics, *J Clin Endocrinol Metab*. **71**: pp. 712-717.

Berrettini W., Hoehe M., Ferraro T., DeMaria P., Gottheil E., 1997, Human μ opioid receptor gene polymorphisms and vulnerability to substance abuse, *Addict Biol.* **2**:303-308.

Bond C., LaForge K., Tian M., Melia D., Zhang S., Borg L. et al., 1998, Single-nucleotide polymorphism in the human μ opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction, *Proc Natl Acad Sci*. **95**:9608-9613.

Bosron W.F., Li T-K., 1986, Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism, *Hepatology*. **6**:502-510.

Brady K.T., Sonne S.C., 1999, The role of stress in alcohol use, alcoholism treatment, and relapse, *Alcohol Res Health.* **23** (4):263-71.

Buck K.J., Metten P., Belknap J.K., et al., 1997, Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice, *J Neurosci*. **17**: 3946-3955.

Chen S.Y., Liu T.Y., Shun C.T., Wu M.S., Lu T.H., Lin J.T., Sheu J.C., Santella R.M., Chen C.J., 2004, Modification effects of GSTM1, GSTT1 and CYP2E1 polymorphisms on associations between raw salted food and incomplete intestinal metaplasia in a high-risk area of stomach cancer, *Int J Cancer*. Feb **10**;108(4):606-12.

Cheung H.H., Gurd J.W., 2001, Tyrosine phosphorylation of the N-methyl_D-aspartate receptor by exogenous and postsynaptic density-associated Src-family kinases, *J Neurochem.* **78**: 524-534.

Chick J., Anton R., Checinski K., Croop R., Drummond D.C., Farmer R. et al., 2000, A multicentre, randomized, double-blind, placebo-controlled trial of naltrexone in the treatment of alcohol dependence or abuse, *Alcohol Alcoholism*. **35**:587-593.

Choi J.Y., Abel J., Neuhaus T., Ko Y., Harth V., Hamajima N., Tajima K., Yoo K.Y, Park S.K., Noh D.Y., Han W., Choe K.J., Ahn S.H., Kim S.U., Hirvonen A., Kang D., 2003, Role of alcohol and genetic polymorphisms of CYP2E1 and ALDH2 in breast cancer development, *Pharmacogenetics*. Feb;**13** (2):67-72.

Cohen C., Perrault G., Voltz C., Steinberg R. and Soubrie P., 2002, SR141716, a central cannabinoid (CB (1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats, *Behavioral Pharmacology.* **13**:5-6, pp. 451-463.

Collingridge G.L., Lester R.A.,1989, Excitatory amino acid receptors in the vertebrate central nervous system, *Pharmacol Rev*. **41**:143-210.

Comings D.E., Blum K., 2000, Reward deficiency syndrome: genetic aspects of behavioral disorders, *Prog Brain Res*. **126**:325-41.

Copper J.R., Bloom F.E., Roth R.H., 1991, The biochemical basis of Neuropharmacology. 6th edn., Oxford University Press, New York.

Costa A., Bono G., Martignoni E., Merlo P., Sances G. and Nappi G., 1996, An assessment of hypothalamo-pituitary-adrenal axis functioning in non-depressed, early abstinent alcoholics, *Psychoneuroendocrinology.* **21**, pp. 263-275.

Crowley J., Oslin D., Patkar A., Gottheil E., DeMaria P., O'Brien C. et al., 2003, A genetic association study of the μ opioid receptor and severe opioid dependence, *Psychiatr Genet*. Sep; **13**(3):169-73.

Deadwyler SA., Hampson RE., Mu J., Whyte A., Childers S., 1995, Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. *J Pharmacol Exp Ther*. **273**(2):734-43.

Di Chiara G.,1995, The rule of dopamine in drug abuse viewed from the perspective of its role in motivation, *Drug Alc Dependence*. **38**:95-137.

Di Chiara G.,1997, Alcohol and Dopamine, *Alcohol Health und Research World*. **21**: 108-113.

Ehrenreich H., Schuck J., Stender N., Pilz J., Gefeller O., Schilling L. et al., 1997, Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence, *Alcohol Clin Exp Res.* **21**, pp. 1285-1293.

Enomoto N., Takada A. and Date T., 1991, Genotyping of the aldehyde dehydrogenase 2 (ALDH2) gene using the polymerase chain reaction: Evidence for single point mutation in the ALDH2 gene of ALDH2 deficiency, *Gastroenterol Jpn*. **26**:440-447.

Fábián G., 2001, Heterotrimeric G-proteins and their role in opioid receptor function, *Acta Biol Szeged*. **45**:13-21.

Fehr C., Rademacher B.L., Buck K.J., 2003, Evaluation of the glutamate decarboxylase genes Gad1 and Gad2 as candidate genes for acute ethanol withdrawal severity in mice, *Prog Neuropsychopharmacol Biol Psychiatry*. **27**:467-472.

Fils-Aime M.L., Eckhardt M.J., George D.T., et al., 1996, Early-onset alcoholics have lower cerebrospinal fluid 5-hydroxyindolacetic acid levels than late-onset alcoholics, *Arch Gen Psychiatry*. **53**:211-216.

Forth W., 1996, Allgemeine und spezielle Pharmakologie und Toxikologie, 7th edn., *Spektrum Verlag Heidelberg*, Germany.

Franke P., Wang T., Nöthen M., Knapp M., Neidt H., Albrecht S. et al., 2001, Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence, *Am J Med Genet.* **105**:114-119.

Froehlich J.C.,1997, Opioid Peptides, *Alcohol Health Res. World* **21**:132-136.

Gelernter J., Kranzler H., Cubells J., 1999, Genetics of two μ opioid receptor gene (OPRM1) exon 1 polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects, *Mol Psychiatry.* **4**:476-483.

Gilligan S.B., Reich T., Cloninger C.R., 1987, Etiologic heterogeneity in alcoholism, *Genet Epidemiol*. **4** (6):395-414.

Goedde H., and Agarwal D., 1987. Polymorphism of aldehyde dehydrogenase and alcohol sensitivity, *Enzyme*. **37**, pp. 29-44.

Goedde H.W., Agarwal D.P., Fritze G., Meier-Tachmann D., Singh S., Beckmann G., Bhatia K., Chen L.Z., Fang B., Lisker R., et al., 1992, Distribution of ADH2 and ALDH2 genotypes in different populations. *Hum Genet* **88**:344-346.

Goldman D., 1995, Candidate genes in alcoholism, *Clin Neurosci*. **3**(3):174-81.

Grosch S., Niederberger E., Lotsch J., Skarke C., Geisslinger G., 2001, A rapid screening method for a single nucleotide polymorphism (SNP) in the human MOR gene*, Br J Clin Pharmacol*. Dec; **52**(6):711-4.

Gscheidel N., Sander T., Wendel B., Heere P., Schmidt L.G., Rommelspacher H., Hoehe M.R., Samochowiec J., 2000, Five exon 1 variants of μ opioid receptor and vulnerability to alcohol dependence, *Pol J Pharmacol*. Jan-Feb; 52(1):27-31.

Harada S., Agarwal D.P., Goedde H.W., 1981, Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese, *Lancet*. **2**:982.

Hashimoto Y., Nakayama T., Futamura A., Omura M., Nakarai H., Nakahara K., 2002, Relationship between genetic polymorphisms of alcohol-metabolizing enzymes and changes in risk factors for coronary heart disease associated with alcohol consumption, *Clin Chem*. Jul; **48**(7):1043-8.

Hayashi S., Watanabe J., Kawajiri K., 1991, Genetic polymorphisms in the 5' flanking region change transcriptional regulation of the human cytochrome p450 IIEI gene, *J Biochem.* **110**:559-65.

Heinz A., 2000, Das dopaminerge Verstärkungssystem: Funktion, Interaktion mit anderen Neurotransmittersystemen und psychopathologische Korrelate. In: *Hippius H, Sass H, Sauer H (Hrsg) Monographien aus dem Gesamtgebiet der Psychiatrie*. Steinkopff, Darmstadt.

Heinz A., Goldman D., Jones D.W., Palmour R., Hommer D., Gorey J.G., Lee K.S., Linnoila M., Weinberger D.R., 2000, Genotype influences *in vivo* dopamine transporter availability in human striatum, *Neuropsychopharmacology*. **22**:133-9.

Heinz A., Batra A., 2003, Neurobiologie der Alkoholabhängigkeit. In: Brandt T., Cohen R., Helmchen H., Schmidt L.R. (Hrsg) *Psychiatrie, Neurologie, Klinische Psychologie Grundlagen, Methoden, Ergebnisse*. Kohlhammer, Stuttgart.

Heinz A., Dettling M., Kuhn S., et al., 1995, Blunted growth hormone response is associated with early relapse in alcohol-dependent patients, *Alc Clin Exp Res*. **19**:62-65.

Hernandez-Avila C., Wand G., Luo X., Gelernter J., Kranzler H., 2003, Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the mu-opioid receptor locus (OPRM1), *Am J Med Genet*. **118B**:60-65.

Herz A.,1997, Endogenous opioid systems and alcohol addiction, *Psychopharmacology*. **129**:99-111.

Hoehe M.R., Kopke K., Wendel B., Rohde K., Flachmeier C., Kidd K.K .et al., 2000, Sequence variability and candidate gene analysis in complex disease: association of μ opioid receptor gene variation with substance dependence. **9**: 895-908.

Hsu L.C., Tani K., Fujiyoshi T., Kurachi K., Yoshida A., 1985, Cloning of cDNAs for human aldehyde dehydrogenases 1 and 2. *Proc Natl Acad Sci USA*. **82**:3771-3775.

Hubbell C.L., Abelson M.L., Burkhardt C.A., Herlands S.E., Reid L.D., 1988, Constant infusions of morphine and intakes of sweetened ethanol solution among rats, *Alcohol*. Sep-Oct; **5**(5):409-15.

Inder W.J., Joyce P.R., Ellis M.J., Evans M.J., Livesey J.H and Donald R.A., 1995, The effects of alcoholism on the hypothalamic-pituitary-adrenal axis: Interaction with endogenous opioid peptides, *Clin Endocrinol.* **43** pp. 283-290.

Johnson B.A., Ait-Daoud N., Prihoda T. J., 2000, Combining ondansetron and naltrexone effectively treats biologically predisposed alcoholics: from hypotheses to preliminary clinical evidence. *Alcohol Clin Exp Res*. **24**(5):737-42.

Johnson R., Nagoshi C., Schwitters S., Bowman K., Ahern F. and Wilson J., 1984, Further investigation of racial/ethnic differences and of familial resemblances in flushing response to alcohol, *Behavior Genetics.* **14**, pp. 171-178.

Kessova I., Cederbaum A.I., 2003, CYP2E1: biochemistry, toxicology, regulation and function in ethanol-induced liver injury, *Curr Mol Med*. Sep; **3** (6):509-18.

King A.C., Volpicelli J.R., Frazer A., O'Brien C.P., 1997, Effect of naltrexone on subjective alcohol response in subjects at high and low risk for future alcohol dependence, *Psychopharmacology*. **129**:15-22.

Knudsen G.M., Christensen H., Berild D., Melgaard B., Kirkegaard C. and Hasselbalch H., 1987, Discordance between the cortisol response to insulin-hypoglycemia and 30-minute ACTH stimulation test in chronic alcoholic men, *Alcohol Clin Exp Res*. **11**, pp. 323-325.

Koob G.F., Le Moal M., 1997, Drug abuse: hedonic homeostatic dysregulation, *Science.* **278**:52-58.

Kranzler H., Modesto-Lowe V., VanKirk J., 2000, Naltrexone vs nefazadone for treatment of alcohol dependence, *Neuropsychopharmacology*. **22**:493-503.

Kranzler H.R., Gelernter J., O'Malley S., Hernandez-Avila C.A., Kaufman D., 1998, Association of alcohol or other drug dependence with alleles of the μ–Opioid Receptor Gene (OPRMI), *Alcoholism: Clin Exp Res.* **22**:1359-1362.

Kranzler H.R., Pierucci-Lagha A., Feinn R., Hernandez-Avila C., 2003, Effects of ondansetron in early- versus late-onset alcoholics: a prospective, open-label study, *Alcohol Clin Exp R*es. Jul; **27**(7):1150-5.

Kranzler H.R., Wesson D.R., Billot L., 2004, DrugAbuse Sciences Naltrexone Depot Study Group. Naltrexone depot for treatment of alcohol dependence: a multicenter, randomized, placebo-controlled clinical trial*, Alcohol Clin Exp* **Res**. Jul; **28**(7):1051-9.

Kreek M.J.,1996, Opiates, opioids and addiction, *Mol Psychiatry.* **1**:232-254.

Kruger H., Wilce P.A., Shanley B.C., 1993, Ethanol and protein kinase C in rat brain, *Neurochem Int.* **22**:575-581.

Krystal J.H., Cramer J.A., Krol W.F., Kirk G.F., Rosenheck R.A., 2001, Veterans Affairs Naltrexone Cooperative Study G (2001). Naltrexone in the treatment of alcohol dependence, *New Engl J Med*. **345**:1734-1739.

Kumari M., Ticku M.K., 2000, Regulation of NMDA receptors by ethanol, *Prog Drug Res.* **54**: 152-189.

LaForge K.S., Shick V., Spangler R., Proudnikov D., Yuferov V., Lysov Y., Mirzabekov A., Kreek M.J., 2000, Detection of single nucleotide polymorphisms of the human μ opioid receptor gene by hybridization or single nucleotide extension on custom oligonucleotide gelpad microchips: potential in studies of addiction, *Am J Med Genet*. Oct 9; **96**(5):604-15.

Lappalainen J., Long J.C., Eggert M., et al., 1998, Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations, *Arch Gen Psychiatry*. **55**:989-994.

Lawford B.R., Young R.M., Rowell J.A., Qualichefski J., Fletcher B.H., Syndulko K., Ritchie T., Noble E.P., 1995, Bromocriptine in the treatment of alcoholics with the D2 dopamine receptor A1 allele, *Nat Med.* Apr; **1**(4):337-41.

Le Foll B., Goldberg SR., 2004, Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences, *Neuroreport*. Sep 15;**15**(13):2139-43.

Le Foll B., Schwartz J.C., Solokoff P., 2000, Dopamine D3-Receptor agents as potenzial new medication for drug addiction. *Eur Psychiatry*. **15**: 140-146.

Lesch K.P., Bengel D., Heils A., et al., 1996, Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region, *Science*. **274**:1527-1531.

Lotsch J., Skarke C., Grosch S., Darimont J., Schmidt H., Geisslinger G., 2002b, The polymorphism A118G of the human mu-opioid receptor gene decreases the pupil constrictory effect of morphine-6-glucuronide but not that of morphine, *Pharmacogenetics*. Jan;**12**(1):3-9.

Lotsch J., Zimmermann M., Darimont J., Marx C., Dudziak R., Skarke C., Geisslinger G., 2002a, Does the A118G polymorphism at the mu-opioid receptor gene protect against morphine-6-glucuronide toxicity?, *Anesthesiology*. Oct;**97**(4):814-9.

Lovallo W.R., Dickensheets S.L., Myers D.A., Thomas D.L. and. Nixon S.J, 2000, Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men, *Alcohol Clin Exp Res*. **24** pp. 651–658.

Lovinger D.M., 1997, Serotonin´s role in alcohol´s effects on the brain, *Alcohol Health & Research World*. **21**: 114-120.

Lovinger D.M., White G. and Weight F.F., 1989, Ethanol inhibits NMDA-activated ion current in hippocampal neurons, *Science*. **243**:1721-1724.

Lovinger D.M., Zhou Q., 1994, Alcohols potentiate ion current mediated by recombinant 5-HT3RA receptors expressed in a mammalian cell line, *Neuropharmacology.* **33**:1567-1572.

Madeira and Paula-Barbosa M.M., 2002, Effects of alcohol on the synthesis and expression of hypothalamic peptides, *Brain Res Bull*. **48** pp. 3-2.

Mansvelder H.D., McGehee D.S., 2002, Cellular and synaptic mechanisms of nicotine addiction, *J Neurobiol*. **53**:606-617.

Mascia M.P., Trudell J.R., Harris R.A., 2000, Specific binding sites for alcohol and anesthetics on ligand-gated ion channels, *Proc Natl Acad Sci USA* . **97**:9305-9310.

Matsuda L.A., Lolait S.J., Brownstein M.J., Young A.C., Bonner T.I., 1990, Structure of a cannabinoid receptor and functional expression of the cloned cDNA, *Nature*. **346**:561-4.

McKernan R.M., Whiting P.J., 1996, Which $GABA_A$-receptor subtypes really occur in the brain?, *Trends in Neuroscience.* **19**:139–143.

Michaelis E.K., 1998, Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging, *Prog Neurobiol*. **54**:369-415.

Mihic S., Harris A.., 1997, GABA and the $GABA_A$ - receptor. *Alcohol Health and Research World* . **21**:127-131.

Monteiro M.G., 2001, A World Health Organization perspective on alcohol and illicit drug use and health, *Eur Addict Res*. Aug; **7**(3):98-103.

Monteiro M.G., Klein J.L. and Schuckit M.A., 1991, High levels of sensitivity to alcohol in young adult Jewish men: A pilot study, *J Stud Alcohol.* **52:** 464-469.

Monti P.M., Rohsenow D.J., Swift R.M., Gulliver S.B., Colby S.M., Mueller T.I. et al., 2001, Naltrexone and cue exposure with coping and communication skills training for alcoholics: treatment process and 1-year outcomes, *Alcoholism: Clin Exp Res*. **25**: 1634-1647.

Morris P.L., Hopwood M., Whelan G., Gardiner J., Drummond E., 2001, Naltrexone for alcohol dependence: a randomized controlled trial, *Addiction*. **96**:1565-1573.

Naranjo C.A., Knoke D.M., 2001, The role of selective serotonin reuptake inhibitors in reducing alcohol consumption, *J Clin Psychiatry*. **62** Suppl 20:18-25.

Nevo I., Hamon M., 1995, Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism, *Neurochem. Int*. **26**:305-336.

Nie Z., Madamba S.G., Siggins G.R., 1994, Ethanol inhibits glutamatergic neurotransmission in nucleus accumbens neurons by multiple mechanisms*, J. Pharmacol. Exp. Ther*. **271**: 1566-1573.

Nille U., 2000, Zur Neurobiologie des Alkoholismus, *Abhängigkeiten.* **3**:27-31.

Nomura F., Itoga S., Uchimoto T., Tomonaga T., Nezu M., Shimada H., Ochiai T., 2003, Transcriptional activity of the tandem repeat polymorphism in the 5'-flanking region of the human CYP2E1 gene, *Alcohol Clin Exp Res*. Aug; **27**(8 Suppl):42S-46S.

O'Malley S.S., Jaffe A.J., Chang G., Schottenfeld R.S., Meyer R.E., Rounsaville B., 1992, Naltrexone and coping skills therapy for alcohol dependence: a controlled study. *Arch Gen Psychiatry*. **49**:881-887.

Oslin D., Liberto J.G., O'Brien J., Krois S., Norbeck J., 1997, Naltrexone as an adjunctive treatment for older patients with alcohol dependence, *Am J Geriatr Psychiatry*. **5**:324-332.

Oslin D.W., Berrettini W., Kranzler H.R., Pettinati H., Gelernter J., Volpicelli J.R., O'Brien C.P., 2003, A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients, *Neuropsychopharmacology.* **28**(8):1546-52.

Pandey S.C., 1998, Neuronal signaling systems and ethanol dependence, *Mol Neurobiol*. **17**:1-15.

Pastino G.M., Flynn E.J., Sultatos L.G., 2000, Genetic polymorphisms in ethanol metabolism: issues and goals for physiologically based pharmacokinetic modeling, *Drug Chem Toxicol*. **23**:179-201.

Raimondi S., Benhamou S., Coutelle C., Garte S., Hayes R., Kiemeney L., Lazarus P., Marchand L.L., Morita S., Povey A., Romkes M., Zijno A., Taioli E., 2004, Association of metabolic gene polymorphisms with alcohol consumption in controls, *Biomarkers*. Mar-Apr; **9**(2):180-9.

Rhee MH, Bayewitch M, Avidor-Reiss T, Levy R, Vogel Z., 1998, Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. *J Neurochem*. Oct; **71**:1525-34.

Robinson T.E., Berridge K.C., 1993, The neural basis of drug craving: anincentiv-sensitization theory od addiction, *Brain Res Rev*. **18**:247-291.

Roivainen R., Hundle B., Messing R.O., 1994, Proteinkinase C and adaption to ethanol. In: Jansson B, Jornvall H, Rydberg U, Terenius L, Vallee BL (Hrsg) *Toward a Molecular Basis of Alcohol Use and Abuse*. Birkhäuser, Basel 29-38.

Rommelspacher H., Raeder C., Kaulen P., et al., 1992, Adaptive changes of dopamine-D2-Receptors in rat brain following ethanol withdrawal: a quantitative autoradiographic investigation, *Alcohol.* **9**:1-8.

Rommelspacher H., Smolka M., Schmidt L., Samochowiec J., Hoehe M.R., 2001, Genetic analysis of the mu-opioid receptor in alcohol-dependent individuals, *Alcohol.* **24**:129-135.

Samochowiec J., Kucharska-Mazur J., Kaminski R., Smolka M., Rommelschpacher H., Wernicke C., Tymicz A., Schimdt L.G., 2002, Norepinephrine transporter gene polymorphism is not associated with susceptibility to alcohol dependence. *Psychiatry Res*. Aug 30;**111** (2-3):229-33.

Sander T., Gscheidel N., Wendel B., Samochowiec J., Smolka M., Rommelspacher H. et al., 1998, Human mu-opioid receptor variation and alcohol dependence, *Alcoholism: Clin Exp Res.* **22**:2108-110.

Sander T., Harms H., Dufeu P., et al., 1998, Serotonin transporter gene variants in alcohol-dependent subjects with dissocial personality disorder, *Biol Psychiatry*. **43**:908-912.

Schinka J., Town T., Abdullah L., Crawford F., Ordorica P., Francis E. et al., 2002, A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances, *Mol Psychiatry.* **7**:224-228.

Schuckit M.A., 1995, Alcohol-related disorders, in *Comprehensive Textbook of Psychiatry* 6th ed. (Kaplan HI and Sadok BJ eds) pp. 775-791, Plenum Press, New York.

Schuckit M.A., Mazzanti C., Smith T.L., 1999, elective genotyping for the role of 5-HT_{2A}, 5-HT_{2C}, und $GABA_{A\alpha6}$ receptors and the serotonin transporter in the level of response to alcohol: A pilot study, *Biol. Psychiatry*. **45**:647-651.

Schumann G., Rujescu D., Kissling C., et al., 2003c, Analysis of genetic variations of protein tyrosine kinase fyn and their association with alcohol dependence in two independent cohorts, *Biol Psychiatry*. **54**:1422-1426.

Schumann G., Rujescu D., Szegedi A., et al., 2003a, No association of alcohol dependence with a NMDA-receptor 2B gene variant, *Mol Psychiatry*. **8**:11-2.

Schwitters S.Y., Johnson R.C., Johnson S.B., Ahern F.M., 1982b, Familial resemblances in flushing following alcohol use, *Behavior Genetics*. **12**:349-352.

Schwitters S.Y., Johnson R.C., McClearn G.E., Wilson J.R., 1982a, Alcohol use and the flushing response in different racial groups, *Journal of Studies on Alcohol.* **43**:1259-1262.

Shi J., Hui L., Xu Y., Wang F., Huang W., Hu G., 2002, Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin, *Hum Mutat*. Apr; **19** (4):459-60.

Shippenberg T.S., Herz A., 1987, Place preference conditioning reveals the involvment of D1-dopamine receptors, in the motivational properties of μ- and κ-opioid agonists, *Brain Res.* **436**:169-172.

Slater S.J., Cox K.J.A., Lombardi J.V., et al., 1993, Inhibition of protein kinase C by alcohols and anaesthetics, *Nature*. **364**:82-84.

Smith A.P., Lee N.M., 2003, Opioid receptor interactions: Local and nonlocal, symmetric and asymmetric, physical and functional, *Life Sciences*. **73**:1873-1893.

Smolka M., Sander T., Schmidt L.G., Samochowiec J., Rommelspacher H., Gscheidel N., Wendel B., Hoehe M.R., 1999, Mu-opioid receptor variants and dopaminergic sensitivity in alcohol withdrawal, *Psychoneuroendocrinology*. Aug; **24**(6):629-38.

Spanagel R., Bienkowski P., 2002, Glutamatergic mechanisms in alcohol dependence and addiction. in: *Therapeutic Potential of Ionotropic Glutamate Receptor Antagonists and Modulators.* In: Lodge D., Danysz W., Parsons F.P. (Hrsg) Graham Publishing Co., 375-403.

Spanagel R., Herz A., Shippenberg T.S., 1992, Opposing tonically active endogeneous opioid systems modulate the mesolimbic dopaminergic pathway, *Proc Natl Acad Sci USA.* **89**: 2046-2050.

Strowig A.B., 2000, Relapse determinants reported by men treated for alcohol addiction: the prominence of depressed mood, *J Subst Abuse Treat*. Dec; **19**(4):469-74.

Szeto C., Tang M., Lee D., Stadlin A., 2001, Association between μ opioid receptor gene polymorphisms and Chinese heroin addicts, *NeuroReport*. **12**:1103-1106.

Takeshita T., Morimoto K., Mao X.Q., Hashimoto T., Furuyama J., 1994, Characterization of the three genotypes of low Km aldehyde dehydrogenase in a Japanese population, *Hum Genet*. **94**:217-223.

Tan E.C., Tan C.H., Karupathivan U., Yap E.P., 2003 , μ opioid receptor gene polymorphisms and heroin dependence in Asian populations, *Neuroreport*. Mar 24; **14**(4):569-72.

Town T., Abdullah L., Crawford F., Schinka J., Ordorica P., Francis E. et al., 1999, Association of a functional mu-opioid receptor allele (+118A) with alcohol dependency, *Am J Med Genet*. **88**:458-461.

Tsai G., Coyle J.T., 1998, The role of glutamatergic neurotransmission in the pathophysiology of alcoholism, *Annu Rev Med*. **49**:173-84.

Türker T., Sodmann R., Goebel U., et al., 1998, High ethanol tolerance in young adults is associated with the low-activity variant of the promoter of the human serotonin transporter gene, *Neurosci Lett*. **248**:147-150.

Tyndale R.F., 2003, Genetics of Alcoholism and Tobacco Use in Humans, *Annals of Medicine*. **35**: 94-121.

Verheul R., Van Den Brink W., Geerlings P., 1999, A three-pathway psychobiological model of craving for alkohol, *Alcohol Alcohol*. **34**:197-222.

Verlaan M., Te Morsche R.H., Roelofs H.M., Laheij R.J., Jansen J.B., Peters W.H., Drenth J.P., 2004, Genetic polymorphisms in alcohol-metabolizing enzymes and chronic pancreatitis, *Alcohol Alcohol*. Jan-Feb; **39**(1):20-4.

Vidali M., Stewart S.F., Rolla R., Daly A.K., Chen Y., Mottaran E., Jones D.E., Leathart J.B., Day C.P., Albano E., 2003, Genetic and epigenetic factors in autoimmune reactions toward cytochrome P4502E1 in alcoholic liver disease, *Hepatology*. Feb; **37**(2):410-9.

Volpicelli J.R., 1987, Uncontrollable events and alcohol drinking, *Br J Addict*. **82**:381-392.

Volpicelli J.R., Alterman A.I., Hayashida M., O'Brien C.P., 1992, Naltrexone in the treatment of alcohol dependence, *Arch Gen Psychiatry*. **49**:876-880.

Volpicelli J.R., O'Brien C.P., Alterman A.I., Hayashida M., 1990, Naltrexone and the treatment of alcohol dependence: initial observations. In: Reid LD (ed) *Opioids, Bulimia, and Alcohol Abuse & Addiction*. Springer-Verlag: New York. pp 195-214.

Volpicelli J.R., Watson N.T., King A.C., Sherman C.E., O'Brien C.P.,1995, Effect of naltrexone on alcohol 'high' in alcoholics, *Am J Psychiatry*. **152**: 613-615.

Von Bardeleben U., Heuser I.J.E. and Holsboer F., 1989, Human CRH stimulation response during acute withdrawal and after medium-term abstention from alcohol abuse, *Psycho-neuroendocrinology.* **14** pp. 441-449.

Wand G.S., Mangold D., El Deiry S., McCaul M.E., Hoover D., 1998, Family history of alcoholism and hypothalamic opioidergic activity, *Arch Gen Psychiatry.* **55**:1114-1119.

Wand G.S., McCaul M., Yang X., Reynolds J., Gotjen D., Lee S. et al., 2002, The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade, *Neuropsychopharmacology*. **26**:106-114.

Wang D., Quillan J.M., Winans K., Lucas J.L., Sadee W., 2001, Single nucleotide polymorphisms in the human μ opioid receptor gene alter basal G protein coupling and calmodulin binding, *J Biol Chem*. Sep 14; **276**(37):34624-30.

Wang J.B., Imai Y., Eppler C.M., Gregor P., Spivak C.E., Uhl G.R., 1993, μ opiate receptor: cDNA cloning and expression, *Proc Natl Acad Sci USA.* Nov 1; **90** (21):10230-4.

Weed M.R., Wolverton W.L ,1995, The reinforcing effects of dopamine D1-Receptor agonists in the rhesus monkeys, *J Pharmacol Exp Ther*. **275**:1367-1374.

Wernicke C., Samochowiec J., Schmidt L.G., Winterer G., Smolka M., Kucharska-Mazur J., Horodnicki J., Gallinat J., Rommelspacher F., Polymorphisms in the N-methyl-D-aspartate receptor 1 and 2B subunits are associated with alcoholism-related traits, *Biol Psychiatry*. 2003 Nov 1; **54**(9):922-8.

Wise R.A., 1988, The neurobiology of craving: Implications for the understanding of addiction, *J Abnorm Psychol*. **97**:118-132.

Yoshida A., Ikawa M., Hsu L.C., Tani K., 1985, Molecular abnormality and cDNA cloning of human aldehyde dehydrogenases. *Alcohol*. **2**:103-10.

8. EATING DISORDERS AND OBESITY

Mariken de Krom, Annemarie van Elburg, Pierre M. Zelissen and Roger A.H. Adan*

1. INTRODUCTION

Obesity is a major problem for developing and developed countries since it provides a risk factor for cardiovascular disease, type 2 diabetes mellitus, some forms of cancer and osteoarthritis. Eating disorders such as anorexia and bulimia nervosa are much rarer, but are severe and sometimes life-threatening disorders (with a 10% mortality rate in anorexia nervosa). The last decade, the knowledge on the neural circuitry underlying regulation of food intake and energy balance, has increased enormously with the discovery of leptin and its downstream targets. This has opened new avenues towards the identification of drug targets to treat eating disorders and obesity. Here, we will briefly outline the major pathways in the brain that have been implicated in regulation of energy balance. Genes in these pathways have been tested for association with eating disorders and with obesity. Linkage studies indicate that there are still genes to be discovered that play an important role in the aetiology of eating disorders and obesity. Genetic variability between patients may underlie differences in responsiveness to drugs used in the treatment of obesity, such as sibutramine, and drugs used in the treatment of eating disorders, such as olanzapine. Therefore these drugs are discussed in the context of the neural circuitry involved in regulation of energy balance.

* Mariken de Krom, Annemarie van Elburg and Roger A.H. Adan, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, 3508 AB Utrecht, The Netherlands. Pierre M. Zelissen, Department of Internal Medicine/Endocrinology, University Medical Center Utrecht, 3508 AB Utrecht, The Netherlands.

2. THE NEURAL CIRCUITRY OF REGULATION OF ENERGY BALANCE

2.1. The discovery of leptin

One major breakthrough in understanding body weight regulation was the discovery of leptin. This discovery was the result of decades of research into mouse strains with heritable forms of obesity. Five such spontaneous mouse mutants displaying obesity have been described until now: ob, db, tub, fat and A^y. Surprisingly, the genes mutated in these mice, can all be placed into the same anatomical and signalling route.

Ob/ob and db/db mice have identical phenotypes, each weighing three times more than normal mice with a fivefold increase in body fat content. Data from cross-circulation (parabiosis) experiments suggested that the ob gene was responsible for the generation of a circulating factor that regulated energy balance and that the db gene encoded the receptor for this factor (Friedman and Halaas, 1998).

The cloning and characterization of the ob gene showed that it encodes a hormone, named leptin, that is expressed abundantly in adipose tissue. The db gene encodes the receptor for leptin. Levels of leptin are proportional to adipose tissue mass. The discovery of leptin was received with great enthusiasm, since it was thought that a drug mimicking leptin could treat the obesity epidemic. The clinical phenotype of human congenital leptin deficiency is very similar to that seen in the ob/ob mouse. Both leptin-deficient humans and mice have early-onset obesity, increased food intake, hypo gonadism, hyper-insulinaemia and defective function of the hypothalamo-pituitary thyroidal axis. Thus, leptin plays a similar role in mice and humans. Indeed treatment with leptin of these rare cases of obese individuals carrying mutations in the leptin gene, is very successful to normalise body weight and neuroendocrine functioning (Farooqi *et al.*, 2002). However, most obese people have plenty of leptin, and it is therefore thought that obese people are leptin-resistant. Consequently, research has focused on the downstream effector pathway of leptin, which was expected to malfunction in obese individuals.

2.2. The neural circuitry mediating leptin's effect

Besides some peripheral tissues, such as adrenal cortex, liver and pancreas, leptin receptors have been found in several hypothalamic nuclei that are involved in the regulation of energy balance, which include the arcuate nucleus, the ventromedial hypothalamus (VMH), the lateral hypothalamus (LH), the dorsomedial hypothalamus (DMH) and the paraventricular nucleus (PVN). Leptin receptors have also been found in brainstem nuclei such as the nucleus of the tractus solitarius, the dorsal motor nucleus of the vagus nerve, the lateral parabrachial nucleus, and the central gray.

The leptin-responsive hypothalamic nuclei express one or more neuropeptides and neurotransmitters that regulate food intake and/or body weight. The arcuate nucleus has a large density of leptin receptors. In the arcuate

nucleus there are at least two different populations of neurons that are oppositely regulated by leptin. Neurons expressing pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-related transcript (CART) are activated by high plasma leptin levels. POMC is the precursor of the melanocortins α-, β- and γ-melanocyte stimulating hormone (MSH) and β-endorphin. α-MSH, when injected into the brain, decreases food intake and body weight. Neurons expressing Agouti-related protein (AgRP) and Neuropeptide Y (NPY) are activated when plasma levels are low, such as during starvation. The importance of the medial hypothalamus, which includes the arcuate nucleus, was already known for decades, since lesions of this area result in obesity, and electrical stimulation inhibits food intake. With the discovery of leptin and its direct effector pathways in the arcuate nucleus, the neuropeptides that probably mediated these effects were identified. Now that important parts of the neural circuitry underlying regulation of energy balance have been discovered, the validation of new drug targets within these neural circuits can be explored for the treatment of eating disorders and obesity.

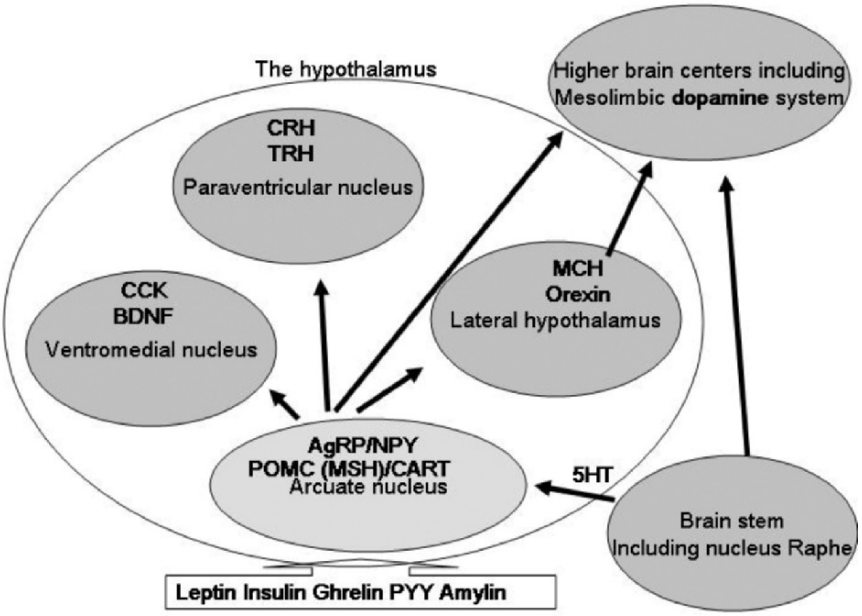


Figure 1. The neural circuitry regulating energy balance.

Major identified pathways involved in the regulation of energy balance are depicted. Note: 1. the central position of the arcuate nucleus, where signals from the periphery (such as leptin) reach the brain; 2. serotonergic and dopaminergic pathways super-imposed on the hypothalamic centers involved in regulation of energy balance.

One of the neuropeptides acting downstream of leptin, NPY, is the most potent orexigenic agent known when administered in the brain. Further analysis of which aspect of feeding behavior is influenced by NPY revealed that NPY stimulates energy intake when rats can go to food, but not when food is administered intra-orally (Ammar *et al.*, 2000). This suggest that NPY is involved in the appetitive phase, but not in the consumatory phase of food intake. Detailed analysis of how NPY regulates food intake in animal models is important to understand the role of NPY in feeding behavior in humans.

NPY mRNA is increased in ob/ob mice and decreased after leptin treatment or following starvation. Therefore, it was expected that increased NPY signalling was responsible for the obesity observed in ob/ob mice. Since in knockouts for NPY and leptin (NPY-/-; ob/ob) the obesity of ob/ob mice was only partially inhibited, it was realised that also other neuropeptides might play a role (Erickson *et al.*, 1996).

In viable yellow mice (A^y) such another pathway, acting in parallel to NPY, was identified. A^y mice have ectopic overexpression of Agouti protein. Agouti is normally only expressed in skin where it acts as an antagonist at the melanocortin MC1 receptor, resulting in the switch from eumelanin (dark pigment) to phaeomelanin (pale pigment) synthesis. Expressed in the brain as in A^y mice, Agouti antagonises the melanocortin MC4 receptor. The activity of the MC4 receptor is activated by MSH, which is an agonist, and inhibited by AgRP (the homolog of Agouti expressed normally in brain), an inverse agonist (Adan and Kas, 2003). Thus, Agouti disrupts the leptin down-stream effector pathway by blocking the effect of POMC neurons releasing MSH. A^y mice therefore have a similar phenotype as melanocortin-MC4-knockout mice: obesity and leptin resistance.

Thus, ob, db and A^y mice have deficits in the same genetic pathway. How about the other two spontaneous obese mouse mutants fat and tubby? The gene defect underlying obesity in the fat mouse is a mutation in the carboxypeptidase E gene (Naggert *et al.*, 1995). Carboxypeptidase E is involved in the processing of neuropeptides like POMC but also of insulin in secretory granules. Impaired processing of POMC could explain the obesity observed in the fat mouse (Berman *et al.*, 2001). The tubby gene, a transcription factor which is mutated in the tub mouse, is expressed in the hypothalamus. In tubby mice levels of expression of POMC and NPY are altered (Guan *et al.*, 1998). Thus, all five naturally occurring obese mouse mutants have defects in leptin or its downstream effector pathways. The primary leptin-responsive neurons are located in the arcuate nucleus. From here, POMC/CART and AgRP/NPY neurons have wide-spread projections to other areas of the brain involved in regulation of food intake and energy balance. Important regions include the ventromedial hypothalamus (VMH), the lateral hypothalamus (LH), the paraventricular nucleus (PVN) and the mesolimbic system. In these areas so-called second order leptin responsive neurons are present. The PVN is an important projection area for leptin-responsive arcuate nucleus neurons. Thyroid-releasing hormone (TRH) and corticotrope-releasing hormone (CRH) neurons have been implicated in the leptin downstream signalling cascade (Legradi *et al.*, 1997; Masaki *et al.*, 2003). TRH influences energy balance via

the pituitary-thyroid axis, which is involved in regulating metabolic rate. CRH, when injected in the brain, suppresses food intake.

The VMH has been implicated in the regulation of food intake, it senses blood glucose levels, controls digestive system functions and regulates glucagon and insulin levels. The VMH expresses amongst other neuropeptides TRH and cholecystokinin (CCK), and the neurotrophin brain-derived neurotrophic factor (BDNF). CCK is a satiety factor released from duodenal mucosa, stimulated by lipids from digestion (Noble and Roques, 2002). Injected into the brain CCK induces satiety and anxiety (Moran and Schwartz, 1994). Therefore, the CCK system is interesting with regard to eating disorders, since in anorexia nervosa anxiety for ingesting food is a major characteristic.

Mice carrying mutations in BDNF signalling are hyperphagic and hyperactive. It was shown that BDNF acts downstream of the MC4 receptor. BDNF suppressed food intake when injected in the brain, and MC4 receptor knockouts have lower VMH BDNF levels (Xu *et al.*, 2003). Thus, also BDNF has been implicated in the downstream signalling pathway of leptin.

The lateral hypothalamus contains at least of two interesting populations of neurons that produce either orexin or melanin-concentrating hormone (MCH). Orexins are involved in the sleep-wake cycling and stimulate food intake during the night in nocturnal species (Hara *et al.*, 2001). MCH also stimulates food intake. Transgenic mice overexpressing MCH in the lateral hypothalamus are mildly obese and display insulin-resistance (Ludwig *et al.*, 2001).

2.3. Modifiers of the leptin neural circuitry

The arcuate nucleus in the hypothalamus is one of the brain regions that lack a clear blood brain barrier. Besides receptors for leptin, the arcuate nucleus senses changes in other plasma derived factors that have been identified in regulation of energy balance, such as insulin, amylin, PYY and Ghrelin. Insulin acts on most of the brain centers that also have leptin receptors, and inhibits food intake. Amylin, co-released with insulin from β cells of the pancreas, also inhibits food intake (Rushing, 2003). PYY is post-prandially released from intestine acts at pre-synaptic, autoinhibitory NPY-2 receptors expressed on NPY/AgRP neurons in the arcuate nucleus. PYY inhibits the activity of these orexigenic neurons and thus decreases food intake (Batterham *et al.*, 2002). Ghrelin, mainly produced in the stomach in particular during starvation, but also in the hypothalamus itself, activates growth–hormone-secretagogue receptors (GHS-R) expressed on AgRP/NPY neurons in the arcuate nucleus, resulting in increased activity of these neurons, which results in increased food intake (Cowley *et al.*, 2003).

The hypothalamus also receives input from other brain areas. Serotonin has been implicated in regulation of food intake. Serotonin 2C receptor knockout mice are hyper-phagic and obese. Fenfluramine, a serotonin reuptake inhibitor which has been on the market to treat obesity, requires serotonin 2C receptors in order to inhibit food intake (Heisler *et al.*, 2002), expressed on arcuate nucleus neurons expressing POMC. Atypical antipsychotics such as olanzapin, may thus increase food intake via antagonism of the serotonin 2C receptor.

The mesolimbic dopamine system is innervated by both primary and secondary leptin responsive neurons. Dopamine deficient mice display decreased motor activity and reduced food intake. Restoration of dopamine signaling in the caudate putamen, but not in the nucleus accumbens, normalizes food intake (Szczypka *et al.*, 2001).

Thus, genes (in particular receptor genes) constituting the serotonergic and dopaminergic systems are expressed in primary and secondary leptin responsive brain nuclei that affect energy balance, and may via this route be involved in eating disorders and obesity and its treatments.

2.4. Mouse mutants with anorexia

Although five spontaneous mutations have been identified resulting in obesity, there is only one spontaneous mouse mutant identified for anorexia: the anx mutant. Anx/anx mice suffer from a autosomal recessive mutation resulting in poor appetite, reduced body weight, emaciated appearance, body tremors, head weaving, hyperactivity, and uncoordinated way of walking. These mice have significantly reduced serum leptin levels. By performing immunohistochemical studies it has been demonstrated that neuropeptide Y (NPY) and agouti-related peptide (AGRP) accumulate in the cell bodies rather than in the dendritic extensions in the arcuate nucleus (Broberger *et al.*, 1999). These studies also showed decreased levels of pro-opiomelanocortin (POMC), neuropeptide receptors and cocaine and amphetamine-related transcript (CART) mRNAs in the arcuate nucleus. Anx/anx mice have been shown to have an increase in serotonergic fibers in the forebrain and arcuate nucleus. Serotonin appears to play a role both in abnormal behaviour and in appetite. The anx gene has been localized on mouse chromosome 2 and cloning and elucidation of the biochemical function of this gene will help to understand more about the regulation of food intake and the alterations that occur in eating disorders (Siegfried *et al.*, 2003).

Dopamine-deficient (DD) mice were generated by deletion of the tyrosine hydroxylase gene specifically in dopaminergic neurons. DD mice are born normal but gradually become hypoactive and hypophagic and die at 3-4 weeks of age. DD mice can be rescued by daily treatment with L-DOPA or by introducing a liquid diet directly into the mouth, which indicated that these mice die due to lack of nutrients. Szczypka *et al.* (Szczypka *et al.*, 2001) believe that the anorexia of DD mice is not exclusively a consequence of motor deficits because they do move as much or more than wild-type mice under certain conditions. Also their ingestive behaviour in response to novel food is initially indistinguishable from that of normal mice.

M3 muscarinic receptor knockout mice (M3R $^{-/-}$) display a significant decrease in food intake, reduced body weight and peripheral fat deposits, and very low levels of leptin and insulin (Yamada *et al.*, 2001). Both decreased MCH expression and reduced responsiveness to AGRP are major contributing factors to the hypophagic phenotype in M3R $^{-/-}$ mice.

MCH knockouts have a hypophagic and lean phenotype and an increased metabolic rate (Shimada *et al.*, 1998). After 24h starvation, weight loss was

greater in MCH $^{-/-}$ than in controls and after 48h of fasting, and 75% of the MCH $^{-/-}$ mice died, while no control mice died.

Corticotropin releasing hormone receptor 2 (Crhr2) knockouts have anxiety-like behaviour and are hypersensitive to stress (Bale *et al.*, 2000). After 24 h of food deprivation mutant mice consumed 75% of wild-type food levels in the 24 h period following food deprivation. However their body weights did not differ from those of wild type mice. It is not clear whether this is a direct effect of metabolism, or if the stress of fasting alters the anxiety state of the animals, and thus affects appetite or metabolism.

Cannabinoid receptor 1 (CB1) knockouts have significantly lower food intake than wild-type mice after 18h of fasting, although no difference was found in weight and food consumption (Di Marzo *et al.*, 2001). Studies using a selective CB1 antagonist indicated that endogenous cannabinoids acting at CB1 receptor may be involved in maintaining food intake in mice made hyperphagic by food deprivation

3. EATING DISORDERS AND OBESITY

3.1. The obesity phenotype

Obesity is defined by the WHO as a chronic disease characterised by an excess of body fat in such a degree that it leads to significant health risks (2000). The body mass index (BMI), calculated as body weight (in kg) divided by height^2 (in m), is widely used as a measure of the degree of overweight and is excellently correlated to body fat mass. In Caucasian populations a BMI between 18.5 and 25 is considered to be normal; overweight is defined as a BMI between 25 and 30 and obesity as a BMI above 30. Not only the total amount of body fat is important to predict the health risks, but also fat distribution: intra-abdominal (visceral) fat is much more associated with co-morbidity than subcutaneous, femorogluteal fat. The intra-abdominal fat mass can easily be estimated by measuring the waist circumference: a waist circumference ≥ 94 cm (males) or ≥ 88 cm (females) carries a significantly increased health risk.

Co-morbidity, associated with obesity, consists primarily of the metabolic syndrome (insulin resistance, eventually resulting in type 2 diabetes, dyslipidemia and hypertension among others), leading to an increased prevalence of cardiovascular disease. In addition, certain types of cancer, osteoarthritis, gout, gallstones, liver steatosis, obstructive sleep-apnea syndrome, gastro-esophageal reflux, disturbances in gonadal hormonal function, depression, psychosocial dysfunctioning and considerable loss of quality of life are clearly associated with obesity. It has also been established that obesity, through its co-morbidity, definitely leads to increased mortality (Calle *et al.*, 1999).

The increasing prevalence of overweight and obesity in the Western world is truly alarming and fighting this epidemic poses one of the greatest challenges for health caretakers. Presently, 10-25% of the adult populations in Western countries are obese and 40-65% are overweight. Although numerous research

has pointed to the existence of a genetic predisposition to obesity, clearly the enormous increase in obesity prevalence during the last decades has to be caused by our obesogenic environment, characterised by abundance of easily available, energy-dense food on one hand and our sedentary lifestyle with lack of physical activity on the other hand.

According to basal thermodynamic laws, excessive fat deposition, as is the case in obesity, is the consequence of a longstanding positive energy balance in which energy intake has been greater than energy expenditure. It is important to realise that only a small, but structural, energy excess is needed to cause obesity: a structural daily excess of not more than 100 kcal will lead to an average weight gain of more than 4 kg after one year! Considering the wide daily variation in both our energy intake and energy expenditure, it is in fact more remarkable that most people are able to keep their weight stable. The neural and hormonal mechanisms which are involved in this energy homeostasis are only partly unravelled and it is very well conceivable that these mechanisms are somewhat less finely tuned in people predisposed to obesity, possibly leading to altered hunger and satiety signals and/or metabolic rate. In addition, an altered functioning of hormones, neurotransmitters and receptors in the parts of the central nervous system involved in eating behaviour may also be related to some different phenotypes in obese subjects which can be encountered (for instance "stress eaters" versus people who primarily respond to external food cues in an exaggerate way). Clarification of these mechanisms could lead to more effective and more individualised pharmacological intervention in obesity.

The most important targets in obesity management are weight loss followed by longstanding weight maintenance and treatment of co-morbid conditions. It should always and primarily consist of lifestyle changes directed to a healthier eating behaviour and more physical activity. Longstanding maintenance of a lower weight appears to be the most difficult and challenging part of obesity management as contraregulatory mechanisms (such as decrease of basal metabolic rate after weight loss a.o.) are operating in a (frequently successful) attempt to increase body weight again to the original (or even higher) level. Apart from bariatric surgery (gastroplasty, gastric banding or gastric bypass), which is only available for selected patients with morbid obesity (BMI > 40), the results of nearly all lifestyle and pharmacological interventions directed to restoration of a (near-)normal weight on the long-term have been disappointing (success rate < 10%). Nowadays the goal of treatment has changed from weight normalisation towards a moderate, but longstanding weight loss of about 10% of the original weight. This moderate weight loss is associated with a clear decrease in cardiovascular risk factors.

3.2. Eating disorder phenotypes

People diagnosed with anorexia nervosa are at the total opposite site of the weight spectrum. One of the diagnostic criteria of anorexia is a BMI lower then 17.5 kg/m2. Anorectic people have a very severe and selective food intake and do not eat sufficient amounts of dietary fat and sugars. Someone is diagnosed anorexic when the following phenotypic observations are made: overevaluation

of shape and weight, active maintenance of a very low BMI and amenorrhoea in postmenarcheal women. Anorexia is predominantly seen in adolescent females. Character traits like low self-esteem and perfectionism are also very often observed in these women.

Phenotypic descriptions of anorexia and bulimia nervosa: The first descriptions of anorexia nervosa were done by the Frenchman Lasègue (Lasèque, 1873) and the English physician Gull in the 1870's (Gull, 1874). They described 'a morbid mental state' that causes an illness characterized by a reduction of food intake, emaciation, amenorrhea, hyperactivity, hypothermia and lack of insight, that typically strikes young females. 130 years later this picture is still very accurate, over the years many theories about the influence of society, families, early traumatic experience etc. have come and gone and treatment has tried to counter those problems, resulting in a range of psychologically based treatment programs.

Nowadays, eating disorders are viewed as classic examples of complex psychiatric phenotypes with both genetic and environmental determinants (Devlin *et al.*, 2002). Anorexia nervosa is characterized by pathologic eating behaviour and the relentless pursuit of thinness, resulting in extreme emaciation. In adults, a weight that leads to a Body Mass Index below 17.5 kg/m² is considered to be the cut-off between normal and anorectic weight, in children and adolescents it is the failure to make the expected weight gain during a period of growth, leading to a bodyweight less that 85% of expected (Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM- IV), 1994). Patients exhibit an overevaluation of shape and weight and often a distorted body image and in addition postmenarcheal women with this disorder are amenorrheic. Abnormally high activity levels and overexercising are seen in 30-80% of patients (Hebebrand *et al.*, 2003), mainly the so-called restrictors or RAN (resticting-type anorexia nervosa), (subjective) binges, laxative abuse and vomiting characterize the purging anorectic patients or BAN (bingeing-type anorexia nervosa). General issues as low self-confidence and self-esteem, and co-morbid anxiety and depressive symptomatology are seen in the majority of cases.

Psychometric studies have consistently linked RAN in particular to a stereotypic cluster of (moderately heritable (Heath *et al.*, 1994)) personality traits that are found with great consistency. These include emotional restraint, avoidance of novelty, anxious worry and self-doubt, compliancy, obsessionality, perfectionism and perseverance in the face of non-reward. Many of these traits are exaggerated by starvation but retrospective accounts suggest that they often predate the onset of the eating disorder (Deep *et al.*, 1995) and persist long after the normalization of weight and menses (Srinivasagam *et al.*, 1995). Considered temperamental risk factors they support the possibility that susceptibility is influenced. Increased serotonergic activity that persists after recovery (Kaye *et al.*, 1991) may be one an important cause.

The presentation of Bulimia Nervosa is less consistent but there are certain common personality and behavioural traits that may play an important biologically mediated etiological role in the development of the disorder. Characteristic traits are thrill seeking and excitability and a tendency to dysphoria in response to rejection or non-reward (Bulik *et al.*, 1995).

4. GENETIC STUDIES IN HUMANS WITH OBESITY OR EATING DISORDERS

4.1. Association studies and obesity

Since it became clear in mouse models that mutations in genes of the neuronal circuitry underlying energy balance can cause obesity many associations studies have been performed to identify the relevance of these genes for obesity in humans. In this part we will go into detail concerning the association studies, which show functional sequence changes, done for the genes involved in the leptin signalling pathway as described before.

For the leptin gene itself, although indicated as the obesity gene after the ob/ob mouse, few mutations and polymorphisms have been found, which makes the link between leptin and overweight in humans uncertain. Although a number of studies have investigated the leptin gene and its role in obesity, only one study reports on polymorphisms present in the leptin gene in obese individuals and not in average weight people. In this study both the leptin gene itself and its regulatory element were genotyped in 125 extreme obese and 86 average weight females. Three allelic variants at +19, –188 and –633 from the initiation site of transcription, which had different allelic frequencies between the extreme obese and average weight females, were found. It is thought that these three polymorphisms may influence the regulation of the leptin gene and predict the degree of obesity in the obese women (Li *et al.*, 1999). Two other studies also indicate that the level of leptin is indeed an indicator for obesity. Although in these studies no significant linkage between a polymorphism in the leptin gene and BMI was found, the studies do describe differences in leptin levels in obese people who are either homozygous for the polymorphism, heterozygous or homozygous wildtype. In case of an A/G polymorphism in exon 1 of the leptin gene, being homozygous for the G allele results in lower leptin levels whereas being heterozygous or homozygous for the A allele (Hager *et al.*, 1998). A polymorphism in the promoter of the leptin gene (-2548 G/A), does the opposite, being homozygous for the A allele results in higher levels of leptin (Hoffstedt *et al.*, 2002). These studies indicate that polymorphisms in the leptin gene can differently influence the concentration of serum leptin and thereby the degree of obesity. Furthermore a frameshift mutation, deletion of glycine 133, and a missense mutation, C105T, have been reported in two different studies in obese individuals with congenital leptin deficiency (Montague *et al.*, 1997); (Strobel *et al.*, 1998). These studies indicate that polymorphisms in the leptin gene can differently influence the concentration of serum leptin and thereby the degree of obesity.

Besides the leptin gene itself, also its receptor was an interesting gene to investigate. Two studies showed significant association between polymorphisms in the receptor and

Table 1. Summary of all positive association studies for candidate genes in the central nervous system for obesity

Candidate gene	Polymorphism	Associated with phenotype	Reference
Leptin	+19, -188, and -633	BMI > or = 40 kg/m^2	Li *et al*., 1999
	A/G exon 1 +19	lower leptin concentrations	Hager *et al*., 1998
	G/A -2548	Higher leptin levels	Hoffstedt *et al*., 2002
Leptin receptor	Q223R	body mass index higher then normal controls (P = 0.015)	Yiannakouris *et al*., 2001 van Rossum *et al*., 2003
	K109R	Higher leptin levels	van Rossum *et al*., 2003
POMC	S7T; S9L; R236G	Higher leptin levels	del Giudice *et al*., 2001; Challis *et al*., 2002
CART	T/C –156	BMI > or = 30 kg/m^2	Yamada *et al*., 2002
	L34F	Severe obesity	del Giudice *et al*., 2001
AgRP	A67T (G/A +199)	Abdominal obesity in people over 50 years of age	Argyropoulos *et al*., 2002
Melanocortin 4 receptor	4 bp insertion at 732	Extreme obesity	Yeo *et al*., 1998
	4 bp deletion at codon 211	Extreme obesity	Vaisse *et al*., 1998
	Y35stop; D35V; S30F; G252S; P78L; T112M; R165W; I317T	Extreme obesity	Hinney *et al*., 1999; Gu *et al*., 1999
	S58C; I102S; I170V; V50M	Extreme obesity	Dubern *et al*., 2001
CCK	V351I	Obesity	Marchal-Victorion *et al*., 2002

obesity (van Rossum *et al.*, 2003); (Yiannakouris *et al.*, 2001). In the study of Yiannakouris three common polymorphisms in the receptor (K109R, Q223R, and K656N) were compared on their influence on bodyweight. A total of 118 people were genotyped and checked for phenotypic parameters for obesity. It appeared that there was prevalence for the R223 allele in the homozygous form among overweight people. With a significance of P = 0.015 this allele is a good indicator for body mass index and overweight. The other polymorphisms were not significantly associated with obesity (Yiannakouris *et al.*, 2001). In the 2003 study by Van Rossum *et al*., the Arg223 allele was also found to be more often present in overweight people as in normal weight people. Besides the Arg223 polymorphism they also measured an other polymorphism, the Lys109Arg, which they also found to be associated with obesity. Carriers of these polymorphisms were found to have higher levels of leptin then non-carriers, suggesting that leptin resistance plays a role in the development of obesity (van Rossum *et al.*, 2003).

Four neuropeptides are the next step in the leptin pathway, POMC and CART which are activated when leptin levels are high and Agrp and NPY which are activated when levels are low. Except for NPY these have also been screened in obese subjects to see if mutations in these neuropeptides could be associated with obesity.

Although linkage studies already did indicate POMC as player in the obesity phenotype, no mutation or association studies have been able to confirm this until two recent studies (Miraglia *et al.*, 2001); (Challis *et al.*, 2002). In these studies indications were found that mutations in the POMC gene could be associated with obesity. In the Miraglia del Giudice study 87 Italian obese children were genotyped, which resulted in the identification of three mutations, Ser7Thr, Ser9Leu and Arg236Gly and a 9bp insertion between 6997 and 6998. All these patients showed significantly higher leptin levels then children who were homozygous for the wild-type alleles. This result provides some evidence that mutations in the POMC gene do play a role in the development of obesity. The Arg236Gly polymorphism was further investigated and it was shown that indeed the Gly236 allele was more frequently present in the obese population as in the average weight population, 0.88% versus 0.22% (Challis *et al.*, 2002). This mutation causes the production of an aberrant fusion protein. This protein can still bind to the melanocortin 4 receptor, but it fails to really activate the receptor. This leads to a stop in the leptin signalling cascade and thus in the obesity phenotype (Challis *et al.*, 2002).

The other anorexigenic neuropeptide CART has been investigated and in two studies mutations have been shown to be associated with obesity (Miraglia del Giudice *et al.*, 2001); (Yamada *et al.*, 2002). A SNP, -156 T/C, was shown to be significantly associated with BMI and was significantly more often present in obese subjects with a BMI over 30 compared to non-obese individuals (Yamada *et al.*, 2002). Furthermore a missense mutation, leading to an amino acid substitution, Leu34Phe, was found to cosegregate with a severe obesity phenotype in a family over three generations. The mutation is thought to alter the susceptibility to proteolysis of CART and thereby the effect of CART on

thermogenesis and energy expenditure resulting in an obese phenotype (Miraglia del Giudice *et al.*, 2001).

For the orexigenic neuropeptides only one positive association has been described. A polymorphism was identified in the third exon of hAGRP, 199G/A, which results in a nonconservative amino acid substitution, Ala67Thr. When this polymorphism was studied it was observed that the presence of the polymorphism had no effect in individuals with a mean age of 25. However, in the parental population with a mean age of 53 it was significantly associated with fatness and abdominal adiposity. This polymorphism thus seems to play a role in the development of obesity in an age-dependent manner (Argyropoulos *et al.*, 2002). The second polymorphism has been found in the minimal promoter of AgRP, a C38T SNP. This polymorphism has been associated with both obesity and type II diabetes. The C38C genotype of this polymorphism results in higher promoter activity and affinity for transcription factors (Mayfield *et al.*, 2001).

Since the melanocortin 4 receptor is activated by MSH, one of the cleavage products of POMC, and is inhibited by Agrp it is an important downstream target in the leptin pathway and thus studied for possible associations with obesity. Thus far, the MC4 receptor is the most frequent form of monogenetic obesity that has been identified, although only 4% of the cases of extreme obesity can be explained by a mutation in this receptor. Today at least 14 mutations, varying from insertions, deletions to SNPs, have been described in the receptor and all seem to be associated with extreme obesity. The first described mutations were respectively a 4 bp insertion at nucleotide 732 and a 4 bp deletion at codon 211, both resulted in a non-functional promoter and thereby a block in the leptin signaling pathway causing extreme obesity (Yeo *et al.*, 1998); (Vaisse *et al.*, 1998). These studies were followed by an extensive study in which the coding region of the MC4 receptor was screened in 306 extremely obese children and adolescents. A total of nine changes in the MC4 receptor were detected in this population compared to the normal weight control population. All seem to be associated with obesity although most of them were only detected in a few individuals (Hinney *et al.*, 1999). Two of the nine changes were also detected in an other study, indicating that they are really associated with obesity (Gu *et al.*, 1999). Four other missense mutations were detected in a study with 63 obese children. All these mutations could be associated with the obese phenotype and it appeared that the obese phenotype was variable in mutation-positive family members (Dubern *et al.*, 2001). All the observed mutations in the MC4 receptor lead to impaired signalling. In vivo this results in leptin resistance and an obesity phenotype.

For all the other peptides in the neuronal circuitry underlying energy balance like TRH, CRH, CCK, BDNF, orexins, MCH, ghrelin and serotonin receptors as mentioned before, only for CCK a positive association study has been found in a human population. One polymorphism was found in an obese population, Val351Ile, in CCK1 receptor. This mutation results in a decreased level of expression of CCK 1 receptor and its efficacy to stimulate inositol phosphates. This decrease in receptor expression may affect the CCK-induced regulation of satiety and therefore play a role in the development of obesity

(Marchal-Victorion *et al.*, 2002). A second polymorphism, C128T, in the CCK receptor has been reported to be associated with higher percentage of body fat and higher levels of leptin and insulin, but not directly with obesity (Funakoshi *et al.*, 2000).

4.2. Association studies and anorexia

In case of anorexia the association studies have been focused for a long time on the 5-HT transporter gene. Changes in the serotonin system have been associated with anorexia nervosa. Altered serotonin neuronal pathway activity persists after recovery from an eating disorder and supports the possibility that these psychobiological alterations might contribute to traits, such as increased anxiety or extremes of impulse control, that, in turn, may contribute to a vulnerability to the development of an eating disorder (Barbarich *et al.*, 2003). Specifically, after recovery, anorexics have increased levels of 5-HIAA, the major metabolite of serotonin, in the cerebrospinal fluid (CSF).

Table 2. Summary of all positive association studies for candidate genes in the central nervous system for anorexia

Candidate gene	Polymorphism	Reference
POMC	D80N; C3832T	Hinney *et al*., 1998
AgRP	G760A; G526A	Vink *et al*., 2001
Melanocortin receptor 4	V193V	Hinney *et al*., 1999
BDNF	V66M	Ribases *et al*., 2003

(Kaye, 1997). Furthermore, reduced mesial temporal cortex 5HT2A receptor binding persists after recovery from AN (Frank *et al.*, 2002). One hypothesis put forward by Walter Kaye, is that patients reduce their food intake in order to decrease tryptophane intake, which is the precursor of serotonin. This would suppress increased serotonin activity in the brain. Indeed, dietary-induced reduction of tryptophane, is associated with decreased anxiety in people with AN. Restricting dietary intake may represent a mechanism through which individuals with AN modulate a dysphoric mood (Kaye *et al.*, 2003).

Although different studies at first noted that there was association between anorexia and the polymorphism G1438A in the 5-HT transporter gene, other studies could not confirm this. A large study, in which samples from different studies of different European centers were included and compared, was performed to put an end to this controversy. In this study no significant association could be established between anorexia and polymorphisms in the 5-HT transporter gene (Gorwood *et al.*, 2002) and references therein). Other genes have also been studied and we will focus here, as for the obesity phenotype, on genes that can be found in the neuronal circuitry underlying food intake and energy balance and have been shown to be positively associated with anorexia. In case of anorexia much less positive associations have been found as

compared to obesity. Four of the genes studied of the leptin pathway have been found to be positively associated with anorexia; the orexigenic neuropeptide Agrp, the anorexigenic neuropeptide POMC, their receptor MC4 and the BDNF gene.

The POMC gene and polymorphisms in the gene have mainly been indicated to be associated with causing leptin resistance and obesity. However, in a large study encompassing obese, healthy and underweight individuals, which were screened for mutations in their POMC gene, two mutations were found in underweight individuals. These mutations, one missense (Asp80Asn) and one silent (C3832T), could only be detected in the underweight individuals (Hinney *et al.*, 1998). This provides some evidence that mutations in the POMC gene can change the effect of the gene on the MC4 receptor such that the leptin response is not accurate anymore. This may enhance the susceptibility to anorexia.

In case of AgRP two polymorphisms were found to be significantly associated with risk for developing anorexia nervosa, the G760A and the G526A single nucleotide polymorphisms, which are in linkage desequilibrium. These variants were detected in 11% of the 145 anorexic patients screened compared to 4.5% of the 244 controls (Vink *et al.*, 2001). These mutant forms of Agrp probably cause a defective suppression of the MC4 receptor, when leptin levels are low. This leads to a decreased feeding signal and thus an increasing risk for developing anorexia (Vink *et al.*, 2001).

The MC4 receptor has also been investigated for association with anorexia. A silent mutation (Val-193-Val) was detected in a male underweight individual (Hinney *et al.*, 1999). Although one such finding does not definitely prove the association between MC4 receptor and anorexia, it encourages to explore this further.

A new player in the field of energy balance and eating disorders is the brain derived neurotrophic factor (BDNF), which acts downstream of the MC4 receptor. The BDNF gene is expressed in the hypothalamus nuclei associated with weight regulation and feeding. The BDNF gene has been screened for functional polymorphisms that can be associated with anorexia. Three mutations were detected in individuals with anorexia (-347A/T, -256G/A and Val66Met). The first two were only detected in two patients. The Val66Met, however, was shown to be significantly associated ($p = 0.002$) with anorexia in a sample group of 143 patients with eating disorders compared to a control group of 112 persons (Ribases *et al.*, 2003). These findings confirm the idea that BDNF may be one of the genes playing a role in the development of eating disorders such as anorexia.

For the other genes of the neuronal circuitry underlying energy balance, mentioned in the other parts of this chapter, no evidence for positive or significant association with anorexia exists yet.

4.3. Linkage studies

Besides association studies, also linkage studies have been performed for obesity and recently also for anorexia nervosa. Linkage studies can only be performed, when a trait runs in a family or in other words when a trait is inherited from parent to child. Twin studies were used to check whether disorders as obesity and anorexia have such a genetic component. In such studies mono-zygotic and di-zygotic twins are compared. Such a comparison gives a clear indication if a certain trait has a genetic/heritable component or not.

Several independent studies compared mono-zygotic and di-zygotic twins on parameters which are used as obesity indicators such as leptin levels, Body Mass Index (BMI) and Percentage Body Fat (PBF) to see whether heritability plays a role in obesity. Below a couple of studies in which these parameters have been measured will briefly be described.

In a group of 58 mono-zygotic and 74 di-zygotic twins leptin levels were measured. These levels showed individual based variances, but the variance in levels between mono-zygotic twins was 66% less as the variance seen between di-zygotic twins. This indicates a level of heritability in the expression of leptin (Kaprio *et al.*, 2001).

Two studies, using completely different groups of twins, have measured the genetic influence on BMI. One study compared 3636 4-year old twins on BMI (Koeppen-Schomerus *et al.*, 2001), while the other study compared a group of adolescent twins (Pietilainen *et al.*, 1999). Both indicate a large genetic effect of over 80% for the variation in BMI.

For the third parameter mentioned here, PBF, a clear genetic component has been established. A study of 66 twin pairs, containing both mono-zygotic and di-zygotic twins, showed that between 75% and 80% of the phenotypic variation obtained between the twins can be accounted for by genetic influences (Faith *et al.*, 1999).

A total of 18 twin studies have been performed for obesity, which led to the final conclusion that heritability does play a role. A heritability between 40 and 70% with a concordance of 0.7-0.9 between mono-zygotic twins and a concordance of 0.35-0.45 between di-zygotic twins has now been estimated (reviewed by (Maes *et al.*, 1997) and (Allison *et al.*, 1996). Twin studies to determine the heritability of anorexia have mainly focused on questionnaires, either self-reports and/or data from the Eating Disorders Questionnaire (EDI). These give information about characteristics of anorexia, such as weight loss and length of amenorrhoea. On the basis of these data in three studies the genetic component of anorexia has been addressed. The comparison of 672 twins led to the conclusion that 74% of the variance in anorexia can be accounted for by genetic components and 26% by a non-shared environment (Klump *et al.*, 2001). Data-analysis of 25 mono-zygotic twins and 20 di-zygotic twins showed a concordance of respectivily 56% and 5%. This study shows that genetic factors play an important role in the predisposition for anorexia (Holland *et al.*, 1988). And finally in a study encompassing 30 twins, it was shown on basis of the questionnaires that 9/16 mono-zygotic twins and 1/14 di-zygotic twins were concordant for anorexia (Holland *et al.*, 1984).

Combining all twin studies done for anorexia the heritability for anorexia has been estimated at 58% with a concordance of 0.55 for mono-zygotic twins and 0.05 for di-zygotic twins (Wade *et al.*, 2000).

Once heritability was confirmed for obesity and anorexia linkage studies were performed. These linkage studies have resulted in the discovery of genomic areas which may be involved in the predisposition or development of either obesity or anorexia. These studies have also shown that the disorders can appear as monogenetic disorders or as part of a complex phenotype as often seen in clinical syndromes, especially in the case of obesity. Since many areas have been linked to obesity but also to anorexia we will focus on those areas that have shown the strongest links or in other words the highest LOD-scores.

For obesity about 26 linkage studies have been performed in families of different populations (obesity gene map database at http://obesitygene.pbrc.edu/). Some of these studies have only indicated areas on chromosomes, which could be involved in obesity. Other studies have also indicated candidate genes within linkage areas or linkage between candidate genes and phenotypic parameters of obesity. One clear example of a chromosomal area that has been linked to obesity is 7q. This is also the region where the structural locus for leptin is located, which is thought of as a very important candidate gene for obesity. Three studies report a linkage between this region of the leptin gene and obesity characteristics. One study in a Mexican-American population shows linkage between the leptin gene and waist/hip ratio ($p = 0.010$) (Bray *et al.*, 1999). The other two studies show linkage between the 7q region and BMI, a clear indicator of obesity (Feitosa *et al.*, 2002); (Platte *et al.*, 2003). In the Feitosa study over 3000 subjects were screened with different markers and a clear linkage was shown between BMI and chromosome 7q32.3 with a LOD score of 4.7 (Feitosa *et al.*, 2002). Platte *et al*. tested several markers for linkage with BMI and BMI-percentile and it appeared that those located on 7q were significantly linked with both ($P < \text{or} = 0.001$) (Platte *et al.*, 2003). Besides 7q, two other chromosomal areas have been linked to BMI using linkage analysis. Using several markers BMI was linked to 3q27 with a LOD score of 4.3 (Luke *et al.*, 2003) and to 13q14 with a LOD score of 3.2 (Feitosa *et al.*, 2002). Because of the high LOD scores, both regions are of potential importance in the search for obesity candidate genes.

Linkage studies have also linked genes to obesity and thereby confirmed their status of candidate genes. Genes like NPY, the leptin receptor, the melanocortin 5 receptor and the beta-3 adrenergic receptor, have been linked with BMI, blood glucose levels or simply obesity. NPY and MC5 have been linked to BMI (respectively $p = 0.020$ and $p = 0.001$) and the leptin receptor has been linked to fasting blood glucose ($p = 0.018$) (Bray *et al.*, 1999); (Chagnon *et al.*, 1997). Finally, in 10 large multigenerational families linkage between obesity and the chromosomal region of the beta-3 adrenergic receptor has been established with a LOD score of 3.21 (Mitchell *et al.*, 1999). Although only a fraction of the linkage studies performed have been mentioned here, it already shows that there are many chromosomal areas and with it many genes, that do play a role in obesity. It also shows that there are still many areas linked to obesity in which the genes still have to be discovered or linked to obesity.

The studies described above have been focused on obesity as single disorder but not yet on obesity as part of a complex phenotype. A total of 16 chromosomal areas have been identified in which obesity is part of a complex heritable syndrome. These areas linked to obesity contain genes, which play a role in different syndromes. These syndromes all have obesity as one of the phenotypic characteristics. The Prader-Willi syndrome is the most common of these pleiotropic obesity syndromes. It is an autosomal disorder and is characterised by diminished foetal activity, obesity, hypotonia, mental retardation, short stature, hypogonadotropic hypogonadism, and small hands and feet (Bray *et al.*, 1983); (Butler, 1990). Other examples are the Bardet-Biedel syndrome, characterised by mental retardation, pigmentary retinopathy, polydactyly, obesity, and hypogenitalism (Katsanis *et al.*, 2001) and the Wilson-Turner syndrome, which is characterised by mental retardation, tapering fingers and gynaecomastia (Wilson *et al.*, 1991). An overview of all the pleiotropic obesity syndromes and their clinical features can be found in table 3.

For anorexia not many linkage studies have been performed, only three studies report on linkage between chromosome areas and anorexia (Grice *et al.*, 2002); (Bergen *et al.*, 2003); (Koronyo-Hamaoui *et al.*, 2002). All these studies indicate linkage between chromosome 1 and anorexia. Two studies used the same sample group of American, Canadian, English, German and Swiss families. Both found significant linkage between anorexia and chromosome 1p. The first study established marker linkage on 1p with a LOD score of 3.45. In this region the glutamate receptor 7, hypocretin (orexin) receptor 1 and the leptin receptor are located, all genes indicated to be involved in anorexia (Grice *et al.*, 2002). The other study established linkage between chromosome 1p33-36, including the serotonin 1D and delta opiod receptor, and anorexia. At 72 cM a LOD score for both genes of 3.91 was found. When this area was narrowed down to 49 cM for serotonin 1D receptor and to 55.7 cM for the delta opioid receptor, LOD scores decreased respectively to 2 and 3.05 (Bergen *et al.*, 2003). The third study has been performed in Israeli families. In this study linkage between 1q21.3 and anorexia has been established with a significance of $p = 0.005$. In this region the hSKCa3 potassium channel gene is located. This gene has been indicated as candidate gene since it has been found to be associated with mental disorders (Koronyo-Hamaoui *et al.*, 2002).

Table 3. Human pleiotropic obesity syndromes

Obesity syndromes	Clinical features	Mode of inheritance	Locus	References
Ulnar-mammary	ulnar defects, delayed puberty, hypoplastic nipples	Autosomal dominant	12q24.1	Bamshad *et al*., 1995
Prader-Willi	diminished foetal activity, obesity, hypotonia, mental retardation, short stature, hypogonadotropic hypogonadism, and small hands and feet	Autosomal dominant	15q11-12	Butler, 1990
Alstrom	retinal dystrophy, neurosensory deafness, diabetes	Autosomal recessive	2p13	Collin *et al*., 1997 and Collin *et al*., 2002
Cohen	prominent central incisors, opthalmopathy, microcephaly	Autosomal recessive	8q22	Tahvanainen *et al*., 1994
Bardet-Biedl	mental retardation, pigmentary retinopathy, polydactyly, obesity, and hypogenitalism	Autosomal recessive	16q21, 15q22.3-23, 1q13, 3p13-12, 2q31, 20p12	Katsanis *et al*., 2001
Borjeson-Forssman-Lehmann	mental retardation, hypogonadism, large ears	X-linked	Xq26	Turner *et al*., 1989
Mehmo	mental retardation, epilepsy, hypogonadism, microcephaly	X-linked	Xp22.13	Steinmuller *et al*., 1998
Simpson-Golabi-Behmel	craniofacial defects, skeletal and visceral abnormalities	X-linked	Xp22	Brzustowicz *et al*., 1999
Wilson-Turner	mental retardation, tapering fingers,	X-linked	Xp21.2	Wilson *et al*., 1991

	gynaecomastia		
Abdominal obesity-metabolic syndrome	abdominal obesity and including glucose intolerance, dyslipidemia, and high blood pressure	3q27, 17p12	Reaven, 1988

5. PERSPECTIVES FOR PHARMACOGENETIC RESEARCH INTO EATING DISORDERS AND OBESITY

5.1. Obesity drugs

Drugs which have been available and licensed for obesity treatment until now are only moderately effective and may have serious side-effects. There is, however, great need for drugs which are on the long term both safe and effective with regard to weight loss and longstanding weight maintenance. Presently, only two drugs are licensed in most Western countries for long term use in obesity management: orlistat and sibutramine.

Orlistat is an inhibitor of gastric and pancreas lipase, resulting in a 30% decrease of the resorption of ingested fats and a similar increase of fecal fat excretion. The drug is virtually not absorbed and thus systemic side effects are absent. The side effects are directly related to the degree of steatorrhea which in turn is dependent of the amount of ingested fat.

Sibutramine is a selective inhibitor of the presynaptical reuptake of serotonin and norepinephrine, leading to enhanced central serotonin and norepinephrine neurotransmission which is associated with decreased sensations of hunger and increased feeling of satiety; besides, sibutramine leads to a slightly enhanced basal metabolic rate (Hansen *et al.*, 1999). Sibutramine has the following side effects: xerostomia, insomnia, obstipation and a mild increase of blood pressure and heart rate.

In a number of randomized, placebo-controlled trials of 1-2 year duration, both drugs are approximately equally effective, leading to a 3-5% more weight loss than placebo, significantly better weight maintenance and a significantly greater number of patients achieving at least 10% of weight loss (Yanovski and Yanovski, 2002); (Haddock *et al.*, 2002).

Other drugs, in some countries licensed for short-term use in obesity management, are amphetamine, its derivatives and related compounds: dexamphetamine, metamphetamine, benzamphetamine, diethylpropion, clobenzorax, fenpropex, phen-dimetrazine, phentermine and mazindol. These anorectic drugs increase the release and action of norepinephrine and dopamine and carry a serious risk of drug dependence and abuse and are therefore not approved in many countries.

Fenfluramine and its dextro-isomer dexfenfluramine both enhance release and inhibit reuptake of serotonin. Despite reasonable efficacy, both drugs have been taken from the market because of serious side-effects (carcinoid-like

valvular heart disease and pulmonary hypertension). Other serotonin reuptake inhibitors, such as fluoxetine, are not licensed for obesity treatment, because of insufficient efficacy.

Drugs currently under clinical investigation for use in obesity management are selective β3-sympathicomimetic drugs, the cannabinoid receptor antagonist rimonabant, the anticonvulsant drug topiramate, a NPY-receptor antagonist and a cholecystokinine-A receptor agonist.

5.2. Eating disorder drugs

Sibutramine has been shown to be effective in treating obesity and binge eating (Appolinario *et al.*, 2002); (Smith and Goulder, 2001); (Finer *et al.*, 2000). Brain sites producing serotonin (the nucleus raphe) and norepinephrine (mainly the locus ceruleus), project widely through the brain, including centers involved in regulation of energy balance. Since another serotonin-reuptake inhibitor, fenfluramine, which has been on the market to treat obesity, requires 5HT2C receptors (Vickers *et al.*, 1999) probably located in POMC neurons (Heisler *et al.*, 2002), one hypothesis might be that sibutramine also acts via this serotonergic system.

The antidepressant drug Fluoxetine, a more selective serotonin reuptake inhibitor, is generally well tolerated and may be an effective treatment option for adolescents with bulimia nervosa. (Kotler *et al.*, 2003). Treatment with fluoxetine in patients with bulimia nervosa improved outcome and decreased the likelihood of relapse. (Romano *et al.*, 2002). Fluoxetine may also prevent relapse in AN. It dimishes associated symptoms of anorexia nervosa following adequate weight restoration (Kim, 2003). Thus, for the treatment response to fluoxetine, genes constituting the serotonin system may play a role. Since serotonin 2C receptors expressed on leptin-sensitive POMC cells mediate at least some of the effects of the reuptake inhibitor fenfluramine on eating behaviour, also the neuropeptidergic genes implicated in the leptin response, are candidate genes for the treatment responsiveness of this class of drugs.

Olanzapine has been evaluated as drug therapy in anorexia nervosa in open label trials. Olanzapine administration was associated with weight gain and maintenance as well as reduced agitation and resistance to treatment (La Via *et al.*, 2000); (Powers *et al.*, 2002; Boachie *et al.*, 2003). Although olanzapine is an atypical antipsychotic that antagonises multiple receptors, it has a relatively high affinity for the serotonin 2C receptor, and may via this route influence energy balance.

Topiramate, an anticonvulsant drug, has anorectic effects. Topiramate may be an effective treatment for binge-eating disorder (Shapira *et al.*,2000). Topiramate treatment improves multiple behavioral dimensions of bulimia nervosa. Binge and purge behaviors are reduced, and treatment is associated with improvements in self-esteem, eating attitudes, anxiety, and body image(Hedges *et al.*, 2003).

The mechanism of drug action is poorly understood but topiramate has been reported to interact with various ion channel types, including AMPA/kainate receptors and in particular GluR5 kainate receptors (Gryder and Rogawski,

2003). These receptors are widely distributed in brain. It is not known how altered activity of these channels by toporimate could play a role in treating eating disorders.

6. CONCLUDING REMARKS

Pharmacogenetic studies have not been performed yet for the drugs that are prescribed for the treatment of obesity and eating disorders. Only orlistat and sibutramine are accepted pharmacotherapies, whereas for eating disorders drug treatment is still in an exploratory phase. The identification of the neural circuitry involved in regulation of energy balance has now implicated several genes, many of which can be targeted with drugs. These genes are also candidate genes to modify drug responsiveness. Genetic variability (including functional polymorphisms) for many of these candidate genes are known, thus making pharmacogenetic studies feasible.

Genes influence susceptibility for obesity and eating disorders via traits within these complex disorders. Indeed for some of these traits, such as perfectionism, a strong genetic component has been identified. Refinement of (behavioural) traits within complex behaviours is an essential step in order to understand better how genes affect development of eating disorders and obesity (Kas *et al.*, 2003). Identification of genes underlying (behavioural) traits within eating disorders and obesity will paven the way towards refinement in classification of eating disorders and obesity endophenotypes. Different endophenotypes may require different pharmacotherapies. One clear example is the treatment with leptin which is successful only in obese individuals carrying mutations in the leptin gene. Once we understand better how the genes described in this chapter affect human feeding behavior and energy balance, new drug treatment strategies can be developed and pharmacogenetic studies will be helpful to select patients for these drug treatments.

7. REFERENCES

Report: Obesity: preventing and managing the global epidemic. 2000, Geneva, World Health Organization. WHO Tech Rep Ser 894.

Report: Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition (DSM-IV). 2004, Washington DC, American Psychiatric Association.

Adan, R. A, and Kas, M. J., 2003, Inverse agonism gains weight, *Trends Pharmacol. Sci.* **24**: 315-321.

Allison, D. B., Kaprio, J., Korkeila, M., Koskenvuo, M., Neale, M. C., and Hayakawa, K., 1996, The heritability of body mass index among an international sample of monozygotic twins reared apart, *Int. J. Obes. Relat Metab Disord.* **20**:501-506.

Ammar, A. A., Sederholm, F., Saito, T. R., Scheurink, A. J., Johnson, A. E., and Sodersten, P., 2000, NPY-leptin: opposing effects on appetitive and consummatory ingestive behavior and sexual behavior, *Am. J. Physiol Regul. Integr. Comp Physiol* **278**:R1627-R1633.

Appolinario, J. C., Godoy-Matos, A., Fontenelle, L. F., Carraro, L., Cabral, M., Vieira, A., and Coutinho, W., 2002, An open-label trial of sibutramine in obese patients with binge-eating disorder, *J. Clin. Psychiatry* **63**:28-30.

Argyropoulos, G., Rankinen, T., Neufeld, D. R., Rice, T., Province, M. A., Leon, A. S., Skinner, J. S., Wilmore, J. H., Rao, D. C., and Bouchard, C., 2002, A polymorphism in the human

agouti-related protein is associated with late-onset obesity, *J. Clin. Endocrinol. Metab* **87**:4198-4202.

Bale, T. L., Contarino, A., Smith, G. W., Chan, R., Gold, L. H., Sawchenko, P. E., Koob, G. F., Vale, W. W., and Lee, K. F., 2000, Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress, *Nat. Genet.* **24**:410-414.

Bamshad, M., Krakowiak, P. A., Watkins, W. S., Root, S., Carey, J. C., and Jorde, L. B., 1995, A gene for ulnar-mammary syndrome maps to 12q23-q24.1, *Hum. Mol. Genet.* **4**: 1973-1977.

Barbarich, N. C., Kaye, W. H., and Jimerson, D., 2003, Neurotransmitter and imaging studies in anorexia nervosa: new targets for treatment, *Curr. Drug Target CNS. Neurol. Disord.* **2**:61-72.

Bergen, A. W., van den Bree, M. B., Yeager, M., Welch, R., Ganjei, J. K., Haque, K., Bacanu, S., Berrettini, W. H., Grice, D. E., Goldman, D., Bulik, C. M., Klump, K., Fichter, M., Halmi, K., Kaplan, A., Strober, M., Treasure, J., Woodside, B., and Kaye, W. H., 2003, Candidate genes for anorexia nervosa in the 1p33-36 linkage region: serotonin 1D and delta opioid receptor loci exhibit significant association to anorexia nervosa, *Mol. Psychiatry.* **8**:397-406.

Berman, Y., Mzhavia, N., Polonskaia, A., and Devi, L. A., 2001, Impaired prohormone convertases in Cpe(fat)/Cpe(fat) mice, *J. Biol. Chem.* **276**:1466-1473.

Boachie, A., Goldfield, G. S., and Spettigue, W., 2003, Olanzapine use as an adjunctive treatment for hospitalized children with anorexia nervosa: case reports, *Int. J. Eat. Disord.* **33**: 98-103.

Bray, G. A., Dahms, W. T., Swerdloff, R. S., Fiser, R. H., Atkinson, R. L., and Carrel, R. E., 1983, The Prader-Willi syndrome: a study of 40 patients and a review of the literature, *Medicine (Baltimore)* **62**:59-80.

Bray, M. S., Boerwinkle, E., and Hanis, C. L., 1999, Linkage analysis of candidate obesity genes among the Mexican-American population of Starr County, Texas, *Genet. Epidemiol.* **16**:397-411.

Broberger, C., Johansen, J., Brismar, H., Johansson, C., Schalling, M., and Hokfelt, T., 1999, Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus, *J. Neurosci.* **19**:7130-7139.

Brzustowicz, L. M., Farrell, S., Khan, M. B., and Weksberg, R., 1999, Mapping of a new SGBS locus to chromosome Xp22 in a family with a severe form of Simpson-Golabi-Behmel syndrome, *Am. J. Hum. Genet.* **65**:779-783.

Bulik, C. M., Sullivan, P. F., Weltzin, T. E., and Kaye, W. H., 1995, Temperament in eating disorders, *Int. J. Eat. Disord.* **17**:251-261.

Butler, M. G., 1990, Prader-Willi syndrome: current understanding of cause and diagnosis, *Am. J. Med. Genet.* **35**:319-332.

Calle, E. E., Thun, M. J., Petrelli, J. M., Rodriguez, C., and Heath, C. W., Jr., 1999, Body-mass index and mortality in a prospective cohort of U.S. adults, *N. Engl. J. Med.* **341**: 1097-1105.

Chagnon, Y. C., Chen, W. J., Perusse, L., Chagnon, M., Nadeau, A., Wilkison, W. O., and Bouchard, C., 1997, Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity-related phenotypes in the Quebec Family Study, *Mol. Med.* **3**:663-673.

Challis, B. G., Pritchard, L. E., Creemers, J. W., Delplanque, J., Keogh, J. M., Luan, J., Wareham, N. J., Yeo, G. S., Bhattacharyya, S., Froguel, P., White, A., Farooqi, I. S., and O'Rahilly, S., 2002, A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism, *Hum. Mol. Genet.* **11**:1997-2004.

Collin, G. B., Marshall, J. D., Cardon, L. R., and Nishina, P. M., 1997, Homozygosity mapping at Alstrom syndrome to chromosome 2p, *Hum. Mol. Genet.* **6**:213-219.

Collin, G. B., Marshall, J. D., Ikeda, A., So, W. V., Russell-Eggitt, I., Maffei, P., Beck, S., Boerkoel, C. F., Sicolo, N., Martin, M., Nishina, P. M., and Naggert, J. K., 2002, Mutations in ALMS1 cause obesity, type 2 diabetes and neurosensory degeneration in Alstrom syndrome, *Nat. Genet.* **31**:74-78.

Cowley, M. A., Smith, R. G., Diano, S., Tschop, M., Pronchuk, N., Grove, K. L., Strasburger, C. J., Bidlingmaier, M., Esterman, M., Heiman, M. L., Garcia-Segura, L. M., Nillni, E. A.,

Mendez, P., Low, M. J., Sotonyi, P., Friedman, J. M., Liu, H., Pinto, S., Colmers, W. F., Cone, R. D., and Horvath, T. L., 2003, The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis, *Neuron* **37**:649-661.

Deep, A. L., Nagy, L. M., Weltzin, T. E., Rao, R., and Kaye, W. H., 1995, Premorbid onset of psychopathology in long-term recovered anorexia nervosa, *Int. J. Eat. Disord.* **17**: 291-297.

Devlin, B., Bacanu, S. A., Klump, K. L., Bulik, C. M., Fichter, M. M., Halmi, K. A., Kaplan, A. S., Strober, M., Treasure, J., Woodside, D. B., Berrettini, W. H., and Kaye, W. H., 2002, Linkage analysis of anorexia nervosa incorporating behavioral covariates, *Hum. Mol. Genet.* **11**:689-696.

Di Marzo., V, Goparaju, S. K., Wang, L., Liu, J., Batkai, S., Jarai, Z., Fezza, F., Miura, G. I., Palmiter, R. D., Sugiura, T., and Kunos, G., 2001, Leptin-regulated endocannabinoids are involved in maintaining food intake, *Nature.* **410**:822-825.

Dubern, B., Clement, K., Pelloux, V., Froguel, P., Girardet, J. P., Guy-Grand, B., and Tounian, P., 2001, Mutational analysis of melanocortin-4 receptor, agouti-related protein, and alpha-melanocyte-stimulating hormone genes in severely obese children, *J. Pediatr.* **139**: 204-209.

Erickson, J. C., Hollopeter, G., and Palmiter, R. D., 1996, Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y, *Science.* **274**:1704-1707.

Faith, M. S., Rha, S. S., Neale, M. C., and Allison, D. B., 1999, Evidence for genetic influences on human energy intake: results from a twin study using measured observations, *Behav. Genet.* **29**:145-154.

Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S. A., Perna, F., Fontana, S., Lechler, R. I., DePaoli, A. M., and O'Rahilly, S., 2002, Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency, *J. Clin. Invest.* **110**:1093-1103.

Feitosa, M. F., Borecki, I. B., Rich, S. S., Arnett, D. K., Sholinsky, P., Myers, R. H., Leppert, M., and Province, M. A., 2002, Quantitative-trait loci influencing body-mass index reside on chromosomes 7 and 13: the National Heart, Lung, and Blood Institute Family Heart Study, *Am. J. Hum. Genet.* **70**:72-82.

Finer, N., Bloom, S. R., Frost, G. S., Banks, L. M., and Griffiths, J., 2000, Sibutramine is effective for weight loss and diabetic control in obesity with type 2 diabetes: a randomised, double-blind, placebo-controlled study, *Diabetes Obes. Metab.* **2**:105-112.

Frank, G. K., Kaye, W. H., Weltzin, T. E., Perel, J., Moss, H., McConaha, C., and Pollice, C., 2001, Altered response to meta-chlorophenylpiperazine in anorexia nervosa: support for a persistent alteration of serotonin activity after short-term weight restoration, *Int. J. Eat. Disord.* **30**:57-68.

Frank, G. K., Kaye, W. H., Meltzer, C. C., Price, J. C., Greer, P., McConaha, C., and Skovira, K., 2002, Reduced 5-HT2A receptor binding after recovery from anorexia nervosa, *Biol. Psychiatry.* **52**:896-906.

Friedman, J. M. and Halaas, J. L., 1998, Leptin and the regulation of body weight in mammals, *Nature.* **395**:763-770.

Funakoshi, A., Miyasaka, K., Matsumoto, H., Yamamori, S., Takiguchi, S., Kataoka, K., Takata, Y., Matsusue, K., Kono, A., and Shimokata, H., 2000, Gene structure of human cholecystokinin (CCK) type-A receptor: body fat content is related to CCK type-A receptor gene promoter polymorphism, *FEBS Lett.* **466**:264-266.

Gorwood, P., Ades, J., Bellodi, L., Cellini, E., Collier, D. A., Di Bella, D., Di Bernardo, M., Estivill, X., Fernandez-Aranda, F., Gratacos, M., Hebebrand, J., Hinney, A., Hu, X., Karwautz, A., Kipman, A., Mouren-Simeoni, M. C., Nacmias, B., Ribases, M., Remschmidt, H., Ricca, V., Rotella, C. M., Sorbi, S., and Treasure, J., 2002, The 5-HT(2A) -1438G/A polymorphism in anorexia nervosa: a combined analysis of 316 trios from six European centres, *Mol. Psychiatry.* **7**:90-94.

Grice, D. E., Halmi, K. A., Fichter, M. M., Strober, M., Woodside, D. B., Treasure, J. T., Kaplan, A. S., Magistretti, P. J., Goldman, D., Bulik, C. M., Kaye, W. H., and Berrettini, W. H., 2002, Evidence for a susceptibility gene for anorexia nervosa on chromosome 1, *Am. J. Hum. Genet.* **70**:787-792.

Gryder, D. S, and Rogawski, M. A., 2003, Selective antagonism of GluR5 kainate-receptor-mediated synaptic currents by topiramate in rat basolateral amygdala neurons, *J. Neurosci.* **23**:7069-7074.

Gu, W., Tu, Z., Kleyn, P. W., Kissebah, A., Duprat, L., Lee, J., Chin, W., Maruti, S., Deng, N., Fisher, S. L., Franco, L. S., Burn, P., Yagaloff, K. A., Nathan, J., Heymsfield, S., Albu, J., Pi-Sunyer, F. X., and Allison, D. B., 1999, Identification and functional analysis of novel human melanocortin-4 receptor variants, *Diabetes.* **48**:635-639.

Guan, X. M., Yu, H., and Van der Ploeg, L. H., 1998, Evidence of altered hypothalamic pro-opiomelanocortin/ neuropeptide Y mRNA expression in tubby mice, *Brain Res. Mol. Brain Res.* **59**:273-279.

Gull, W. W., 1874, Anorexia Nervosa (apepsia hysterica, anorexia hysterica), *Transact. Clin. Soc. London.* **7**:22-28.

Haddock, C. K., Poston, W. S., Dill, P. L., Foreyt, J. P., and Ericsson, M., 2002, Pharmacotherapy for obesity: a quantitative analysis of four decades of published randomized clinical trials, *Int. J. Obes. Relat Metab Disord.* **26**:262-273.

Hager, J., Clement, K., Francke, S., Dina, C., Raison, J., Lahlou, N., Rich, N., Pelloux, V., Basdevant, A., Guy-Grand, B., North, M., and Froguel, P., 1998, A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels, *Int. J. Obes. Relat Metab Disord.* **22**:200-205.

Halaas, J. L., and Friedman, J. M., 1997, Leptin and its receptor, *J. Endocrinol.* **155**:215-216.

Hansen, D. L., Toubro, S., Stock, M. J., Macdonald, I. A., and Astrup, A., 1999, The effect of sibutramine on energy expenditure and appetite during chronic treatment without dietary restriction, *Int. J. Obes. Relat Metab Disord.* **23**:1016-1024.

Hara, J., Beuckmann, C. T., Nambu, T., Willie, J. T., Chemelli, R. M., Sinton, C. M., Sugiyama, F., Yagami, K., Goto, K., Yanagisawa, M., and Sakurai, T., 2001, Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity, *Neuron* **30**:345-354.

Heath, A. C., Cloninger, C. R., and Martin, N. G., 1994, Testing a model for the genetic structure of personality: a comparison of the personality systems of Cloninger and Eysenck, *J. Pers. Soc. Psychol.* **66**:762-775.

Hebebrand, J., Exner, C., Hebebrand, K., Holtkamp, C., Casper, R. C., Remschmidt, H., Herpertz-Dahlmann, B., and Klingenspor, M., 2003, Hyperactivity in patients with anorexia nervosa and in semistarved rats: evidence for a pivotal role of hypoleptinemia, *Physiol. Behav.* **79**:25-37.

Hedges, D. W., Reimherr, F. W., Hoopes, S. P., Rosenthal, N. R., Kamin, M., Karim, R., and Capece, J. A., 2003, Treatment of bulimia nervosa with topiramate in a randomized, double-blind, placebo-controlled trial, part 2: improvement in psychiatric measures, *J. Clin. Psychiatry.* **64**:1449-1454.

Heisler, L. K., Cowley, M. A., Tecott, L. H., Fan, W., Low, M. J., Smart, J. L., Rubinstein, M., Tatro, J. B., Marcus, J. N., Holstege, H., Lee, C. E., Cone, R. D., and Elmquist, J. K., 2002, Activation of central melanocortin pathways by fenfluramine, *Science.* **297**: 609-611.

Hinney, A., Becker, I., Heibult, O., Nottebom, K., Schmidt, A., Ziegler, A., Mayer, H., Siegfried, W., Blum, W. F., Remschmidt, H., and Hebebrand, J., 1998, Systematic mutation screening of the pro-opiomelanocortin gene: identification of several genetic variants including three different insertions, one nonsense and two missense point mutations in probands of different weight extremes, *J. Clin. Endocrinol. Metab.* **83**:3737-3741.

Hinney, A., Schmidt, A., Nottebom, K., Heibult, O., Becker, I., Ziegler, A., Gerber, G., Sina, M., Gorg, T., Mayer, H., Siegfried, W., Fichter, M., Remschmidt, H., and Hebebrand, J., 1999, Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans, *J. Clin. Endocrinol. Metab.* **84**:1483-1486.

Hoffstedt, J., Eriksson, P., Mottagui-Tabar, S., and Arner, P., 2002, A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin, *Horm. Metab Res.* **34**:355-359.

Holland, A. J., Hall, A., Murray, R., Russell, G. F., and Crisp, A. H., 1984, Anorexia nervosa: a study of 34 twin pairs and one set of triplets, *Br. J. Psychiatry.* **145**:414-419.

Holland, A. J., Sicotte, N., and Treasure, J., 1988, Anorexia nervosa: evidence for a genetic basis, *J. Psychosom. Res.* **32**:561-571.

Kaprio, J., Eriksson, J., Lehtovirta, M., Koskenvuo, M., and Tuomilehto, J., 2001, Heritability of leptin levels and the shared genetic effects on body mass index and leptin in adult Finnish twins, *Int. J. Obes. Relat. Metab. Disord.* **25**:132-137.

Kas, M. J., van Elburg, A. A., van Engeland, H., and Adan, R. A., 2003, Refinement of behavioural traits in animals for the genetic dissection of eating disorders, *Eur. J. Pharmacol.* **480**: 13-20.

Katsanis, N., Lupski, J. R., and Beales, P. L., 2001, Exploring the molecular basis of Bardet-Biedl syndrome, *Hum. Mol. Genet.* **10**:2293-2299.

Kaye, W. H., Gwirtsman, H. E., George, D. T., and Ebert, M. H., 1991, Altered serotonin activity in anorexia nervosa after long-term weight restoration. Does elevated cerebrospinal fluid 5-hydroxyindoleacetic acid level correlate with rigid and obsessive behavior?, *Arch. Gen. Psychiatry.* **48**:556-562.

Kaye, W. H., 1997, Anorexia nervosa, obsessional behavior, and serotonin, *Psychopharmacol. Bull.* **33**:335-344.

Kaye, W. H., Barbarich, N. C., Putnam, K., Gendall, K. A., Fernstrom, J., Fernstrom, M., McConaha, C. W., and Kishore, A., 2003, Anxiolytic effects of acute tryptophan depletion in anorexia nervosa, *Int. J. Eat. Disord.* **33**:257-267.

Kim, S. S., 2003, Role of fluoxetine in anorexia nervosa, *Ann. Pharmacother.* **37**:890-892.

Klump, K. L., Miller, K. B., Keel, P. K., McGue, M., and Iacono, W. G., 2001, Genetic and environmental influences on anorexia nervosa syndromes in a population-based twin sample, *Psychol. Med.* **31**:737-740.

Koeppen-Schomerus, G., Wardle, J., and Plomin, R., 2001, A genetic analysis of weight and overweight in 4-year-old twin pairs, *Int. J. Obes. Relat Metab Disord.* **25**:838-844.

Koronyo-Hamaoui, M., Danziger, Y., Frisch, A., Stein, D., Leor, S., Laufer, N., Carel, C., Fennig, S., Minoumi, M., Apter, A., Goldman, B., Barkai, G., Weizman, A., and Gak, E., 2002, Association between anorexia nervosa and the hsKCa3 gene: a family-based and case control study, *Mol. Psychiatry.* **7**:82-85.

Kotler, L. A., Devlin, M. J., Davies, M., and Walsh, B. T., 2003, An open trial of fluoxetine for adolescents with bulimia nervosa, *J. Child Adolesc. Psychopharmacol.* **13**:329-335.

La Via, M. C., Gray, N., and Kaye, W. H., 2000, Case reports of olanzapine treatment of anorexia nervosa, *Int. J. Eat. Disord.* **27**:363-366.

Lasèque, C., 1873, De l'anorexie hystérique, *Arch. Gen. Medecine.* **1**:385-403.

Legradi, G., Emerson, C. H., Ahima, R. S., Flier, J. S., and Lechan, R. M., 1997, Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus, *Endocrinology.* **138**: 2569-2576.

Li, W. D., Reed, D. R., Lee, J. H., Xu, W., Kilker, R. L., Sodam, B. R., and Price, R. A., 1999, Sequence variants in the 5' flanking region of the leptin gene are associated with obesity in women, *Ann. Hum. Genet.* **63**:227-234.

Ludwig, D. S., Tritos, N. A., Mastaitis, J. W., Kulkarni, R., Kokkotou, E., Elmquist, J., Lowell, B., Flier, J. S., and Maratos-Flier, E., 2001, Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance, *J. Clin. Invest.* **107**:379-386.

Luke, A., Wu, X., Zhu, X., Kan, D., Su, Y., and Cooper, R., 2003, Linkage for BMI at 3q27 region confirmed in an African-American population, *Diabetes.* **52**:1284-1287.

Maes, H. H., Neale, M. C., and Eaves, L. J., 1997, Genetic and environmental factors in relative body weight and human adiposity, *Behav. Genet.* **27**:325-351.

Marchal-Victorion, S., Vionnet, N., Escrieut, C., Dematos, F., Dina, C., Dufresne, M., Vaysse, N., Pradayrol, L., Froguel, P., and Fourmy, D., 2002, Genetic, pharmacological and functional analysis of cholecystokinin-1 and cholecystokinin-2 receptor polymorphism in type 2 diabetes and obese patients, *Pharmacogenetics.* **12**:23-30.

Masaki, T., Yoshimichi, G., Chiba, S., Yasuda, T., Noguchi, H., Kakuma, T., Sakata, T., and Yoshimatsu, H., 2003, Corticotropin-releasing hormone-mediated pathway of leptin to regulate feeding, adiposity, and uncoupling protein expression in mice, *Endocrinology.* **144**:3547-3554.

Mayfield, D. K., Brown, A. M., Page, G. P., Garvey, W. T., Shriver, M. D., and Argyropoulos, G., 2001, A role for the Agouti-Related Protein promoter in obesity and type 2 diabetes, *Biochem. Biophys. Res. Commun.* **287**:568-573.

Miraglia, D. G., Cirillo, G., Santoro, N., D'Urso, L., Carbone, M. T., Di Toro, R., and Perrone, L., 2001, Molecular screening of the proopiomelanocortin (POMC) gene in Italian obese children: report of three new mutations, *Int. J. Obes. Relat. Metab. Disord.* **25**:61-67.

Mitchell, B. D., Cole, S. A., Comuzzie, A. G., Almasy, L., Blangero, J., MacCluer, J. W., and Hixson, J. E., 1999, A quantitative trait locus influencing BMI maps to the region of the beta-3 adrenergic receptor, *Diabetes.* **48**:1863-1867.

Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., Sewter, C. P., Digby, J. E., Mohammed, S. N., Hurst, J. A., Cheetham, C. H., Earley, A. R., Barnett, A. H., Prins, J. B., and O'Rahilly, S., 1997, Congenital leptin deficiency is associated with severe early-onset obesity in humans, *Nature.* **387**:903-908.

Moran, T. H., and Schwartz, G. J., 1994, Neurobiology of cholecystokinin, *Crit Rev. Neurobiol.* **9**: 1-28.

Naggert, J. K., Fricker, L. D., Varlamov, O., Nishina, P. M., Rouille, Y., Steiner, D. F., Carroll, R. J., Paigen, B. J., and Leiter, E. H., 1995, Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity, *Nat. Genet.* **10**:135-142.

Noble, F., and Roques, B. P., 2002, Phenotypes of mice with invalidation of cholecystokinin (CCK(1) or CCK(2)) receptors, *Neuropeptides.* **36**:157-170.

Pietilainen, K. H., Kaprio, J., Rissanen, A., Winter, T., Rimpela, A., Viken, R. J., and Rose, R. J., 1999, Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons, *Int. J. Obes. Relat. Metab. Disord.* **23**:107-115.

Platte, P., Papanicolaou, G. J., Johnston, J., Klein, C. M., Doheny, K. F., Pugh, E. W., Roy-Gagnon, M. H., Stunkard, A. J., Francomano, C. A., and Wilson, A. F., 2003, A study of linkage and association of body mass index in the Old Order Amish, *Am. J. Med. Genet.* **121C**:71-80.

Powers, P. S., Santana, C. A., and Bannon, Y. S., 2002, Olanzapine in the treatment of anorexia nervosa: an open label trial, *Int. J. Eat. Disord.* **32**:146-154.

Reaven, G. M., 1988, Banting lecture 1988. Role of insulin resistance in human disease, *Diabetes.* **37**:1595-1607.

Ribases, M., Gratacos, M., Armengol, L., de Cid, R., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez, F., and Estivill, X., 2003, Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type, *Mol. Psychiatry* **8**:745-751.

Romano, S. J., Halmi, K. A., Sarkar, N. P., Koke, S. C., and Lee, J. S., 2002, A placebo-controlled study of fluoxetine in continued treatment of bulimia nervosa after successful acute fluoxetine treatment, *Am. J. Psychiatry.* **159**:96-102.

Rushing, P. A., 2003, Central amylin signaling and the regulation of energy homeostasis, *Curr. Pharm. Des.* **9**:819-825.

Shapira, N. A., Goldsmith, T. D., and McElroy, S. L., 2000, Treatment of binge-eating disorder with topiramate: a clinical case series, *J. Clin. Psychiatry.* **61**:368-372.

Shimada, M., Tritos, N. A., Lowell, B. B., Flier, J. S., and Maratos-Flier, E., 1998, Mice lacking melanin-concentrating hormone are hypophagic and lean, *Nature.* **396**:670-674.

Siegfried, Z., Berry, E. M., Hao, S., and Avraham, Y., 2003, Animal models in the investigation of anorexia, *Physiol. Behav.* **79**:39-45.

Smith, I. G, and Goulder, M. A., 2001, Randomized placebo-controlled trial of long-term treatment with sibutramine in mild to moderate obesity, *J. Fam. Pract.* **50**:505-512.

Srinivasagam, N. M., Kaye, W. H., Plotnicov, K. H., Greeno, C., Weltzin, T. E., and Rao, R., 1995, Persistent perfectionism, symmetry, and exactness after long-term recovery from anorexia nervosa, *Am. J. Psychiatry.* **152**:1630-1634.

Steinmuller, R., Steinberger, D., and Muller, U., 1998, MEHMO (mental retardation, epileptic seizures, hypogonadism and -genitalism, microcephaly, obesity), a novel syndrome: assignment of disease locus to xp21.1-p22.13, *Eur. J. Hum. Genet.* **6**:201-206.

Strobel, A., Issad, T., Camoin, L., Ozata, M., and Strosberg, A. D., 1998, A leptin missense mutation associated with hypogonadism and morbid obesity, *Nat. Genet.* **18**:213-215.

Szczypka, M. S., Kwok, K., Brot, M. D., Marck, B. T., Matsumoto, A. M., Donahue, B. A., and Palmiter, R. D., 2001, Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice, *Neuron.* **30**:819-828.

Tahvanainen, E., Norio, R., Karila, E., Ranta, S., Weissenbach, J., Sistonen, P., and de la, C. A., 1994, Cohen syndrome gene assigned to the long arm of chromosome 8 by linkage analysis, *Nat. Genet.* **7**:201-204.

Turner, G., Gedeon, A., Mulley, J., Sutherland, G., Rae, J., Power, K., and Arthur, I., 1989, Borjeson-Forssman-Lehmann syndrome: clinical manifestations and gene localization to Xq26-27, *Am. J. Med. Genet.* **34**:463-469.

Vaisse, C., Clement, K., Guy-Grand, B., and Froguel, P., 1998, A frameshift mutation in human MC4R is associated with a dominant form of obesity, *Nat. Genet.* **20**:113-114.

van Rossum, C. T., Hoebee, B., van Baak, M. A., Mars, M., Saris, W. H., and Seidell, J. C., 2003, Genetic variation in the leptin receptor gene, leptin, and weight gain in young Dutch adults, *Obes. Res.* **11**:377-386.

Vickers, S. P., Clifton, P. G., Dourish, C. T., and Tecott, L. H., 1999, Reduced satiating effect of d-fenfluramine in serotonin 5-HT(2C) receptor mutant mice, *Psychopharmacology (Berl)* **143**:309-314.

Vink, T., Hinney, A., van Elburg, A. A., van Goozen, S. H., Sandkuijl, L. A., Sinke, R. J., Herpertz-Dahlmann, B. M., Hebebrand, J., Remschmidt, H., van Engeland, H., and Adan, R. A., 2001, Association between an agouti-related protein gene polymorphism and anorexia nervosa, *Mol. Psychiatry.* **6**:325-328.

Wade, T. D., Bulik, C. M., Neale, M., and Kendler, K. S., 2000, Anorexia nervosa and major depression: shared genetic and environmental risk factors, *Am. J. Psychiatry.* **157**: 469-471.

Wilson, M., Mulley, J., Gedeon, A., Robinson, H., and Turner, G., 1991, New X-linked syndrome of mental retardation, gynecomastia, and obesity is linked to DXS255, *Am. J. Med. Genet.* **40**:406-413.

Xu, B., Goulding, E. H., Zang, K., Cepoi, D., Cone, R. D., Jones, K. R., Tecott, L. H., and Reichardt, L. F., 2003, Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor, *Nat. Neurosci.* **6**:736-742.

Yamada, K., Yuan, X., Otabe, S., Koyanagi, A., Koyama, W., and Makita, Z., 2002, Sequencing of the putative promoter region of the cocaine- and amphetamine-regulated-transcript gene and identification of polymorphic sites associated with obesity, *Int. J. Obes. Relat Metab Disord.* **26**:132-136.

Yamada, M., Miyakawa, T., Duttaroy, A., Yamanaka, A., Moriguchi, T., Makita, R., Ogawa, M., Chou, C. J., Xia, B., Crawley, J. N., Felder, C. C., Deng, C. X., and Wess, J., 2001, Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean, *Nature* **410**:207-212.

Yanovski, S. Z. and Yanovski, J. A., 2002, Obesity, *N. Engl. J. Med.* **346**:591-602.

Yeo, G. S., Farooqi, I. S., Aminian, S., Halsall, D. J., Stanhope, R. G., and O'Rahilly, S., 1998, A frameshift mutation in MC4R associated with dominantly inherited human obesity, *Nat. Genet.* **20**:111-112.

Yiannakouris, N., Yannakoulia, M., Melistas, L., Chan, J. L., Klimis-Zacas, D., and Mantzoros, C. S., 2001, The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability, *J. Clin. Endocrinol. Metab* **86**:4434-4439.

9. NEUROPSYCHOPHARMACOGENETICS: 'STIMULATING' RATIONALE THERAPY IN ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

Pharmacogenetics of psychostimulants in ADHD

Mario Masellis, Vincenzo S. Basile, and James L. Kennedy*

1. INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent childhood behavioural disease affecting approximately 3-5% of school-age children (A.P.A., 1994). It also poses a major problem for adults who were either first diagnosed later in life or have suffered from persisting symptoms from childhood (Faraone et al., 2000). The cardinal symptoms of ADHD may be classified into three major clusters – inattentiveness, hyperactivity, and impulsivity (A.P.A., 1994). These symptom clusters may coexist or may occur individually and must significantly

* Mario Masellis, Departments of Neurology and Psychiatry, University of Toronto, Neuroscience Research, Sunnybrook & Women's College Health Science Centre, Cognitive Neurology Unit, Room A 421, 2075 Bayview Avenue, Toronto, Ontario, Canada M4N 3M5; Tel: 416-480-6100 ext 2449; Fax: 416-480-4552; E-mail: mario.masellis@utoronto.ca. Vincenzo S. Basile, Departments of Neurology and Psychiatry, University of Toronto, Toronto, Canada. James L. Kennedy, Department of Psychiatry, University of Toronto, Centre for Addiction and Mental Health, Toronto, Canada.

interfere with academic, family, and social functioning of the afflicted individual. Furthermore, onset must be ascertained to be prior to age seven. ADHD is a complex, multifactorial illness of unknown etiology with evidence suggesting a strong genetic component as determined by family, twin and adoption studies (Faraone et al., 1994; Gillis et al., 1992; Hechtman, 1994; Pauls, 1991).

Approximately 60 years ago, Bradley (1937) published the first report suggesting that psychostimulants have a unique calming effect on the behaviour of hyperactive children. Since then, there has been a wealth of research confirming the effectiveness of psychostimulant medications in controlling the hyperactive, impulsive, and inattentive symptoms of ADHD, and much work has been invested in determining their mechanism of action. As with all drugs, understanding the mechanism through which psychostimulants produce their therapeutic effects begins with the study of two important pharmacological parameters – pharmacokinetics (PK) and pharmacodynamics (PD).

In order for a drug to exert its biological effect, it must first accumulate in the tissue(s) where its pharmacological 'target(s)' is/are located. Pharmacokinetics explains how a drug achieves this by examining the drug concentration versus time relationships in an organism through mathematical formulations of its absorption from site of administration, its distribution in tissues throughout the body, its metabolism by various enzyme systems, and its excretion from the body (ADME framework) (Greenblatt et al., 1995; Rowland et al., 1995). Once a drug is concentrated in the tissue, it interacts with the 'target(s)' through which the final biological effects are elicited (pharmacodynamic interaction). Specifically, pharmacodynamics describes the drug concentration versus effect relationships in an organism (Greenblatt et al., 1995; Rowland et al., 1995). The targets may include a variety of proteins, such as receptors, enzymes, transporters, ion channels, second messengers, among others. Alternatively, a drug may directly or indirectly interact with deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) to produce its biological effects.

We will now briefly review the pharmacokinetics and pharmacodynamics of methylphenidate (Ritalin®), the most commonly prescribed psychostimulant used in over 90% of children diagnosed with ADHD in the U.S. (Kimko et al., 1999; Safer et al., 1988). This will be followed by a discussion of neuropsychopharmacogenetics and its application to ADHD using methylphenidate response as an example. Other less commonly used psychostimulants include dextroamphetamine and pemoline, which have been reviewed elsewhere (Greenhill et al., 2002; Markowitz et al., 2001).

1.1. Pharmacokinetics of methylphenidate

Absorption of methylphenidate is complete and rapid with time to peak plasma concentrations (t_{max}) after oral administration being between 1 and 3 hours (Kimko et al., 1999). In hyperactive children, there is considerable person-to-person

variability in the absorption of methylphenidate [rate constant: $1.83 \pm 1.5\ h^{-1}$ for 0.34 mg/kg and $2.07 \pm 1.0\ h^{-1}$ for 0.65 mg/kg] (Shaywitz et al., 1982). Methylphenidate is highly lipid soluble and the extent of protein binding is low, so it undergoes rapid distribution throughout the body (Kimko et al., 1999). It is thus possible to attain high methylphenidate concentrations in the central nervous system (CNS) over a short duration of time.

In terms of metabolism and excretion in healthy adults and in children with ADHD, methylphenidate undergoes extensive stereoselective and first-pass clearance (Hubbard et al., 1989; Srinivas et al., 1993), with plasma concentrations of *d*-methylphenidate being greater than those of the *l*-isomer. The *d*-enantiomer is responsible for the clinical effects of the drug (Srinivas et al., 1992). The predominant metabolic pathway is de-esterification of methylphenidate to form the corresponding carboxylic acid metabolites, *d*- and *l*-ritalinic acid, which have no known pharmacological activity (Kimko et al., 1999). These stereospecific metabolites undergo extensive urinary excretion.

Initially, it was thought that non-hepatic hydrolytic esterases, which are ubiquitously distributed throughout the body, were responsible for the de-esterification of methylphenidate (Kimko et al., 1999). More recent evidence suggests that the carboxylesterase isoenzyme, CES1A1, which is highly expressed in hepatic and gastrointestinal tissue, has high catalytic efficiency for both the *d*- and *l*-enantiomers of methylphenidate; the catalytic efficiency for *l*-methylphenidate is 3.6- to 6-fold higher than for the *d*-enantiomer (Sun et al., 2004). The high catalytic efficiency of this enzyme together with the stereoselective and first-pass clearance of methylphenidate suggests a major role for CES1A1 in the metabolism of this drug. Microsomal oxidation and conjugation appear to be minor pathways in the metabolism of methylphenidate accounting for less than 2% of the overall variance observed in its disposition (Kimko et al., 1999). Therefore, the role of microsomal cytochrome-P450 enzymes, responsible for the phase I metabolism of the majority of xenobiotics, appears to be minimal in the disposition of methylphenidate. This premise is supported by evidence suggesting that the polymorphic CYP2D6 enzyme does not play a prominent role in the metabolism of methylphenidate (DeVane et al., 2000).

1.2. Pharmacodynamics of methylphenidate

Although the precise mechanism of action of psychostimulants is not understood, they are believed to act primarily on the dopaminergic and norepinephrinergic systems. Methylphenidate is an indirect agonist of central catecholaminergic pathways. It facilitates the action of endogenous dopamine and norepinephrine at their respective pre- and post-synaptic receptor sites. This occurs through three mechanisms: inhibition of catecholamine reuptake, facilitation of catecholamine release into the synaptic cleft, and inhibition of monoamine oxidase (MAO) activity (Solanto, 1998). Specifically, methylphenidate has high affinity for and inhibits both

the dopamine and norepinephrine transporters, thereby blocking reuptake of the respective neurotransmitter (Seeman and Madras, 1998). Methylphenidate also promotes the release of dopamine from reserpine-sensitive storage pools (Solanto, 1998). Ultimately, methylphenidate alters the activity of the nigrostriatal and mesocorticolimbic dopaminergic systems, in addition to norepinephrinergic projections from the locus coeruleus to the cortex, all of which are thought to underlie improvement in ADHD symptoms. For a comprehensive review of the mechanisms of action of psychostimulants, refer to Seeman and Madras (1998), and Solanto (1998).

2. NEUROPSYCHOPHARMACOGENETICS: A SYNOPSIS

Before examining the molecular genetic basis underlying a variable trait such as response to methylphenidate, it is usually necessary to establish the existence of a heritable component. This is accomplished by undertaking epidemiologically based family, twin, and adoption studies from which estimates of trait heritability are derived. For a discussion of these approaches, refer to Gottesman (1991). These epidemiologically based methods are generally applied to determine whether genetic factors play a role in persistent phenotypes (Kalow et al., 1998).

In attempting to identify a heritable component for methylphenidate response, several considerations must be made. For pharmacological traits, it is important to keep in mind that 1) these are induced by the presence of the drug, and 2) these are temporary, for the most part, existing when the drug is present in the patient's body. The latter premise does not always hold true as exemplified by the persistence of tardive dyskinesia in patients who have developed this side effect secondary to neuroleptic treatment, even after discontinuation of the offending agent (Kane et al., 1982). Furthermore, it is very difficult to find well-characterized families, all affected members of which have been taking the drug in question. Despite these challenges, evidence for heritability of methylphenidate response can be accrued from several sources. Extrapolation to humans can be based on animal studies demonstrating that genetically different rat strains have variable responses to acute and chronically administered methylphenidate (Swerdlow et al., 2003; Yang et al., 2003). In addition, a small study in humans demonstrated that behavioural, biochemical and neuroendocrine responses to amphetamine were more highly correlated in monozygotic twins versus dizygotic twins suggesting heritability of these traits for a related psychostimulant (Nurnberger et al., 1982).

Kalow et al. (1998) have proposed that a comparison of both between and within variabilities in drug (e.g., methylphenidate) response among unrelated individuals could potentially be used to derive heritability estimates of response. In applying their proposed methods, they suggest that large-scale, family-based epidemiological studies may no longer be required to estimate the underlying

heritability of a pharmacological trait, such as response to a drug (Kalow et al., 1998).

Given that there is evidence of heritability in response to methylphenidate, how does one go about understanding the genetic mechanisms underlying this inter-individual variability in response? The field of pharmacogenetics seeks to understand the hereditary basis for variability in response and adverse drug reactions (ADRs) to pharmacologic agents among individuals. Recently, molecular genetic techniques have been applied to the study of variability in the effects of neuropsychopharmacotherapy forming the field known as neuropsycho-pharmacogenetics. Genetic variation or polymorphism of the enzymes that metabolize psychotropic drugs may account for variations in efficacy and toxicity observed in a population. A patient expressing a particular enzyme variant for rapid metabolism may display drug concentrations below the therapeutic plasma level; by contrast, a patient with a slowly metabolizing version of this enzyme may accumulate toxic drug concentrations. Alternatively, variations in the target sites for drugs (e.g., receptors, transporters) may be the reason why treatment failure of psychotropic agents occurs in some individuals but not in others with the same neuropsychiatric illness. Knowledge of the relationship between specific polymorphisms in genes encoding proteins involved in both a psychotropic drug's pharmacokinetics and pharmacodynamics, and its clinical effects may lead to better drug design and to individualized pharmacotherapy. It is also important to note that disease severity, environmental, psychosocial and demographic factors may also contribute to variation in both drug efficacy and toxicity.

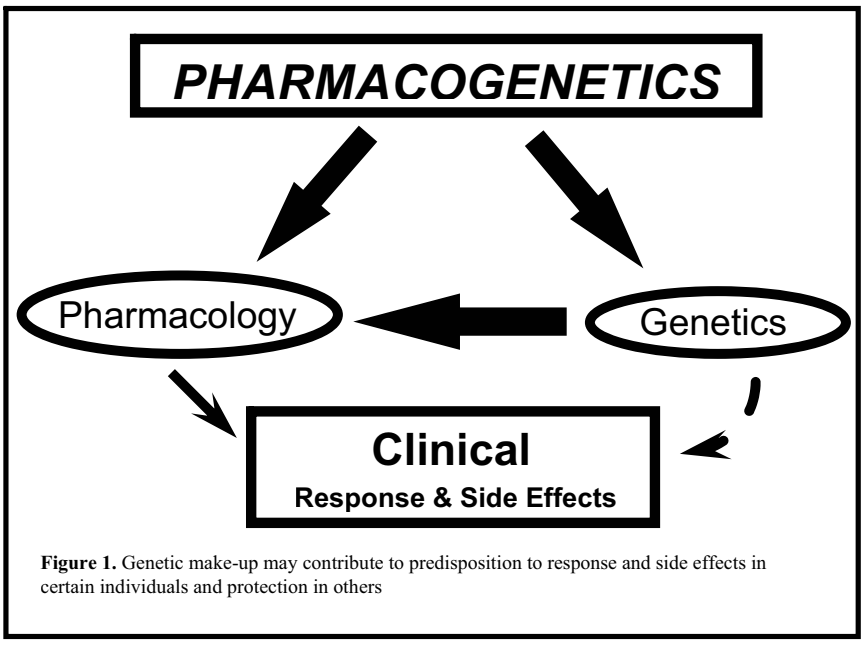


Figure 1. Genetic make-up may contribute to predisposition to response and side effects in certain individuals and protection in others

To date, the majority of studies in the field of neuropsychopharmacogenetics have employed genetic association strategies. This method involves taking a group of unrelated patients with the neuropsychiatric illness in question, who have received appropriate pharmacotherapy, and assessing their degree of therapeutic response or presence of ADRs. Response is typically evaluated using psychometric measures, which provide indices of psychopathology and/or levels of functioning. Quality of life measures may also be used in the assessment of response. Therapeutic response can either be defined categorically based on an appropriate threshold value or it can be treated as a continuous measure. Depending on the type of ADR to the treatment in question, these can also be dichotomously measured (i.e., adverse effect, present or absent) or their severity may be quantified using a validated, reliable and responsive rating instrument. Candidate genes are then selected according to the degree with which their expressed proteins pharmacologically interact with the drug at hand. It is a requirement of the genetic association approach that there is prior evidence suggesting a role for the candidate gene in the phenotype of interest (Crowe, 1993).

Polymorphisms within the candidate genes must then be identified using various techniques [for example, single-stranded conformation polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and/or direct DNA sequencing]. The polymorphisms may alter the amino acid sequence of the protein and therefore affect its function; be located in promoter or regulatory regions of the gene and therefore affect expression of its protein; be located within the third base pair of a codon and therefore may be silent (does not alter amino acid sequence), affect mRNA stability, or introduce a splice site; or be located within an intron and have no known function. Polymorphisms having functional consequences or altering protein levels are obviously more interesting to examine than ones which are silent or non-functional, and in studying the former, one can increase the prior probability of detecting a valid association (Malhotra et al., 1999). The rationale for examining silent or non-functional polymorphisms is that they may be in linkage disequilibrium with functional variants elsewhere in the gene.

Once candidate gene polymorphisms are identified, the group of patients are then genotyped, and differences among the distribution of the genetic variants (alleles) in responders/non-responders or those with/without ADRs may then be statistically compared using the chi-square test or Fisher's Exact Test. Alternatively, the mean changes in psychometric measure or ADR rating instrument, after treatment, may be grouped according to genotype and compared using the parametric analysis of variance (ANOVA) or the non-parametric Kruskall-Wallis H test. Information at the DNA level can potentially be maximised by combining allelic data from several polymorphisms across the gene (haplotype analysis). These methodologies allow the identification of genetic factors which account for some of the variance in the phenotype of drug response or side effect profile.

3. NEUROPSYCHOPHARMACOGENETICS APPLIED TO ADHD

Why examine the pharmacogenetics of response and ADRs to psycho-stimulant therapy? First, there is documented evidence of inter-individual variation in response to psychostimulants. There is an average response rate of about 75% to a given psychostimulant, as identified across several controlled outpatient trials (Solanto, 1998). In a key study conducted at the NIMH, Elia et al. (1991) demonstrated that approximately 25% of individuals have a clinically meaningful response to either methylphenidate or dextroamphetamine. Second, there is substantial variability among individuals in adverse effects to psychostimulants. These include: insomnia, weight loss, decreased appetite, abdominal pain, headache, irritability, anxiety, and proneness to crying, and increases in heart rate, diastolic and systolic blood pressure (Barkley et al., 1990; Kelly et al., 1988). It is unclear whether this variability in response and ADRs is predominantly pharmacokinetic or pharmacodynamic (Kimko et al., 1999). More likely, the inter-individual differences may be accounted for by a combination of both of the aforementioned factors as well as environmental influences. Third, there is evidence of heritability of this trait as discussed in the preceding section. Pharmacogenetic studies of psychostimulant therapy may help to elucidate the extent to which pharmacokinetic and pharmacodynamic factors play a role in predicting this inter-individual variation. Moreover, in identifying pharmacogenetic markers, predicted responders to a particular psychostimulant could be treated preferentially, while those less likely to respond or having propensity to develop adverse effects could be offered other possible treatments.

In considering candidate genes to examine with respect to the pharmacogenetics of methylphenidate, obvious choices include genes which express proteins involved in the pharmacokinetics and pharmacodynamics of this psychostimulant. From the PK perspective, identification of polymorphism within the carboxylesterase enzyme, CES1A1, and association with measures of response to methylphenidate in ADHD will be a priority in the near future. To our knowledge, however, no polymorphisms in this gene have been identified.

Key candidate genes involved in the pharmacodynamics of methylphenidate include numerous players from the dopamine and norepinephrine systems. To this end, a discussion of ‘direct’ and ‘indirect’ candidate genes is warranted. As previously proposed (Masellis et al., 2000), ‘direct’ candidate genes include those which express proteins having a direct pharmacological interaction with the drug in question. In the case of response and ADRs to methylphenidate, a ‘direct’ candidate gene of paramount importance to study is the dopamine transporter (*DAT1*), given that methylphenidate binds to and directly inhibits its expressed protein. In support of *DAT1* as a candidate gene, *DAT1* knockout mice are hyperactive as compared to wild type mice (Giros et al., 1996). Perhaps the strongest evidence comes from humans suffering from ADHD, where Krause et al. (2000) demonstrated using brain Single Photon Emission Computed Tomography (SPECT) that unmedicated patients had an elevation of striatal *DAT1* density which decreased after methylphenidate

treatment. Furthermore, numerous genetic association studies have demonstrated positive associations between ADHD and polymorphism within *DAT1* (Comings et al., 1996; Cook et al., 1995; Daly et al., 1999; Gill et al., 1997; Waldman et al., 1998). From the norepinephrine system, the norepinephrine transporter (*NET*) is also an important 'direct' candidate gene to study given the affinity of methylphenidate and other psychostimulants for its protein product.

Other candidate genes to examine in the phenotype of inter-individual variation in response and ADRs to methylphenidate include those expressing proteins involved in catecholamine biosynthesis and metabolism, as well as those involved in both extraneuronal and intraneuronal signal transduction. Examples include: tyrosine hydroxylase, dopamine β-hydroxylase, monoamine oxidase (MAO), catechol-O-methyl transferase (COMT) and the five dopamine receptor subtypes. For a review, refer to Cook (1999). Genes expressing these proteins may be considered 'indirect' candidate genes according to our current state of knowledge, given that their expressed proteins do not appear to have a 'direct' pharmacological interaction with methylphenidate. Rather they may be 'indirectly' involved in the mechanism of methylphenidate through secondary changes in catecholamine levels following inhibition of the dopamine transporter. It is important to distinguish between 'direct' and 'indirect' candidate genes, as ones directly involved in the mechanism of action of methylphenidate will likely account for a substantial proportion of the variance in response, as well as increasing the prior probability of detecting a valid association (Masellis et al., 2000).

Winsberg and Comings (1999) were the first to examine the relationship between polymorphism in several candidate genes from the dopamine system and inter-individual variation in response to methylphenidate. They examined polymorphisms in *DAT1*, the dopamine D4 receptor gene (*DRD4*), and the dopamine D2 receptor gene (*DRD2*) in a sample of 30 African-American children, who had received DSM-IIIR diagnoses of ADHD and who underwent a prospective, open-label trial of methylphenidate therapy. *DRD4* is an important 'indirect' candidate gene to examine with regard to methylphenidate response in light of replicated evidence suggesting that the 7-repeat allele is associated with ADHD (Barr et al., 2000; LaHoste et al., 1996; Muglia et al., 2000; Rowe et al., 1998; Sunohara et al., 2000; Swanson et al., 1998; Tahir et al., 2000). The three polymorphisms examined were the *Taq1* A1/A2 polymorphism located 3' to *DRD2*, the 48 bp repeat polymorphism located in the coding region of the third intracytoplasmic loop of *DRD4*, and the variable number tandem repeat (VNTR) polymorphism located in the 3'-untranslated region (3'-UTR) of *DAT1*. They described a significant association between the 10/10 genotype of the *DAT1* VNTR and non-response to methylphenidate in their sample. No significant associations were observed between the *DRD2* and *DRD4* polymorphisms and variation in response to methylphenidate, although their sample size did not have adequate power to detect an association with these. This finding further confirms the role of the dopamine transporter as the site of action of methylphenidate. Furthermore, the authors speculated that binding of

methylphenidate to the dopamine transporter might be altered in those carrying the 10/10 genotype versus 9/10, 8/10, and 5/9 genotypes.

Although there were several limitations with this initial study as described in Winsberg and Comings (1999) and Cook (1999), it does provide preliminary evidence suggesting that neuropsychopharmacogenetics can be effectively applied to the pharmacological treatment of childhood psychiatric diseases, which was the topic of discussion in a comprehensive review article published by Anderson and Cook (2000). Since then, several other studies have provided support for this finding. In a naturalistic study of 50 Brazilian youths with ADHD, Roman et al. (2002) also found that the 10/10 genotype predicted a non-response to methylphenidate. Kirley et al. (2003) found an association between transmission of the 10-repeat allele of the *DAT1* VNTR and positive response to methylphenidate in a family-based sample of 119 Irish children with ADHD, who were retrospectively assessed for response. In addition, a recent study presented by Stein et al. (2002) demonstrated that homozygosity for the 9-repeat allele of *DAT1* was associated with poor response to methylphenidate.

Although these studies support an association between *DAT1* and methylphenidate response, the results are not always in the same direction, i.e., the same allele/genotype predicts negative response in one sample and positive response in another. One explanation for this may be that this polymorphism is not causative and is only in linkage disequilibrium with another functional polymorphism in *DAT1*. Alternatively, methodological differences such as power issues, and retrospective versus prospective nature of assessment, among others, may account for the inconsistent findings. These studies also provide support for the concept of distinguishing between 'direct' and 'indirect' candidate genes as contributors to the phenotype of response and ADRs to psychotropic medications, given that the significant associations observed in these studies were between *DAT1* and response to methylphenidate.

Several other studies examining responses to amphetamine also demonstrate significant association with the *DAT1*. Using a double-blind, randomized, cross-over design, Lott et al. (2004) demonstrated that, in 96 healthy controls, subjective responses to *d*-amphetamine in a challenge paradigm were no different from placebo in 9/9 homozygotes for the *DAT1* polymorphism, whereas 9/10 heterozygotes and 10/10 homozygotes described feelings of anxiety and euphoria. Applying similar stringent research-design methodologies to clinical samples of ADHD should improve neuropsychopharmacogenetic studies in the future. Another study demonstrated that nine or fewer repeats of the *DAT1* VNTR predicted persistence of psychosis secondary to methamphetamine for greater than one month after drug discontinuation (Ujike et al., 2003). An important study in a pilot sample of eight boys suffering from ADHD, who were treated with methylphenidate, documented significantly higher regional cerebral blood flow using SPECT in medial frontal and left basal ganglia in 10/10 homozygotes versus those without this genotype (Rohde et al., 2003). All of the aforementioned studies of responsiveness to psychostimulant

therapy and association with the *DAT1* VNTR polymorphism are supported by data suggesting this polymorphism regulates expression of *DAT1* (Fuke et al., 2001; Mill et al., 2002).

Although *DAT1* appears to be one of the critical targets of methylphenidate and other psychostimulants, there is one study to suggest that polymorphism within the norepinephrine transporter (*NET*) also predicts response to methylphenidate in ADHD. In a sample of 35 Chinese Han youths with ADHD, Yang et al. (2004) documented that the G1287A polymorphism in *NET* was associated with improvement in hyperactive-impulsive subscale scores after methylphenidate treatment. Specifically, individuals with the G-allele demonstrated a higher reduction in hyperactive-impulsive symptoms versus those homozygous for the A-allele. Interestingly, none of the other scales demonstrated change according to genotype raising the possibility that endophenotypes ('phenotypes within') such as subscale scores in ADHD may be regulated differentially by genes. The topic of endophenotypes in ADHD will be more comprehensively discussed in the following section.

4. DEFINING RESPONSE: THE CHALLENGES

One of the main reasons for the inconsistent results among neuropsychopharmacogenetic studies is difficulties in what comprises the definition or characterization of response. Phenotypes of response to neuropsychotropic medications are extremely complex to measure because of a number of potential sources of error. First, symptoms of neuropsychiatric disease are subjectively described by either the patient or by a caretaker or observed and interpreted by the clinician. Despite the use of 'objective' psychometric measures that have previously demonstrated validity and reliability in quantifying these symptoms, ultimately the subjective nature of the initial description or interpretation will add 'noise' or error into any subsequent statistical analyses of response. Second, the high variability among clinicians/raters will further confound the determination of response to a neuropsychotropic drug. Third, there are several different psychometric measures available to assess response to a drug and this leads to significant heterogeneity among neuropsychopharmacogenetic studies. To provide a contrast, an example of a medical phenotype will be discussed. There have been numerous studies that have consistently replicated the finding that polymorphism within the β_2-adrenoceptor predicts vascular and airway responses to β_2-agonist therapy (reviewed in Evans et al., 2003). We postulate that the high replicability of these findings is due to the use of standardized investigations that directly provide measures of airway- and vaso-reactivity at baseline and after administration of the drug. Several other confounds contribute to the inconsistent results among neuropsychopharmacogenetic studies and these are reviewed in detail elsewhere (Masellis et al., 2000).

This leads into a discussion of endophenotypes that are being developed to more accurately determine response to methylphenidate in ADHD. As applied to drug response, endophenotypes are latent traits (e.g., physiological, cognitive, or radiographic) that are related to the global phenotype of response to a drug but are also more closely linked to underlying genetic factors (Leboyer et al., 1998). Thus, they may help in 'bridging the gap' between genotype and phenotype and improve both validity and reliability of neuropsychopharmacogenetic studies. To this end, three major topics will be covered including cognitive, neurophysiological and functional neuroradiographic endophenotypes in ADHD.

Two meta-analyses examining neuropsychological features of ADHD in children and adults have been published which demonstrate that overall cognitive ability is diminished in ADHD as compared to normal healthy controls (Frazier et al., 2004; Hervey et al., 2004). Although generalized impairments were noted, the underlying cognitive domains that were most prominently affected include attention, behavioural inhibition, working memory, and executive function (Frazier et al., 2004; Hervey et al., 2004). It is likely that deficits in attention and executive function translate into global deficits in ADHD as these measures are critical for performing most tasks during neuropsychological testing. Therefore, it may be extrapolated that deficits involving frontal-subcortical circuits of attention, behavioural inhibition, working memory, and executive function may be the most responsive measures to psychostimulant therapy. Indeed, these cognitive domains have been explored as cognitive endophenotypes in ADHD (Nigg et al., 2004; Seidman et al., 2000; Slaats-Willemse et al., 2003; Westerberg et al., 2004). Furthermore, Mollica et al. (2004) has successfully applied statistical decision rules to cognitive and behavioural domains in children with ADHD to assess responsiveness to psychostimulant therapy. They demonstrated a high sensitivity and specificity of their methods in determining a favourable response, and methodologies such as these will likely play an important role for future pharmacogenetic studies in ADHD.

From a neurophysiological perspective, several studies have been conducted using electroencephalography (EEG) and event-related potentials as endophenotypes in assessing responsiveness to methylphenidate. Ozdag et al. (2004) demonstrated that auditory event-related potentials assessing parietal P3 latency and amplitude, as well as frontal P3 amplitude were significantly different in children with ADHD versus controls at baseline. These differences normalized after methylphenidate treatment suggesting that abnormalities in signal detection and discrimination as well as information processing in ADHD may be improved by methylphenidate therapy (Ozdag et al., 2004). Another study employing EEG in 36 children with ADHD documented that, in responders to methylphenidate, frontal beta-activity was increased and that this was significantly correlated with improvement on a continuous performance test and parent-rated measures of attention and hyperactivity (Loo et al., 2004). Taking this one step further, Loo et al. (2003) conducted an association study of the *DAT1* VNTR polymorphism which

demonstrated that 10/10 homozygotes performed more poorly on a vigilance task and had increased central and parietal beta-activity, decreased right frontal theta-activity as well as lower theta:beta ratios. This suggested that neurophysiological as well as clinical measures are correlated with each other during treatment with ADHD and that genotype status of the *DAT1* VNTR may contribute to some of the variance in this relationship. This supports the use of neurophysiological endophenotypes of psychostimulant response in pharmacogenetic studies.

Functional neuroradiographic studies also show great promise as an endophenotype in assessing response to psychostimulants. A recent functional magnetic resonance imaging (*f*MRI) study demonstrated that unmedicated adolescents with ADHD recruited the left ventral basal ganglia significantly less than healthy controls during a test of divided attention (Shafritz et al., 2004). Once these patients were given a challenge dose of methylphenidate, there was a notable increase in activity in this brain area which approached that of the control group (Shafritz et al., 2004).

5. FUTURE DIRECTIONS

To date, neuropsychopharmacogenetics has been applied to the study of several phenotypes related to neuropsychopharmacologic therapy. Examples include: serotonin receptor genes and therapeutic response to clozapine in psychosis (Arranz et al., 1998; Masellis et al., 1998; Masellis et al., 2001; Masellis et al., 1995); the serotonin transporter gene and antidepressant treatment response in depression (Smeraldi et al., 1998) and obsessive-compulsive disorder (Billett et al., 1997); and dopamine receptor and cytochrome-P450 genes and predisposition to developing tardive dyskinesia in schizophrenia (Basile et al., 1999; Basile et al., 2000). In reviewing these studies, it is evident that results across studies that have been replicated are, for the most part, inconsistent. This is likely the result of methodological heterogeneity across studies, which is expected given that the field of neuropsychopharmacogenetics is in its early stages. However, in order for the field to progress, methodological consistency must be achieved not only for the basis of comparison among studies, but more importantly to allow for collaborative efforts that combine samples. Sample size limitations that decrease the power of analysis can be overcome through these collaborative efforts. Methodological considerations include the establishment of discrete inclusion/exclusion criteria regarding patient diagnosis and sample characterization; consensus regarding the use of particular psychiatric rating instruments should be established *a priori* (Rietschel et al., 1999). Furthermore, studies should be designed specifically for the identification of genetic susceptibility to pharmacogenetic traits. To date, studies have used samples obtained from prospective or retrospective clinical trials with subsequent analysis of genetic hypotheses. The current efforts of the National

Institutes of Health to fund pharmacogenetic studies are positive steps forward in achieving these goals (NIH, 1998a; NIH, 1998b).

The limitations inherent in genetic association strategies are well described in the literature however the problem of population stratification provides the greatest challenges. Population stratification can be defined as false positive and negative results that occur due to differences in allele frequencies among different subpopulations of the sample in question. The development of family based association strategies (transmission disequilibrium test: TDT), which control for population stratification biases would be useful in pharmacogenetic studies (Thomson, 1995). TDT based approaches are particularly relevant with regard to pharmacogenetic studies of psychostimulant response, and therapy in other childhood psychiatric diseases because of the increased availability of parents in obtaining samples for parent-offspring trios.

Future areas of research will include further clarification of the roles of other attentional circuits in the CNS, namely the norepinephrinergic and cholinergic systems, which are already being targeted in drug discovery for ADHD. Beane and Marrocco (2004) have suggested that there is a reduced level of norepinephrinergic facilitation of the cholinergic system in ADHD. This is proposed to be on the basis of: 1) reduced activity of locus coeruleus neurons, 2) impaired synthesis of norepinephrine, 3) supersensitive or upregulated α-2 epinephrinergic autoreceptors on terminals of locus coeruleus neurons, 4) altered regulation of norepinephrine release, or combinations thereof (reviewed in Beane et al., 2004). Interestingly, one of the newest drugs approved by the FDA in the U.S. for use in ADHD is the non-stimulant, atomoxetine. This drug selectively inhibits the pre-synaptic norepinephrine transporter (*NET*) thus elevating synaptic levels of norepinephrine (Corman et al., 2004). From a pharmacogenetic perspective, it would be important to examine polymorphism within *NET* and also within CYP2D6, since this cytochrome-P450 isoenzyme is the main metabolizer of atomoxetine. There is also preliminary evidence supporting a role for cholinergic-facilitating agents in ADHD. Nicotine patches have shown efficacy in treating symptoms of ADHD (Levin et al., 1996; Shytle et al., 2002). A small case series of treatment-resistant youths with ADHD demonstrated a benefit of the cholinesterase inhibitor, donepezil (Wilens et al., 2000). Furthermore, a double-blind, placebo-controlled, randomized, crossover trial of transdermal ABT-418, a nicotinic analogue, in 32 adults with DSM-IV diagnosed ADHD showed significant improvement over placebo (Wilens et al., 1999). Pharmacogenetic studies targeting candidate genes from the cholinergic system may be useful in further clarifying the mechanisms of action of these potential drugs for ADHD.

Neuropsychopharmacogenetic research has great potential to improve the treatment of childhood, adolescent, and adult neuropsychiatric disease, and thus ultimately the quality of life for patients suffering from these disorders. This line of research may eventually lead to a simple, fast, and inexpensive DNA test to identify response status and predisposition to ADRs of individual patients. In doing so, a decision on

whom to initiate treatment and which drug and dose to use can be made in advance. This may spare the patient significant side effects and maximize response. This research may also aid in elucidating the mechanism of action of psychotropic medications, and thus may help in designing new, more efficacious therapeutic agents.

6. ACKNOWLEDGEMENTS

We are thankful to Professor Sandra E. Black for her insightful comments. This work was supported by a Canadian Institutes of Health Research (MT15007) grant. MM is supported by the Royal College Clinician Investigator/Scientist Program at the University of Toronto. JLK is supported by a NARSAD Independent Investigator Award.

7. REFERENCES

American Psychiatric Association, 1994, *Diagnostic and statistical manual of mental disorder, 4th ed.*

Anderson, G. M., Cook, E. H., 2000, Pharmacogenetics. Promise and potential in child and adolescent psychiatry, *Child Adolesc Psychiatr Clin N Am*. **9**: 23-42.

Arranz, M. J., Munro, J., Sham, P., Kirov, G., Murray, R. M., Collier, D. A., Kerwin, R. W., 1998, Meta-analysis of studies on genetic variation in 5-HT2A receptors and clozapine response, *Schizophr Res*. **32**: 93-9.

Barkley, R. A., McMurray, M. B., Edelbrock, C. S., Robbins, K., 1990, Side effects of methylphenidate in children with attention deficit hyperactivity disorder: a systemic, placebo-controlled evaluation, *Pediatrics*. **86**: 184-92.

Barr, C. L., Wigg, K. G., Bloom, S., Schachar, R., Tannock, R., Roberts, W., Malone, M., Kennedy, J. L., 2000, Further evidence from haplotype analysis for linkage of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder, *Am J Med Genet*. **96**: 262-7.

Basile, V. S., Masellis, M., Badri, F., Paterson, A. D., Meltzer, H. Y., Lieberman, J. A., Potkin, S. G., Macciardi, F., Kennedy, J. L., 1999, Association of the MscI polymorphism of the dopamine D3 receptor gene with tardive dyskinesia in schizophrenia, *Neuropsychopharmacology*. **21**: 17-27.

Basile, V. S., Ozdemir, V., Masellis, M., Walker, M. L., Meltzer, H. Y., Lieberman, J. A., Potkin, S. G., Alva, G., Kalow, W., Macciardi, F. M., Kennedy, J. L., 2000, A functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene: association with tardive dyskinesia in schizophrenia, *Mol Psychiatry*. **5**: 410-7.

Beane, M., Marrocco, R. T., 2004, Norepinephrine and acetylcholine mediation of the components of reflexive attention: implications for attention deficit disorders, *Prog Neurobiol*. **74**: 167-81.

Billett, E. A., Richter, M. A., King, N., Heils, A., Lesch, K. P., Kennedy, J. L., 1997, Obsessive compulsive disorder, response to serotonin reuptake inhibitors and the serotonin transporter gene, *Mol Psychiatry*. **2**: 403-6.

Bradley, C., 1937, The behavior of children receiving benzedrine, *Am J Psychiatry*. **94**: 577-85.

Comings, D. E., Wu, S., Chiu, C., Ring, R. H., Gade, R., Ahn, C., MacMurray, J. P., Dietz, G., Muhleman, D., 1996, Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: the additive and subtractive effect of the three dopaminergic genes–DRD2, D beta H, and DAT1, *Am J Med Genet*. **67**: 264-88.

Cook, E. H., Jr., 1999, The early development of child psychopharmacogenetics, *J Am Acad Child Adolesc Psychiatry*. **38**: 1478-81.

Cook, E. H., Jr., Stein, M. A., Krasowski, M. D., Cox, N. J., Olkon, D. M., Kieffer, J. E., Leventhal, B. L., 1995, Association of attention-deficit disorder and the dopamine transporter gene, *Am J Hum Genet*. **56**: 993-8.

Corman, S. L., Fedutes, B. A., Culley, C. M., 2004, Atomoxetine: the first nonstimulant for the management of attention-deficit/hyperactivity disorder, *Am J Health Syst Pharm*. **61**: 2391-9.

Crowe, R. R., 1993, Candidate genes in psychiatry: an epidemiological perspective, *Am J Med Genet*. **48**: 74-7.

Daly, G., Hawi, Z., Fitzgerald, M., Gill, M., 1999, Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children, *Mol Psychiatry*. **4**: 192-6.

DeVane, C. L., Markowitz, J. S., Carson, S. W., Boulton, D. W., Gill, H. S., Nahas, Z., Risch, S. C., 2000, Single-dose pharmacokinetics of methylphenidate in CYP2D6 extensive and poor metabolizers, *J Clin Psychopharmacol*. **20**: 347-9.

Elia, J., Borcherding, B. G., Rapoport, J. L., Keysor, C. S., 1991, Methylphenidate and dextroamphetamine treatments of hyperactivity: are there true nonresponders?, *Psychiatry Res*. **36**: 141-55.

Evans, W. E., McLeod, H. L., 2003, Pharmacogenomics–drug disposition, drug targets, and side effects, *N Engl J Med*. **348**: 538-49.

Faraone, S. V., Biederman, J., 1994, Genetics of ADHD, *Child Adolesc Psychiatr Clin North America*. **3**: 285-301

Faraone, S. V., Biederman, J., Spencer, T., Wilens, T., Seidman, L. J., Mick, E., Doyle, A. E., 2000, Attention-deficit/hyperactivity disorder in adults: an overview, *Biol Psychiatry*. **48**: 9-20.

Frazier, T. W., Demaree, H. A., Youngstrom, E. A., 2004, Meta-analysis of intellectual and neuropsychological test performance in attention-deficit/hyperactivity disorder, *Neuro-psychology*. **18**: 543-55.

Fuke, S., Suo, S., Takahashi, N., Koike, H., Sasagawa, N., Ishiura, S., 2001, The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression, *Pharmacogenomics J*. **1**: 152-6.

Gill, M., Daly, G., Heron, S., Hawi, Z., Fitzgerald, M., 1997, Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism, *Mol Psychiatry*. **2**: 311-3.

Gillis, J. J., Gilger, J. W., Pennington, B. F., DeFries, J. C., 1992, Attention deficit disorder in reading-disabled twins: evidence for a genetic etiology, *J Abnorm Child Psychol*. **20**: 303-15.

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., Caron, M. G., 1996, Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter, *Nature*. **379**: 606-12.

Gottesman, I. I., 1991, *Schizophrenia Genesis. The Origins of Madness*, W.H. Freeman and Company, New York

Greenblatt, D. J., Harmatz, J. S., von Moltke, L. L., Shader, R. I. (1995). Pharmacokinetics and Pharmaco-dynamics. In *Psychopharmacology: The Fourth Generatiion of Progress*, ed. Bloom, F. E., Kupfer, D. J., pp. 849-58. New York: Raven Press.

Greenhill, L. L., Pliszka, S., Dulcan, M. K., Bernet, W., Arnold, V., Beitchman, J., Benson, R. S., Bukstein, O., Kinlan, J., McClellan, J., Rue, D., Shaw, J. A., Stock, S., 2002, Practice parameter for the use of stimulant medications in the treatment of children, adolescents, and adults, *J Am Acad Child Adolesc Psychiatry*. **41**: 26S-49S.

Hechtman, L., 1994, Genetic and neurobiological aspects of attention deficit hyperactive disorder: a review, *J Psychiatry Neurosci*. **19**: 193-201.

Hervey, A. S., Epstein, J. N., Curry, J. F., 2004, Neuropsychology of adults with attention-deficit/hyperactivity disorder: a meta-analytic review, *Neuropsychology*. **18**: 485-503.

Hubbard, J. W., Srinivas, N. R., Quinn, D., Midha, K. K., 1989, Enantioselective aspects of the disposition of dl-threo-methylphenidate after the administration of a sustained-release formulation to children with attention deficit-hyperactivity disorder, *J Pharm Sci*. **78**: 944-7.

Kalow, W., Tang, B. K., Endrenyi, L., 1998, Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research, *Pharmacogenetics*. **8**: 283-9.

Kane, J. M., Smith, J. M., 1982, Tardive dyskinesia: prevalence and risk factors, 1959 to 1979, *Arch Gen Psychiatry*. **39**: 473-81.

Kelly, K. L., Rapport, M. D., DuPaul, G. J., 1988, Attention deficit disorder and methylphenidate: a multi-step analysis of dose-response effects on children's cardiovascular functioning, *Int Clin Psychopharmacol*. **3**: 167-81.

Kimko, H. C., Cross, J. T., Abernethy, D. R., 1999, Pharmacokinetics and clinical effectiveness of methylphenidate, *Clin Pharmacokinet*. **37**: 457-70.

Kirley, A., Lowe, N., Hawi, Z., Mullins, C., Daly, G., Waldman, I., McCarron, M., O'Donnell, D., Fitzgerald, M., Gill, M., 2003, Association of the 480 bp DAT1 allele with methylphenidate response in a sample of Irish children with ADHD, *Am J Med Genet B Neuropsychiatr Genet*. **121**: 50-4.

Krause, K. H., Dresel, S. H., Krause, J., Kung, H. F., Tatsch, K., 2000, Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography, *Neurosci Lett*. **285**: 107-10.

LaHoste, G. J., Swanson, J. M., Wigal, S. B., Glabe, C., Wigal, T., King, N., Kennedy, J. L., 1996, Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder [see comments], *Mol Psychiatry*. **1**: 121-4.

Leboyer, M., Bellivier, F., Nosten-Bertrand, M., Jouvent, R., Pauls, D., Mallet, J., 1998, Psychiatric genetics: search for phenotypes, *Trends Neurosci*. **21**: 102-5.

Levin, E. D., Conners, C. K., Sparrow, E., Hinton, S. C., Erhardt, D., Meck, W. H., Rose, J. E., March, J., 1996, Nicotine effects on adults with attention-deficit/hyperactivity disorder, *Psychopharmacology (Berl)*. **123**: 55-63.

Loo, S. K., Hopfer, C., Teale, P. D., Reite, M. L., 2004, EEG Correlates of Methylphenidate Response in ADHD: Association With Cognitive and Behavioral Measures, *J Clin Neurophysiol*. **21**: 457-64.

Loo, S. K., Specter, E., Smolen, A., Hopfer, C., Teale, P. D., Reite, M. L., 2003, Functional effects of the DAT1 polymorphism on EEG measures in ADHD, *J Am Acad Child Adolesc Psychiatry*. **42**: 986-93.

Lott, D. C., Kim, S. J., Cook, E. H., de Wit, H., 2004, Dopamine Transporter Gene Associated with Diminished Subjective Response to Amphetamine, *Neuropsychopharmacology*.

Malhotra, A. K., Goldman, D., 1999, Benefits and pitfalls encountered in psychiatric genetic association studies, *Biol Psychiatry*. **45**: 544-50.

Markowitz, J. S., Patrick, K. S., 2001, Pharmacokinetic and pharmacodynamic drug interactions in the treatment of attention-deficit hyperactivity disorder, *Clin Pharmacokinet*. **40**: 753-72.

Masellis, M., Basile, V., Meltzer, H. Y., Lieberman, J. A., Sevy, S., Macciardi, F. M., Cola, P., Howard, A., Badri, F., Nothen, M. M., Kalow, W., Kennedy, J. L., 1998, Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients, *Neuropsychopharmacology*. **19**: 123-32.

Masellis, M., Basile, V. S., Meltzer, H. Y., Lieberman, J. A., Sevy, S., Goldman, D. A., Hamblin, M. W., Macciardi, F. M., Kennedy, J. L., 2001, Lack of association between the T/C 267 serotonin 5-HT6 receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia., *Schizophrenia Research*. **47**: 49-58.

Masellis, M., Basile, V. S., Ozdemir, V., Meltzer, H. Y., Macciardi, F. M., Kennedy, J. L., 2000, Pharmacogenetics of antipsychotic treatment: lessons learned from clozapine, *Biol Psychiatry*. **47**: 252-66.

Masellis, M., Paterson, A. D., Badri, F., Lieberman, J. A., Meltzer, H. Y., Cavazzoni, P., Kennedy, J. L., 1995, Genetic variation of 5-HT2A receptor and response to clozapine, *Lancet*. **346**: 1108.

Mill, J., Asherson, P., Browes, C., D'Souza, U., Craig, I., 2002, Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR, *Am J Med Genet*. **114**: 975-9.

Mollica, C. M., Maruff, P., Vance, A., 2004, Development of a statistical approach to classifying treatment response in individual children with ADHD, *Hum Psychopharmacol*. **19**: 445-56.

Muglia, P., Jain, U., Macciardi, F., Kennedy, J. L., 2000, Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene, *Am J Med Genet*. **96**: 273-7.

Nigg, J. T., Blaskey, L. G., Stawicki, J. A., Sachek, J., 2004, Evaluating the endophenotype model of ADHD neuropsychological deficit: results for parents and siblings of children with ADHD combined and inattentive subtypes, *J Abnorm Psychol*. **113**: 614-25.

NIH, 1998a, Mechanisms underlying variations in drug responses, *www.nih.gov/grants/guide/pa-files/PA-99-016.html pps. 1-10*.

NIH, 1998b, Pharmacogenetics research network and database, *www.nih.gov/grants/guide/rfa-files/RFA-GM-099-004.html pps . 1-18*.

Nurnberger, J. I., Jr., Gershon, E. S., Simmons, S., Ebert, M., Kessler, L. R., Dibble, E. D., Jimerson, S. S., Brown, G. M., Gold, P., Jimerson, D. C., Guroff, J. J., Storch, F. I., 1982, Behavioral, biochemical and neuroendocrine responses to amphetamine in normal twins and 'well-state' bipolar patients, *Psychoneuroendocrinology*. **7**: 163-76.

Ozdag, M. F., Yorbik, O., Ulas, U. H., Hamamcioglu, K., Vural, O., 2004, Effect of methylphenidate on auditory event related potential in boys with attention deficit hyperactivity disorder, *Int J Pediatr Otorhinolaryngol*. **68**: 1267-72.

Pauls, D. L., 1991, Genetic factors in the expression of attention-deficit hyperactivity disorder, *J Child Adolesc Psychopharm*. **1**: 353-60.

Rietschel, M., Kennedy, J. L., Macciardi, F. M., DeLisi, L., Meltzer, H. Y., The Consensus Group for Outcome Measures in Psychoses for Pharmacogenetic Studies, 1999, Pharmacogenetics in psychosis, the first consensus conference, *Schizophrenia Research.* **37**: 191-6.

Rohde, L. A., Roman, T., Szobot, C., Cunha, R. D., Hutz, M. H., Biederman, J., 2003, Dopamine transporter gene, response to methylphenidate and cerebral blood flow in attention-deficit/hyperactivity disorder: a pilot study, *Synapse*. **48**: 87-9.

Roman, T., Szobot, C., Martins, S., Biederman, J., Rohde, L. A., Hutz, M. H., 2002, Dopamine transporter gene and response to methylphenidate in attention-deficit/hyperactivity disorder, *Pharmacogenetics*. **12**: 497-9.

Rowe, D. C., Stever, C., Giedinghagen, L. N., Gard, J. M., Cleveland, H. H., Terris, S. T., Mohr, J. H., Sherman, S., Abramowitz, A., Waldman, I. D., 1998, Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder, *Mol Psychiatry*. **3**: 419-26.

Rowland, M., Tozer, T. N., 1995, *Clinical Pharmacokinetics. Concepts and Applications*, Williams & Wilkins, Philadelphia.

Safer, D. J., Krager, J. M., 1988, A survey of medication treatment for hyperactive/inattentive students, *Jama*. **260**: 2256-8.

Seeman, P., Madras, B. K., 1998, Anti-hyperactivity medication: methylphenidate and amphetamine, *Mol Psychiatry*. **3**: 386-96.

Seidman, L. J., Biederman, J., Monuteaux, M. C., Weber, W., Faraone, S. V., 2000, Neuropsychological functioning in nonreferred siblings of children with attention deficit/hyperactivity disorder, *J Abnorm Psychol*. **109**: 252-65.

Shafritz, K. M., Marchione, K. E., Gore, J. C., Shaywitz, S. E., Shaywitz, B. A., 2004, The effects of methylphenidate on neural systems of attention in attention deficit hyperactivity disorder, *Am J Psychiatry*. **161**: 1990-7.

Shaywitz, S. E., Hunt, R. D., Jatlow, P., Cohen, D. J., Young, J. G., Pierce, R. N., Anderson, G. M., Shaywitz, B. A., 1982, Psychopharmacology of attention deficit disorder: pharmacokinetic, neuroendocrine, and behavioral measures following acute and chronic treatment with methylphenidate, *Pediatrics*. **69**: 688-94.

Shytle, R. D., Silver, A. A., Wilkinson, B. J., Sanberg, P. R., 2002, A pilot controlled trial of transdermal nicotine in the treatment of attention deficit hyperactivity disorder, *World J Biol Psychiatry*. **3**: 150-5.

Slaats-Willemse, D., Swaab-Barneveld, H., de Sonneville, L., van der Meulen, E., Buitelaar, J., 2003, Deficient response inhibition as a cognitive endophenotype of ADHD, *J Am Acad Child Adolesc Psychiatry*. **42**: 1242-8.

Smeraldi, E., Zanardi, R., Benedetti, F., Di Bella, D., Perez, J., Catalano, M., 1998, Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine, *Mol Psychiatry*. **3**: 508-11.

Solanto, M. V., 1998, Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration, *Behav Brain Res*. **94**: 127-52.

Srinivas, N. R., Hubbard, J. W., Korchinski, E. D., Midha, K. K., 1993, Enantioselective pharmacokinetics of dl-threo-methylphenidate in humans, *Pharm Res*. **10**: 14-21.

Srinivas, N. R., Hubbard, J. W., Quinn, D., Midha, K. K., 1992, Enantioselective pharmacokinetics and pharmacodynamics of dl-threo-methylphenidate in children with attention deficit hyperactivity disorder, *Clin Pharmacol Ther*. **52**: 561-8.

Stein, M. A., Sarampote, C., Waldman, I., Seymour, K., Robb, A., Colon, C., et al., 2002, *Dopamine transporter genotype affects stimulant response according to parent ratings.* Presented at 49th Annual Meeting of Child and Adolescent Psychiatry, San Francisco.

Sun, Z., Murry, D. J., Sanghani, S. P., Davis, W. I., Kedishvili, N. Y., Zou, Q., Hurley, T. D., Bosron, W. F., 2004, Methylphenidate is stereoselectively hydrolyzed by human carboxylesterase CES1A1, *J Pharmacol Exp Ther*. **310**: 469-76.

Sunohara, G. A., Roberts, W., Malone, M., Schachar, R. J., Tannock, R., Basile, V. S., Wigal, T., Wigal, S. B., Schuck, S., Moriarty, J., Swanson, J. M., Kennedy, J. L., Barr, C. L., 2000, Linkage of the dopamine D4 receptor gene and attention-deficit/hyperactivity disorder, *J Am Acad Child Adolesc Psychiatry*. **39**: 1537-42.

Swanson, J. M., Sunohara, G. A., Kennedy, J. L., Regino, R., Fineberg, E., Wigal, T., Lerner, M., Williams, L., LaHoste, G. J., Wigal, S., 1998, Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family- based approach, *Mol Psychiatry*. **3**: 38-41.

Swerdlow, N. R., Shoemaker, J. M., Platten, A., Pitcher, L., Goins, J., Crain, S., 2003, Heritable differences in the effects of amphetamine but not DOI on startle gating in albino and hooded outbred rat strains, *Pharmacol Biochem Behav*. **75**: 191-7.

Tahir, E., Yazgan, Y., Cirakoglu, B., Ozbay, F., Waldman, I., Asherson, P. J., 2000, Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children, *Mol Psychiatry*. **5**: 396-404.

Thomson, G., 1995, Mapping disease genes: family-based association studies, *American Journal of Human Genetics*. **57**: 487-98.

Ujike, H., Harano, M., Inada, T., Yamada, M., Komiyama, T., Sekine, Y., Sora, I., Iyo, M., Katsu, T., Nomura, A., Nakata, K., Ozaki, N., 2003, Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis, *Pharmacogenomics J*. **3**: 242-7.

Waldman, I. D., Rowe, D. C., Abramowitz, A., Kozel, S. T., Mohr, J. H., Sherman, S. L., Cleveland, H. H., Sanders, M. L., Gard, J. M., Stever, C., 1998, Association and linkage of the dopamine transporter gene and attention- deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity, *Am J Hum Genet*. **63**: 1767-76.

Westerberg, H., Hirvikoski, T., Forssberg, H., Klingberg, T., 2004, Visuo-spatial working memory span: a sensitive measure of cognitive deficits in children with ADHD, *Neuropsychol Dev Cogn C Child Neuropsychol*. **10**: 155-61.

Wilens, T. E., Biederman, J., Spencer, T. J., Bostic, J., Prince, J., Monuteaux, M. C., Soriano, J., Fine, C., Abrams, A., Rater, M., Polisner, D., 1999, A pilot controlled clinical trial of ABT-418, a cholinergic agonist, in the treatment of adults with attention deficit hyperactivity disorder, *Am J Psychiatry*. **156**: 1931-7.

Wilens, T. E., Biederman, J., Wong, J., Spencer, T. J., Prince, J. B., 2000, Adjunctive donepezil in attention deficit hyperactivity disorder youth: case series, *J Child Adolesc Psychopharmacol*. **10**: 217-22.

Winsberg, B. G., Comings, D. E., 1999, Association of the dopamine transporter gene (DAT1) with poor methylphenidate response, *J Am Acad Child Adolesc Psychiatry*. **38**: 1474-7.

Yang, L., Wang, Y. F., Li, J., Faraone, S. V., 2004, Association of norepinephrine transporter gene with methylphenidate response, *J Am Acad Child Adolesc Psychiatry*. **43**: 1154-8.

Yang, P. B., Amini, B., Swann, A. C., Dafny, N., 2003, Strain differences in the behavioral responses of male rats to chronically administered methylphenidate, *Brain Res*. **971**: 139-52.

10. AUTISM AND AUTISTIC DISORDERS

Stéphane Jamain and Marion Leboyer

1. INTRODUCTION

Autism is a neurodevelopmental disorder affecting more than 1 in 1000 people in general population (Fombonne, 2003). It usually occurs before 3 years of age with variable clinical manifestations, but persistent features define the core phenotype. Affected people classically manifest impairment in the use of nonverbal behaviour, such as facial expression or body posture, fail to develop peer relationship or do not seek to share enjoyments or interests. They also manifest impairment in communication and restricted, repetitive and stereotyped patterns of interests and activities (American Psychiatric Association, 1994). Other syndromes correspond to impairment of the development of reciprocal social interaction, such as Asperger syndrome, but these patients do not meet all the criteria of autism. In these cases, we talk about autism spectrum disorders. Despite clinical manifestations of Asperger syndrome are really close to autism, these patients usually have higher cognitive functions, no delay in the language development and sometimes manifest particularly skills such an impressive memory for details.

Although there is no clear evidence of an increase in the incidence of autism, prevalence estimates of this disorder have gone up over the past 20 years (Fombonne, 2002). Indeed, first epidemiological studies of autism reported rates of 2 to 5 in 10,000, whereas present studies estimate until 60 in 10,000 children. Improvement in diagnostic criteria and growing in public awareness can explain this rise. At present, we can estimate than one in one thousand children is affected with autism with a four-fold increased risk for males, and unfortunately, no pharmacological treatment has been identified yet.

Stephane Jamain, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Strasse 3, 37075 Goettingen, Germany.
Marion Leboyer, INSERM U513, Centre for Adult Psychiatry, Albert Chenevier Hospital and Henri Mondor Hospital, Assistance Publique-Hôpitaux de Paris, 40 rue de Mesly, 94000 Créteil, France.

2. DRUG TREATMENTS FOR AUTISM

Despite many trials to identify drugs that affect social interactions, no medication has been shown to unambiguously modify the core or associated symptoms in individuals with autism. Recent reviews describe drug treatment strategies for various associated symptoms commonly found in autism (Hollander *et al.*, 2003). For instance, α-adrenergic agents, β- blockers and typical and atypical antipsychotic agents are prescribed to decrease aggressive behaviours. Selective serotonin reuptake inhibitors are used for treatment of the anxiety or for stereotypes and repetitive behaviours. Dopamine reuptake blocking agents, stimulants and α-adrenergic agents are used for hyperactive patients, and finally antidepressants, anticonvulsants and melatonin have shown some benefits for sleep disorders. However, none of these treatments can definitely improve the severe symptoms in autistic patients and it is some times necessary to try several of them before to observe a slight effect. Behavioural therapies have also been extensively used to decrease behavioural disorders, but their efficiency depends on how they are integrated in the educational program (Volkmar and Pauls, 2003). To date, the combination of pharmacological agents with a behavioural treatment may be more effective than either treatment alone. For instance, major tranquilisers can reduce hyperactive behaviours and allow participation in educational program. The weak success of drug treatments on symptoms of autism is likely due to the poorness of knowledge about molecular mechanisms involved in this syndrome. However, recent advances in genetic contribution to autism may help to break down these mechanisms and lead to new pharmacological strategies to improve symptoms of affected individuals.

3. AUTISM IS A GENETIC SYNDROME

Evidences in favour of genetic contribution to autism come from familial and twin studies. Indeed, the occurrence risk to be autistic in sibling of affected patients is 45-fold higher than in general population (Jorde *et al.*, 1991). Moreover, twin studies show a 60% to 90% concordance rate in monozygotic twins compare to 0% to 10% rate in dizygotic twins, depending on whether only autism is considered or more generally social or cognitive disorders (Folstein and Rutter, 1977; Bailey *et al.*, 1995). According to these data, we can estimate a heritability rate for autism higher than 90%. However, the difference in concordance rate between monozygotic and dizygotic twins strongly suggests that autism is a polygenic disorder, which is consistent with first findings on genetic diseases associated to autism.

First genes involved in autism have been identified with the characterization of genetic syndromes associated to autism. Indeed, approximately 15% of autism cases are associated with known genetic diseases, such as tuberous sclerosis complex, fragile X syndrome and Rett syndrome.

The tuberous sclerosis complex (TSC) is a dominantly inherited disease clinically characterised by epilepsy, learning difficulties, behavioural disorders, and brain, skin and renal lesions. Between 43% and 86% of TSC patients have developmental disorders similar to autism (Harrison and Bolton, 1997) as well as epilepsy. Moreover, 0.4% to 3% of autistic individuals have TSC, increasing to 14% when only epileptic patients are considered. This association suggests that mutation in *TSC1* or *TSC2*, the genes encoding respectively for hamartin and tuberin and frequently mutated in TSC, or in transduction pathways involving these genes may trigger autistic disorders in affected individuals (Serajee *et al.*, 2003). These proteins are tumour suppressor factors, suggesting that autistic disorders may directly result from functional deficit in hamartin and tuberin or from the number and localisation of tubers in the brain. A significant increase of autism frequency in TSC has been reported in patients with lesions in temporal lobes (Filipek *et al.*, 1999). Moreover, some autistic individuals have hyperplasia of temporal lobes, which could explain macrocephaly observed in 20% of affected individuals. Therefore, deeper studies of brain areas affected in this syndrome may help to describe cerebral structures altered in autistic individuals.

The fragile X syndrome (FXS) is the most common form of inherited mental retardation. People affected with FXS are mainly males with combination of mild to severe cognitive impairment, attention deficit, anxiety, communicative disorders and stereotypic behaviours. Adult males have also machroorchidism, large ears and prominent jaw (Bardoni and Mandel, 2002). In 25% to 40% of cases, affected individuals have also autistic behaviours, doing this syndrome one of the most frequently associated to autism. Moreover, we estimate more than 2% of autistic individuals have mutations in the *FMR1* gene (Wassink *et al.*, 2001a). The fragile X syndrome results from the absence of functional Fragile X Mental Retardation Protein (FMRP), encoding by the *FMR1* gene. This protein binds other partners to form a RNA-binding complex, regulating the translation of at least 80 different target proteins (Miyashiro *et al.*, 2003). The main mutation in this syndrome is an over-amplification of a CGG triplet in the promoter of the *FMR1* gene, preventing its expression and leading to a fragile site on the X chromosome. Thus, the absence of FMRP modifies the expression level of many other proteins, explaining the wide range of phenotypes observed in FXS or autistic patients. Therefore, the characterisation of RNA targets for FMRP may help to identify molecular mechanism involved in autism. For instance, lack of FMRP leads to abnormal synaptic connections with an excess of long, thin and immature spines (Irwin *et al.*, 2001; Irwin *et al.*, 2002). These results converge with our recent data concerning the involvement of neuroligin in autism (Jamain *et al.*, 2003), which are likely involved in synapse formation (Dean *et al.*, 2003). Therefore, we can assume that FMRP may be a translational regulator of neuroligins or partners of the same pathway leading to autistic disorders in patients with FXS.

The Rett syndrome is a neurodegenerative disorder affecting essentially girls. After a period of normal function after birth, affected girls manifest mental

retardation, autistic disorders, microcephaly and loss of purposeful hand skills. In approximately 80% of cases, Rett syndrome is caused by heterozygous mutation in the X-linked methyl-CpG-binding protein 2 (*MECP2*) gene (Amir *et al.*, 1999). Moreover, at least two autistic girls without RTT symptoms have been reported with mutation in *MECP2*, suggesting that this gene may be involved in autism in girls (Ashley-Koch *et al.*, 2001). MECP2 binds to methylated sites in genomic DNA and facilitate gene silencing (Nan *et al.*, 1997; Nan *et al.*, 1998). Again, autism describe in Rett patients may depend on targeted genes for which the expression pattern may be modified by mutations in *MECP2*. There is a continuous increase in abundance of MeCP2 in cortical neurons throughout childhood (Shahbazian *et al.*, 2002). This points to a dynamic regulation of this protein, suggesting a role during synaptic connection (Zoghbi, 2003). In human patients and animal models, presence of synapses is consistent with the idea that MeCP2 is not essential for initiating synaptogenesis, but loss of learning skills during development suggests that it may be critical for maintaining or modulating synapses.

Despite a monogenic aspect for these syndromes, the heterogeneity of the phenotype observed for all of them comes from the involvement of a number of genes, which share the same regulation factor. Therefore, the identification of common target for these regulating protein may help to find genes or molecular mechanisms involved in susceptibility to autism. However, these syndromes explain only a small proportion of autism in population, and the origin of the syndrome is completely ignored in more than 80% of cases. Therefore, it is crucial to determine the genes, which are involved in idiopathic autism in order to better understand the modifications occurring in patient brains and to develop new pharmacological approaches to improve their symptoms.

4. SUSCEPTIBILITY GENES TO IDIOPATHIC AUTISM

4.1. Functional candidate genes

One of the strategies to identify susceptibility genes to autism consists in analysing candidate genes according to their function. In this case, genes are screened since they are responsible for genetic diseases associated to autism, such as *NF1* (involved in neurofibromatosis type 1), *FMR1* or *MECP2*. Some times genes

are also selected since they play a major role in the brain formation or maturation, such as the *EN2* homeogene (Petit *et al.*, 1995) or the *HOXA1* gene (Ingraham and Kety, 2000). Sometimes, genes are selected according to metabolic data of patients. For instance, approximately 30% of autistic patients have an increase in platelet serotonin level (Cook *et al.*, 1993; Leboyer *et al.*, 1999). Moreover, selective serotonin reuptake inhibitors sometimes improve stereotyped and repetitive behaviours (Gordon *et al.*, 1993; McDougle *et al.*, 1996). However, association studies between the gene *SLC6A4*, encoding the serotonin transporter, and autism report contradictory data (Cook *et al.*, 1997; Klauck *et al.*, 1997; Persico *et al.*, 2000; Yirmiya *et al.*, 2001; Betancur *et al.*, 2002). One of the main biases in this approach is it consists in studying mechanisms without to consider whether they are the cause or one of the consequences of physiological processes modified in affected individuals. Therefore, the most promising method to identify susceptibility genes to autism are linkage analyses and characterisation of chromosomal abnormalities reported in autistic subjects.

4.2. Linkage analyses

Although the genetic contribution to autism is well established, the mode of inheritance does not match with one unambiguous model, suggesting the involvement of multiple chromosomal regions and interaction of multiple genes. In accordance with this, non-parametric whole genome screens probably correspond to the best approach to identify susceptibility genes to autism. Using this approach, several independent groups (IMGSAC, 1998; Barrett *et al.*, 1999; Philippe *et al.*, 1999; Risch *et al.*, 1999; Buxbaum *et al.*, 2001; Liu *et al.*, 2001; Auranen *et al.*, 2002; Shao *et al.*, 2002) identified multiple loci containing one or more susceptibility genes (Figure 1). Despite a strong effort to homogenise tools for autism diagnostic, differences in inclusion criteria and differences in ethnical origin may explain the discrepancies among studies. Nevertheless, several promising regions came out these analyses, such as long arms of chromosome 2 and 7 for which a significant linkage has been identified by most of the studies.

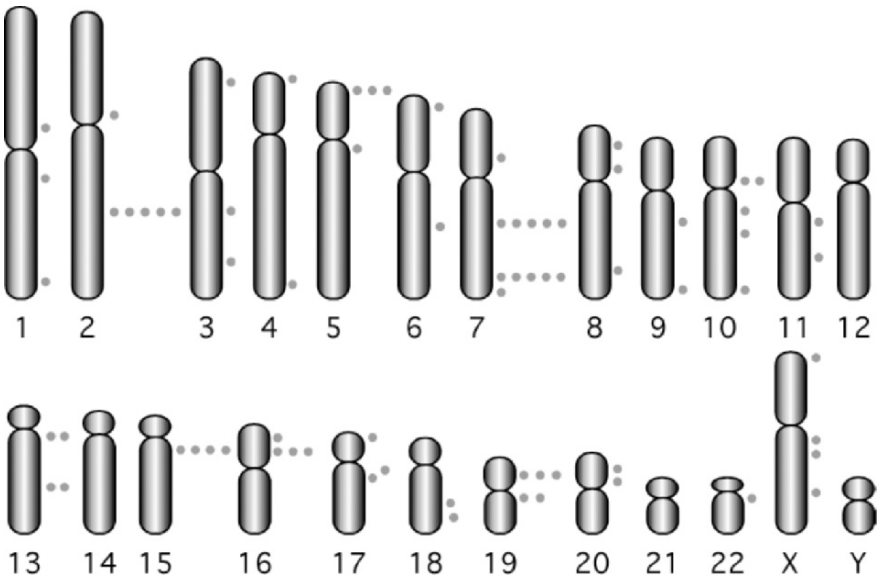


Figure 1. Regions predisposing to autism spectrum disorders. These regions have been identified by genome-wide linkage analyses. Each point corresponds to an independent positive linkage between one loci and autism (maximum lod score ≥ 1). The most consistent results have been obtained for the long arms of chromosome 2, 7 and 15.

4.2.1. Chromosome 2q24-q31

At least three independent genome-wide screens have identified linkage between a broad region of the long arm of chromosome 2 and autism (Buxbaum *et al.*, 2001; IMGSAC, 2001a; Shao *et al.*, 2002). In these studies, the LOD score value increase when only autistic individuals with speech delay are considered. Moreover, several deletions of chromosome 2q37 (Ghaziuddin and Burmeister, 1999; Smith *et al.*, 2001; Wolff *et al.*, 2002) as well as two deletions of 2q32 (Gallagher *et al.*, 2003) and 2q35 (Borg *et al.*, 2002) regions have been reported in autistic individuals. Thus, the susceptibility region on chromosome 2 spans more than 10 cM and contains at least 20 genes. Some of them are involved in neuronal differentiation, in brain development or in neural transmission, such as camp-GEFII (Bacchelli *et al.*, 2003) in which non-synonymous substitutions have been identified in autistic individuals. However, these variations are rare and cannot explain the high linkage found in this region. Moreover, the low frequency of these variations

did not allow the identification of a strongest association with language retardation. Therefore, it is crucial to continue, for this gene and others in the same region, the mutation screening in autistic individuals. It is also important to cluster autistic individuals according to their candidate symptoms, such as speech delay, in order to increase the sample homogeneity.

4.2.2. The long arm of chromosome 7 (7q)

This region of the human genome is the most frequently associated to autism (Folstein and Rosen-Sheidley, 2001). More precisely, fine mappings of this region revealed two susceptibility peaks separated with approximately 25 cM and located in 7q21-q22 and 7q32-q34 (Ashley-Koch *et al.*, 1999; Bradford *et al.*, 2001; IMGSAC, 2001a; IMGSAC, 2001b; Liu *et al.*, 2001). Furthermore, at least 7 chromosomal abnormalities involving one of these two regions have been reported in autistic individuals (Folstein and Rosen-Sheidley, 2001). Several genes located in these two regions have been screened for mutation in autistic individuals and some of them brought good evidence for predisposition to autism. For instance, *RAY1* is directly interrupted by a chromosomal breakpoint in a patient with a translocation involving chromosomes 7 and 13 (Vincent *et al.*, 2000). However, no other mutation has been identified yet in independent individuals with autism.

The *RELN* gene encodes a protein called reelin. This gene is also located on the long arm of chromosome 7. Reelin is a key protein in the development of laminar structure in cerebral cortex, hippocampus, cerebellum and several nuclei of the brainstem. Mutant mice for this gene have abnormal migration of neurons in cortex and cerebellum, leading to disorders in locomotion. Interestingly, people with schizophrenia, bipolar disorder or major depression have a decrease of reelin level in blood (Fatemi *et al.*, 2001). An association study showed a higher number of GGC repeat upstream to the reelin gene in some autistic patients (Persico *et al.*, 2001). This increase may modify the transcription of the *RELN* gene and change the neuronal network in autism. Despite some difficulties in replication of this association study (Krebs *et al.*, 2002), these results are really promising and should be consider for future association studies in other group of patients.

The *WNT2* gene is also a good functional candidate gene in the 7q region. The function of this protein is not really known yet, but the WNT family is involved in the establishment of the central nervous system. These molecules play a crucial role in cell-cell interaction during embryogenesis and mice, mutant for these genes, have developmental abnormalities. Moreover, the WNT signal transduction is dependant of dishevelled family (DVL) for which mutant mice (*Dvl1* knockout mice) have a decrease in social interactions and maternal behaviours (Lijam *et al.*, 1997). Wassink *et al*. identified two amino acid changes in WNT2 in two independent families (Wassink *et al.*, 2001b). In one of them, the father carries the mutation and has language abnormalities, which may reflect an endophenotype associated to this mutation. Despite few positive associations, no other mutation has been identified in

autistic patients. The mutation frequency of WNT2 is too weak to explain the high genetic linkage identified between the long arm of chromosome 7 and autism. However, the mutation screening in autistic patients should be maintained for WNT2, but also for other genes in this region, and it may be relevant to focus on families in which first relatives have speech delay.

According to this hypothesis, Newbury *et al.* screened for mutation the gene encoding the transcription factor FOXP2, the first gene involved in language and speech development (Lai *et al.*, 2001), which is located in this 7q31 region. However, no mutation has been identified, even when children had severe speech and language impairments (Newbury *et al.*, 2002).

Likely due to the heterogeneity of the syndrome, replication studies often reveal contradictory data for these candidate genes. However, this chromosome 7 region is one of the most frequently associated regions to autism and it is decisive to better define it, in order to determine how many autism susceptibility genes are located in this region.

Our team also identified a positive association between specific haplotypes of the gene coding for the glutamate ionotropic receptor 2 (GRIK2) and autism (Jamain *et al.*, 2002). This gene is located in the 6q16-q21 region, corresponding to the highest linkage peak identified in our genome-wide scan (Philippe *et al.*, 1999). We also identified an amino acid change (M867I) with a significant non-random transmission from mother to autistic children (Jamain *et al.*, 2002). These results have been recently strengthened by other linkage analyses and confirmed in an independent population (Shuang *et al.*, 2004). In animal models, Grik2 is involved in sensitivity to seizures and its amount of transcripts has been reported higher in individuals with epileptic seizures. Therefore, this gene may be related to epilepsy observed in 30% of autistic subjects.

Now linkage analyses reported chromosomal regions associated to autism, the next step is to reduce them and search inside for best candidate genes. With the complete sequence of the human genome (Lander *et al.*, 2001), most of the genes have been identified and more and more single nucleotide polymorphisms (SNP), as well as haplotypes, are available (Sachidanandam *et al.*, 2001). These databases will speed up association studies and will allow the identification of susceptibility alleles to autism, even those with a small effect on the phenotype (Risch and Merikangas, 1996).

4.3. Chromosomal abnormalities

Numerous of chromosomal abnormalities have been reported in autistic patients, including deletions, duplications and translocations. Breakpoint characterisation of these rearrangements may help in the identification of susceptibility genes. However, the safest strategy would be the combination of these results with those providing by linkage analyses. Indeed, 5% to 10% of autistic individuals have a chromosomal modification, affecting a part or a whole chromosome. Moreover,

abnormalities of all chromosomes have been reported in autism (Gillberg, 1998; Wassink *et al.*, 2001a). Among all of them, those involving chromosome 15 and sex chromosomes are the most frequent.

4.3.1. Chromosome 15q11-q13

More than 30 autistic individuals have been reported with interstitial duplication of the 15q11-q13 region, which has been also pointed out by linkage analyses (Folstein and Rosen-Sheidley, 2001). Most of these duplications are transmitted by the mother, reminding the parental imprinting described for Angelman and Prader-Willi syndromes in this region. Angelman syndrome is caused by mutations in *UBE3A*, a gene coding for the ubiquitin-protein ligase E3A and involved in degradation of many of other proteins (Matsuura *et al.*, 1997). Despite the lack of association between this gene and autism (Veenstra-VanderWeele *et al.*, 1999), comparison of target proteins for UBE3A with those of FMRP and MeCP2 may help us to identify susceptibility genes for autism.

Several other good candidate genes are located in this region, such as α5, β3 and γ3 subunits of γ-aminobutyric acid receptor ($GABA_A$). GABA is the main inhibitory neurotransmitter in the mammalian brain, controlling excitability and responsible for lot of epilepsies. A desequilibrated transmission of one *GABRB3* allele has been reported in autistic patients (Cook *et al.*, 1998), but without parental origin effect. Other studies showed contradictory results about this receptor, but the Buxbaum's team concluded to a global significant association with autism (Buxbaum *et al.*, 2002). Therefore, further investigations are required in this region to determine whether $GABA_A$, or another gene is this region may be responsible for susceptibility to autism.

4.3.2. Neuroligins: the first susceptibility genes to idiopathic autism

Sex chromosome abnormalities may easily explain the disequilibrated sex ratio between autistic males and females (Fombonne, 1999). This ratio, already reported by Kanner when he described first autistic individuals (Kanner, 1943), increases when only autistic individuals without any physical or cerebral abnormalities are considered (Miles and Hillman, 2000).

Looking for susceptibility genes on the X and Y chromosomes, we identified three genes encoding human neuroligins (NLGNs). *NLGN4* and *NLGN3* are both located on the X chromosome in the Xp22 region, deleted in three autistic females (Thomas *et al.*, 1999) and in Xq13 region, highly associated to autism by two independent genome-wide scans (Auranen *et al.*, 2002; Shao *et al.*, 2002), respectively. *NLGN4Y* is located on the Y chromosome. Neuroligins are cell adhesion molecules (figure 2), which form transsynaptic contacts with pre-synaptic beta-neurexins (Ichtchenko *et al.*, 1995; Ichtchenko *et al.*, 1996; Song *et al.*, 1999). These molecules are thought to be involved in the initiation of synaptogenesis and in

the maturation of the synapse contacts (Scheiffele *et al.*, 2000; Dean *et al.*, 2003). We screened these genes for mutations in autistic individuals and identified a frameshift mutation in *NLGN4*, leading to a truncated protein without transmembrane domain (Jamain *et al.*, 2003). This mutation appeared *de novo* in the mother and has been transmitted to the two affected children, one with autism and one with Asperger syndrome, and not to their healthy brother. Moreover, we also identified an amino acid change in the esterase domain of NLGN3 transmitted from a mother to two affected children also with autism and Asperger syndrome respectively. These two mutations are deleterious since they lead to intracellular retention of the mutated protein and prevent them to their ability to increase the number of synapses (Chih *et al.*, 2004; Comoletti *et al.*, 2004). For the first time in autism, these results have been genetically replicated by an independent team, who also identified a premature stop codon in NLGN4 (Laumonnier *et al.*, 2004). In a five-generation family, this mutation is present in 13 affected males either with autism, mental retardation or other autism spectrum disorders. Even if only a few families are affected by perturbation in this gene family (Gauthier *et al.*, 2004; Vincent *et al.*, 2004), further studies of the neuroligin protein system and its impact on the formation and maturation of the brain may reveal important elements to understand the pathophysiology of autism.

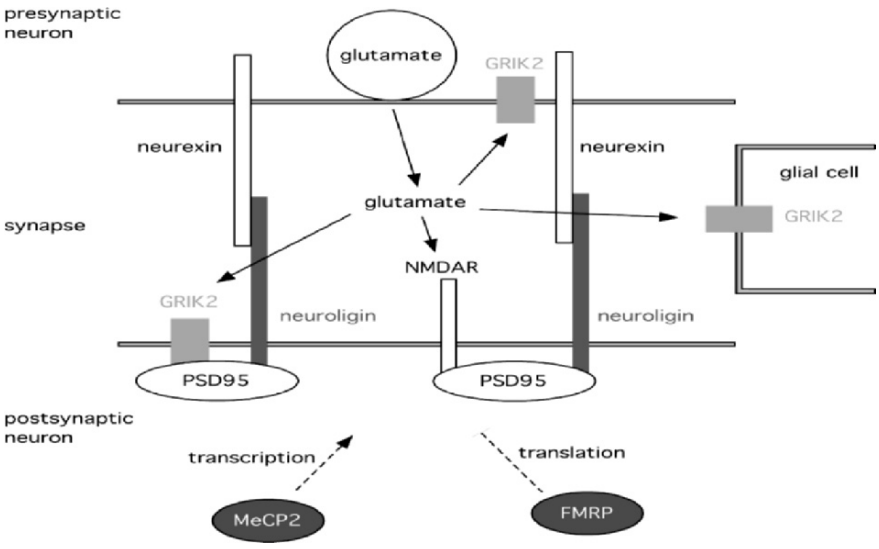


Figure 2. Autism susceptibility molecules at synapse. Hypothetical synapse in which molecules associated to autism are represented as well as their putative partners.

5. CONCLUSION

In the past decade, tremendous progress has been made in understanding of autism and other autism spectrum disorders. A better characterisation of the phenotype associated to a meticulous genetic analysis of the autistic patients have led to the identification of chromosomal regions strongly associated to autism, such as chromosomes 2q, 7q and 15q, and more recently to the identification of the first genes involved in idiopathic autism (NLGNs). These results are really promising and open new avenues in understanding the pathophysiology of autism. First they bring decisive elements to select new functional candidate genes in susceptibility regions to autism, by focusing our attention on molecular partners of neuroligins or proteins involved in the same physiological process (figure 2). In a second hand, the break down of molecular mechanisms, in which neuroligins and associated molecules are involved, will allow the development of new pharmacological strategies to identify new drugs to relieve patients suffering with autism.

6. REFERENCES

American Psychiatric Association., 1994, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. APA, Washington, DC.

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U. and Zoghbi, H. Y., 1999, Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2, *Nat Genet*. **23**: 185-188.

Ashley-Koch, A. E., Wolpert, C. M., Menold, M. M., Zaeem, L., Basu, S., Donnelly, S. L., Ravan, S. A., Powell, C. M., Qumsiyeh, M. B., Aylsworth, A. S., et al., 1999, Genetic studies of autistic disorder and chromosome 7, *Genomics*. **61**: 227-236.

Auranen, M., Vanhala, R., Varilo, T., Ayers, K., Kempas, E., Ylisaukko-Oja, T., Sinsheimer, J. S., Peltonen, L. and Jarvela, I., 2002, A genomewide screen for autism-spectrum disorders: evidence for a major susceptibility locus on chromosome 3q25-27. *Am J Hum Genet*. **71**: 777-790.

Bacchelli, E., Blasi, F., Biondolillo, M., Lamb, J. A., Bonora, E., Barnby, G., Parr, J., Beyer, K. S., Klauck, S. M., Poustka, A., et al., 2003, Screening of nine candidate genes for autism on chromosome 2q reveals rare nonsynonymous variants in the cAMP-GEFII gene. *Mol Psychiatr*. **8**: 916-924.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E. and Rutter, M., 1995, Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med.* **25**: 63-77.

Bardoni, B. and Mandel, J. L., 2002, Advances in understanding of fragile X pathogenesis and FMRP function, and in identification of X linked mental retardation genes. *Curr Opin Genet Dev*. **12**: 284-293.

Barrett, S., Beck, J. C., Bernier, R., Bisson, E., Braun, T. A., Casavant, T. L., Childress, D., Folstein, S. E., Garcia, M., Gardiner, M. B., et al., 1999, An autosomal genomic screen for autism. Collaborative linkage study of autism. *Am J Med Genet*. **88**: 609-615.

Betancur, C., Corbex, M., Spielewoy, C., Philippe, A., Laplanche, J. L., Launay, J. M., Gillberg, C., Mouren-Simeoni, M. C., Hamon, M., Giros, B., et al., 2002, Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. *Mol Psychiatr.* **7**: 67-71.

Borg, I., Squire, M., Menzel, C., Stout, K., Morgan, D., Willatt, L., O'Brien, P. C., Ferguson-Smith, M. A., Ropers, H. H., Tommerup, N., et al., 2002, A cryptic deletion of 2q35 including part of the

PAX3 gene detected by breakpoint mapping in a child with autism and a de novo 2;8 translocation. *J Med Genet.* **39**: 391-399.

Bradford, Y., Haines, J., Hutcheson, H., Gardiner, M., Braun, T., Sheffield, V., Cassavant, T., Huang, W., Wang, K., Vieland, V., et al., 2001, Incorporating language phenotypes strengthens evidence of linkage to autism. *Am J Med Genet*. **105**: 539-547.

Buxbaum, J. D., Silverman, J. M., Smith, C. J., Kilifarski, M., Reichert, J., Hollander, E., Lawlor, B. A., Fitzgerald, M., Greenberg, D. A. and Davis, K. L., 2001, Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. *Am J Hum Genet*. **68**: 1514-1520.

Buxbaum, J. D., Silverman, J. M., Smith, C. J., Greenberg, D. A., Kilifarski, M., Reichert, J., Cook Jr, E. H., Fang, Y., Song, C. Y. and Vitale, R., 2002, Association between a GABRB3 polymorphism and autism. *Mol Psychiatr.* **7**: 11-316.

Chih, B., Afridi, S. K., Clark, L. and Scheiffele, P., 2004, Disorder-associated mutations lead to functional inactivation of neuroligins. *Hum Mol Genet*. **13**: 1471-1477.

Comoletti, D., De Jaco, A., Jennings, L. L., Flynn, R. E., Gaietta, G., Tsigelny, I., Ellisman, M. H. and Taylor, P., 2004, The Arg451Cys-neuroligin-3 mutation associated with autism reveals a defect in protein processing. *J Neurosci*. **24**: 4889-4893.

Cook, E. H., Courchesne, R. Y., Cox, N. J., Lord, C., Gonen, D., Guter, S. J., Lincoln, A., Nix, K., Haas, R., Leventhal, B. L., et al., 1998, Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. *Am J Hum Genet*. **62**: 1077-1083.

Cook, E. H., Jr., Arora, R. C., Anderson, G. M., Berry-Kravis, E. M., Yan, S. Y., Yeoh, H. C., Sklena, P. J., Charak, D. A. and Leventhal, B. L., 1993, Platelet serotonin studies in hyperserotonemic relatives of children with autistic disorder. *Life Sci.* **52**: 2005-2015.

Cook, E. H., Jr., Courchesne, R., Lord, C., Cox, N. J., Yan, S., Lincoln, A., Haas, R., Courchesne, E. and Leventhal, B. L., 1997, Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatr.* **2**: 247-250.

Dean, C., Scholl, F. G., Choih, J., DeMaria, S., Berger, J., Isacoff, E. and Scheiffele, P., 2003, Neurexin mediates the assembly of presynaptic terminals. *Nat Neurosci*. **6**: 708-716.

Fatemi, S. H., Kroll, J. L. and Stary, J. M., 2001, Altered levels of Reelin and its isoforms in schizophrenia and mood disorders. *Mol Psychiatr.* **6**: 129-133.

Filipek, P. A., Accardo, P. J., Baranek, G. T., Cook, E. H., Jr., Dawson, G., Gordon, B., Gravel, J. S., Johnson, C. P., Kallen, R. J., Levy, S. E., et al., 1999, The screening and diagnosis of autistic spectrum disorders. *J Autism Dev Disord*. **29**: 439-484.

Folstein, S. E. and Rutter, M., 1977, Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatr*. **18**: 297-321.

Folstein, S. E. and Rosen-Sheidley, B., 2001, Genetics of autism: Complex aetiology for a heterogeneous disorder. *Nat Rev Genet.* **2**: 943-955.

Fombonne E., 1999, The epidemiology of autism: a review. *Psychol Med.* **29**: 769-786.

Fombonne, E., 2002, Epidemiological trends in rates of autism. *Mol Psychiatr*. 7 Suppl **2**: S4-6.

Fombonne, E., 2003, The prevalence of autism. *JAMA*. **289**: 87-89.

Gallagher, L., Becker, K., Kearney, G., Dunlop, A., Stallings, R., Green, A., Fitzgerald, M. and Gill, M., 2003, Brief report: A case of autism associated with del(2)(q32.1q32.2) or (q32.2q32.3). *J Autism Dev Disord*. **33**: 105-108.

Gauthier, J., Bonnel, A., St-Onge, J., Karemera, L., Laurent, S., Mottron, L., Fombonne, E., Joober, R. and Rouleau, G. A., 2004, NLGN3/NLGN4 gene mutations are not responsible for autism in the Quebec population. *Am J Med Genet*.

Ghaziuddin, M. and Burmeister, M., 1999, Deletion of chromosome 2q37 and autism: a distinct subtype? *J Autism Dev Disord*. **29**: 259-263.

Gillberg, C. 1998, Chromosomal disorders and autism. *J Autism Dev Disord*. **28**: 415-425.

Gordon, C. T., State, R. C., Nelson, J. E., Hamburger, S. D. and Rapoport, J. L., 1993, A double-blind comparison of clomipramine, desipramine, and placebo in the treatment of autistic disorder. *Arch Gen Psychiatr*. **50**: 441-447.

Harrison, J. E. and Bolton, P. F., 1997, Annotation: tuberous sclerosis. *J Child Psychol Psychiatry*. **38**: 603-614.

Hollander, E., Phillips, A. T. and Yeh, C. C., 2003, Targeted treatments for symptom domains in child and adolescent autism. *Lancet*. **362**: 732-734.

Ichtchenko, K., Hata, Y., Nguyen, T., Ullrich, B., Missler, M., Moomaw, C. and Sudhof, T. C., 1995, Neuroligin 1: a splice site-specific ligand for beta-neurexins. *Cell*. **81**: 435-443.

Ichtchenko, K., Nguyen, T. and Sudhof, T. C., 1996, Structures, alternative splicing, and neurexin binding of multiple neuroligins. *J Biol Chem*. **271**: 2676-2682.

IMGSAC., 1998, A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International Molecular Genetic Study of Autism Consortium. *Hum Mol Genet.* **7**: 571-578.

IMGSAC., 2001a, A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet*. **69**: 570-581.

IMGSAC., 2001b, Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. *Hum Mol Genet.* **10**: 973-982.

Ingraham, L. J. and Kety, S. S., 2000, Adoption studies of schizophrenia. *Am J Med Genet*. **97**: 18-22.

Irwin, S. A., Patel, B., Idupulapati, M., Harris, J. B., Crisostomo, R. A., Larsen, B. P., Kooy, F., Willems, P. J., Cras, P., Kozlowski, P. B., et al., 2001, Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am J Med Genet*. **98**: 161-167.

Irwin, S. A., Idupulapati, M., Gilbert, M. E., Harris, J. B., Chakravarti, A. B., Rogers, E. J., Crisostomo, R. A., Larsen, B. P., Mehta, A., Alcantara, C. J., et al., 2002, Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. *Am J Med Genet*. **111**: 140-146.

Jamain, S., Betancur, C., Quach, H., Philippe, A., Fellous, M., Giros, B., Gillberg, C., Leboyer, M., Bourgeron, T. and the Paris Autism Research International Sibpair (PARIS) Study., 2002, Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatr.* **7**: 302-310.

Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, I. C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., et al., 2003, Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet*. **34**: 27-29.

Jorde, L. B., Hasstedt, S. J., Ritvo, E. R., Mason-Brothers, A., Freeman, B. J., Pingree, C., McMahon, W. M., Petersen, B., Jenson, W. R. and Mo, A., 1991, Complex segregation analysis of autism. *Am J Hum Genet*. **49**: 932-938.

Kanner, L., 1943, Autistic disturbances of affective contact. *Nervous Child.* **2**: 217-250.

Klauck, S. M., Poustka, F., Benner, A., Lesch, K. P. and Poustka, A., 1997, Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet.* **6**: 2233-2238.

Krebs, M. O., Betancur, C., Leroy, S., Bourdel, M. C., Gillberg, C., Leboyer, M. and Study, t. P. A. R. I. S., 2002, Absence of association between a polymorphic GGC repeat in the 5' untranslated region of the reelin gene and autism. *Mol Psychiatr.* **7**: 801-804.

Lai, C. S., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F. and Monaco, A. P., 2001, A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature*. **413**: 519-523.

Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., et al., 2001, Initial sequencing and analysis of the human genome. *Nature*. **409**: 860-921.

Laumonnier, F., Bonnet-Brilhault, F., Gomot, M., Blanc, R., David, A., Moizard, M. P., Raynaud, M., Ronce, N., Lemonnier, E., Calvas, P., et al., 2004, X-Linked Mental Retardation and Autism Are Associated with a Mutation in the NLGN4 Gene, a Member of the Neuroligin Family. *Am J Hum Genet*. **74**: 552-557.

Leboyer, M., Philippe, A., Bouvard, M., Guilloud-Bataille, M., Bondoux, D., Tabuteau, F., Feingold, J., Mouren-Simeoni, M. C. and Launay, J. M., 1999, Whole blood serotonin and plasma beta-endorphin in autistic probands and their first-degree relatives. *Biol Psychiatr*. **45**: 158-163.

Lijam, N., Paylor, R., McDonald, M. P., Crawley, J. N., Deng, C. X., Herrup, K., Stevens, K. E., Maccaferri, G., McBain, C. J., Sussman, D. J., et al., 1997, Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. *Cell*. **90**: 895-905.

Liu, J., Nyholt, D. R., Magnussen, P., Parano, E., Pavone, P., Geschwind, D., Lord, C., Iversen, P., Hoh, J., Ott, J., et al., 2001, A genomewide screen for autism susceptibility loci. *Am J Hum Genet*. **69**: 327-340.

Matsuura, T., Sutcliffe, J. S., Fang, P., Galjaard, R. J., Jiang, Y. H., Benton, C. S., Rommens, J. M. and Beaudet, A. L., 1997, De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. *Nat Genet*. **15**: 74-77.

McDougle, C. J., Naylor, S. T., Cohen, D. J., Volkmar, F. R., Heninger, G. R. and Price, L. H., 1996, A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Arch Gen Psychiatr*. **53**: 1001-1008.

Miles, J. H. and Hillman, R. E., 2000, Value of a clinical morphology examination in autism. *Am J Med Genet*. **91**: 245-253.

Miyashiro, K. Y., Beckel-Mitchener, A., Purk, T. P., Becker, K. G., Barret, T., Liu, L., Carbonetto, S., Weiler, I. J., Greenough, W. T. and Eberwine, J., 2003, RNA cargoes associating with FMRP reveal deficits in cellular functioning in Fmr1 null mice. *Neuron*. **37**: 417-431.

Nan, X., Campoy, F. J. and Bird, A., 1997, MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell*. **88**: 471-481.

Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N. and Bird, A., 1998, Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*. **393**: 386-389.

Newbury, D. F., Bonora, E., Lamb, J. A., Fisher, S. E., Lai, C. S., Baird, G., Jannoun, L., Slonims, V., Stott, C. M., Merricks, M. J., et al., 2002, FOXP2 is not a major susceptibility gene for autism or specific language impairment. *Am J Hum Genet*. **70**: 1318-1327.

Persico, A. M., Militerni, R., Bravaccio, C., Schneider, C., Melmed, R., Conciatori, M., Damiani, V., Baldi, A. and Keller, F., 2000, Lack of association between serotonin transporter gene promoter variants and autistic disorder in two ethnically distinct samples. *Am J Med Genet*. **96**: 123-127.

Persico, A. M., D'Agruma, L., Maiorano, N., Totaro, A., Militerni, R., Bravaccio, C., Wassink, T. H., Schneider, C., Melmed, R., Trillo, S., et al., 2001, Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol Psychiatr*. **6**: 150-159.

Petit, E., Herault, J., Martineau, J., Perrot, A., Barthelemy, C., Hameury, L., Sauvage, D., Lelord, G. and Muh, J. P., 1995, Association study with two markers of a human homeogene in infantile autism. *J Med Genet*. **32**: 269-274.

Philippe, A., Martinez, M., Guilloud-Bataille, M., Gillberg, C., Rastam, M., Sponheim, E., Coleman, M., Zappella, M., Aschauer, H., Van Maldergem, L., et al., 1999, Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. *Hum Mol Genet.* **8**: 805-812.

Risch, N. and Merikangas, K., 1996, The future of genetic studies of complex human diseases. *Science*. **273**: 1516-1517.

Risch, N., Spiker, D., Lotspeich, L., Nouri, N., Hinds, D., Hallmayer, J., Kalaydjieva, L., McCague, P., Dimiceli, S., Pitts, T., et al., 1999, A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet*. **65**: 493-507.

Sachidanandam, R., Weissman, D., Schmidt, S. C., Kakol, J. M., Stein, L. D., Marth, G., Sherry, S., Mullikin, J. C., Mortimore, B. J., Willey, D. L., et al., 2001, A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*. **409**: 928-933.

Scheiffele, P., Fan, J., Choih, J., Fetter, R. and Serafini, T., 2000, Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell*. **101**: 657-669.

Serajee, F. J., Nabi, R., Zhong, H. and Mahbubul Huq, A. H., 2003, Association of INPP1, PIK3CG, and TSC2 gene variants with autistic disorder: implications for phosphatidylinositol signalling in autism. *J Med Genet*. **40**: e119.

Shahbazian, M. D., Antalffy, B., Armstrong, D. L. and Zoghbi, H. Y., 2002, Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Hum Mol Genet*. **11**: 115-124.

Shao, Y., Wolpert, C. M., Raiford, K. L., Menold, M. M., Donnelly, S. L., Ravan, S. A., Bass, M. P., McClain, C., von Wendt, L., Vance, J. M., et al., 2002, Genomic screen and follow-up analysis for autistic disorder. *Am J Med Genet*. **114**: 99-105.

Shuang, M., Liu, J., Jia, M. X., Yang, J. Z., Wu, S. P., Gong, X. H., Ling, Y. S., Ruan, Y., Yang, X. L. and Zhang, D., 2004, Family-based association study between autism and glutamate receptor 6 gene in Chinese Han trios. *Am J Med Genet*.

Smith, M., Escamilla, J. R., Filipek, P., Bocian, M. E., Modahl, C., Flodman, P. and Spence, M. A., 2001, Molecular genetic delineation of 2q37.3 deletion in autism and osteodystrophy: report of a case and of new markers for deletion screening by PCR. *Cytogenet Cell Genet*. **94**: 15-22.

Song, J. Y., Ichtchenko, K., Sudhof, T. C. and Brose, N., 1999, Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc Natl Acad Sci U S A*. **96**: 1100-1105.

Thomas, N. S., Sharp, A. J., Browne, C. E., Skuse, D., Hardie, C. and Dennis, N. R., 1999, Xp deletions associated with autism in three females. *Hum Genet*. **104**: 43-48.

Veenstra-VanderWeele, J., Gonen, D., Leventhal, B. L. and Cook, E. H., Jr., 1999, Mutation screening of the UBE3A/E6-AP gene in autistic disorder. *Mol Psychiatr.* **4**: 64-67.

Vincent, J. B., Herbrick, J. A., Gurling, H. M., Bolton, P. F., Roberts, W. and Scherer, S. W., 2000, Identification of a novel gene on chromosome 7q31 that is interrupted by a translocation breakpoint in an autistic individual. *Am J Hum Genet*. **67**: 510-514.

Vincent, J. B., Kolozsvari, D., Roberts, W. S., Bolton, P. F., Gurling, H. M. and Scherer, S. W., 2004, Mutation screening of X-chromosomal neuroligin genes: no mutations in 196 autism probands. *Am J Med Genet*. **129B**: 82-84.

Volkmar, F. R. and Pauls, D., 2003, Autism. *Lancet*. **362**: 1133-1141.

Wassink, T. H., Piven, J. and Patil, S. R., 2001a, Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. *Psychiatr Genet*. **11**: 57-63.

Wassink, T. H., Piven, J., Vieland, V. J., Huang, J., Swiderski, R. E., Pietila, J., Braun, T., Beck, G., Folstein, S. E., Haines, J. L., et al., 2001b, Evidence supporting WNT2 as an autism susceptibility gene. *Am J Med Genet*. **105**: 406-413.

Wolff, D. J., Clifton, K., Karr, C. and Charles, J., 2002, Pilot assessment of the subtelomeric regions of children with autism: detection of a 2q deletion. *Genet Med*. **4**: 10-14.

Yirmiya, N., Pilowsky, T., Nemanov, L., Arbelle, S., Feinsilver, T., Fried, I. and Ebstein, R. P., 2001, Evidence for an association with the serotonin transporter promoter region polymorphism and autism. *Am J Med Genet*. **105**: 381-386.

Zoghbi, H. Y., 2003, Postnatal neurodevelopmental disorders: meeting at the synapse? *Science*. **302**: 826-830.

11. GENETICS OF MONOAMINE METABOLIZING ENZYMES:

Psychopharmacogenetics

Rolando Meloni, Olfa Khalfallah and Nicole Faucon Biguet*

1. INTRODUCTION

The monoamine (MA) neurotransmitters, namely the catecholamines dopamine (DA), norepinephrine (NE) and epinephrine (E) and the indolamine serotonin (5-hydroxytryptamine = 5-HT), play important roles in mood, cognition, learning, motor activity, reward, sleep, appetite, and cardiovascular functions.

1.1. Monoamine brain distribution and pathways

Peripheral MA-ergic cells are mainly present in the adrenal medulla (E and NE), sympathetic ganglia (NE) and myenteric plexus (5-HT). In the brain neurons containing DA, NE and 5-HT have a restricted distribution in specific nuclei mainly in the brainstem from where they form pathways that allow regulation of the activity of large regions of the CNS.

1.1.1. Dopamine

Most DA-ergic neurons are located in the *pars compacta* of the substantia nigra (SN) and in the medially adjacent ventral tegmental area (VTA) in the mesencephalon. They project rostrally in three partially overlapping pathways.

* Rolando Meloni, Olfa Khalfallah and Nicole Faucon Biguet, Laboratoire de Génétique de la Neurotransmission et des Processus Neurodégénératifs (LGN), C.N.R.S. UMR 7091, Hôpital de la Pitié-Salpêtrière, 75013 Paris, France.
Corresponding Author: R. Meloni, LGN C.N.R.S. UMR 7091, Bât. C.E.R.V.I., Hôpital de la Pitié-Salpêtrière, 83 Boulevard de l'Hôpital, 75013 Paris, France.
Tel: 33 (0)142177563; fax: 33 (0)142177533; E-mail: meloni@infobiogen.fr

The nigrostriatal projection (or mesostriatal projection) is from the SN to the caudate nucleus and putamen. The mesolimbic and mesocortical pathways travel from the VTA to several forebrain structures such as the nucleus accumbens, septal area, amygdala, and pre-frontal cerebral cortex. Additional DA-ergic neurons are found in the retina, the olfactory bulb, and the hypothalamus and form local contacts.

1.1.2. Norepinephrine

NE-ergic neurons form the locus ceruleus (LC) in the pons and are also found in the medullary reticular formation, in the solitary nucleus and the dorsal motor nucleus of the vagus in the medulla. They project to most of the CNS with ascending fibres reaching the thalamus, hypothalamus, limbic forebrain structures, the somatosensory cerebral cortex and the cerebellar cortex and deep nuclei while descending fibres project to other parts of the brainstem and to all spinal levels.

1.1.3. Serotonin

5-HT-ergic neurons are found in the brainstem where they are concentrated in the raphe nuclei. They innervate virtually all parts of the CNS with projections from the rostral raphe nuclei to the forebrain. The cortical innervation is most dense in sensory and limbic areas. The caudal raphe nuclei provide most of the projections to the brainstem and spinal cord. Moreover, 5-HT is a precursor for melatonin and is therefore synthesized in high amounts in the pineal gland.

1.2. Monoamine pre-synaptic neurotransmission (synthesis, storage, release, uptake and degradation)

Tyrosine Hydroxylase (TH) and Tryptophan Hydroxylase (TPH) are the rate-limiting enzymes involved in the hydroxylation of the amino acids tyrosine and tryptophan, the first step in the synthesis of, respectively, the catecholamines and serotonin. Thereafter, the Aromatic Amino acid Decarboxylase (AAD or Dihydroxyphenylalanine (DOPA) Decarboxylase) decarbo-xylates the DOPA to DA and the 5-hydroxytryptophan to 5-HT. The Dopamine-ß-Hydroxylase (DBH) further converts DA to NE and the Phenylethanolamine-N-Methyl-Transferase (PNMT) NE to E in the adrenal medulla.

After their synthesis, all monoamines are concentrated in vesicles at the nerve terminal by the specific vesicular monoamine transporters VMAT-1, primarily present in endocrine and paracrine cells of peripheral organs, and VMAT-2, the predominant monoamine vesicular transporter in the CNS (Erickson et al., 1996). These transporters favorize the uptake and protect these molecules from leakage and/or intraneuronal metabolism (Masson et al., 1999) and regulate the release of MA from the vesicle pool (Boehm & Kubista, 2002).

Upon their release, the availability of extracellular MA and, thus, the spread and duration of synaptic excitability at their receptors is limited by

presynaptically localized transporters and/or the action of specific catabolyzing enzymes. The transporters NET, DAT and SERT retrieve the released NE, DA and 5-HT, respectively, allowing these neurotransmitters to be repackaged into synaptic vesicles inside the terminal (Gainetdinov & Caron, 2003). The enzymes monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT) carry out the first step in catecholamine catabolism. Two isoforms of MAO (types A and B), which are encoded by separate genes, can be distinguished by substrate specificity and sensitivity to selective inhibitors. MAO-A appears to be the main enzyme for metabolising 5-HT and NE as substrates, and clorgyline is a selective MAO-A inhibitor, whereas MAO-B prefers phenylethylamine as a substrate, and is inactivated by deprenyl as a selective inhibitor. Both MAO-A and MAO-B oxidize DA. MAO-A is preferentially located in dopaminergic and noradrenergic neurons, while MAO-B appears to be the major form present in serotonergic neurons and glia (Shih et al., 1999; Nagatsu, 2004). COMT is bound to membranes and appears to be located principally in postsynaptic neurons. The degradation of catecholamines by the MAO and the COMT enzymes generates aldehyde intermediates that are reduced to 3,4-dihydroxyphenyl-glycol (DHPG) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by cytosolic aldehyde reductase or oxidized to 3,4-dihydroxy-phenylacetic acid (DOPAC) by mitochondrial aldehyde dehydrogenase. DOPAC may further be catabolized to homovanillic acid (HVA). The MAO and aldehyde reductase enzymes catabolize 5-HT yielding 5-hydroxy indole acetic acid as the end product.

1.2.1. Monoamine functions in the CNS

Each of the MA systems in the brain is consistently associated with distinctive but sometimes overlapping physiological processes whose disturbances are implicated in neuropsychiatric diseases.

The DA systems are important mediators of motor function, mood, reward and cognition (Koob & Nestler, 1997). The loss of the DA neurons in the SN is responsible for Parkinson's disease (Wolters & Calne, 1989). Alterations in DA-ergic neurotransmission are implicated in psychiatric diseases such as bipolar disorder (Schildkraut, 1965), schizophrenia (Carlsson, 1988; Abi-Dargham et al., 1998; Abi-Dargham et al., 2000), attention deficit/hyperactivity disorder (ADHD) (Shastry, 2004), Tourette's syndrome (Segawa, 2003) as well as drug abuse (Koob & Nestler, 1997; Melichar et al., 2001).

The NE system participates in the regulation of arousal, mood, attention and the response to stress suggesting its implication in depression (Schildkraut, 1965; Ressler & Nemeroff, 1999).

The 5-HT system plays a role in mood, aggression, response to alcohol, appetite, sleep, cognition, and sexual and motor activity. 5-HT has been implicated in the etiology of depression mostly based on the pharmacological studies of the mechanism of action on the selective serotonin reuptake inhibitors class of anti-depressant drugs (Vaswani et al., 2003; Gross & Hen, 2004).

2. GENETIC ASPECTS OF MONOAMINE METABOLISM AND NEUROPSYCHIATRIC DISEASES

The role of the MA systems in regulating several biological processes that are disrupted in mental diseases suggests that the imbalance in MA neurotransmission may play a substantial role in the pathophysiology of neuropsychiatric diseases (Grace, 1991; Mallet, 1996; Dreher & Burnod, 2002). The relative abundance and activity of the MA varies in different species and in different cell groups as well as interindividually and between normal and pathological states, albeit it is unclear to what extent any neurobiological findings reflect primary rather than secondary pathology, compensatory mechanisms, or environmental influences. However, the primary source of interindividual differences in MA availability at the synapsis is essentially represented by DNA polymorphisms in the genes encoding the metabolizing machine of the MA systems. Accordingly, the psychiatric genetic studies of the genes encoding these enzymes have highlighted their implication in the genetic predisposition to several neuropsychiatric diseases. The major findings of these studies will be presented focusing on the relevance for behavioral and other complex traits of functional polymorphisms in MA metabolism related genes.

2.1. Tyrosine Hydroxylase

The *TH* gene, encoding the rate limiting enzyme in the synthesis of catecholamines, is a strong candidate gene for neuropsychiatric diseases (Mallet, 1996). A seminal paper showing for the first time a genetic linkage between bipolar disorder in the Amish population and markers at the chromosome 11p15, a region that contains the *TH* locus, strengthened the case for *TH* as a "positional" candidate gene (Egeland et al., 1987). This result was questioned because the lod score method utilized did not take into account genetic heterogeneity that characterizes bipolar disorder and other complex diseases (Hodgkinson et al., 1987). Eventually, further genetic analysis of the Amish or studies of other populations did not confirm this initial result (Detera-Wadleigh et al., 1987; Kelsoe et al., 1989; Ginns et al., 1992; Gerhard et al., 1994; Gershon et al., 1996; Ginns et al., 1996). However, other studies finding significant linkage between markers at the *TH* locus and bipolar disorder, maintained the implication of the *TH* gene in this disease and strengthened the case for genetic heterogeneity (Pakstis et al., 1991; Byerley et al., 1992; Lim et al., 1993; Gurling et al., 1995; Smyth et al., 1996; Malafosse et al., 1997).

In the first of a series of association studies on the *TH* gene, a significant genetic association was found between restriction fragment polymorphism markers at the *TH* locus and bipolar disorder in a French population sample (Leboyer et al., 1990). However, this result has not always been replicated in other studies (Korner et al., 1990; Gill et al., 1991; Inayama et al., 1993; Korner et al., 1994; Kawada et al., 1995).

In order to further investigate the implication of the *TH* gene in the genetic predisposition to bipolar disorder, an association analysis was conducted using the more informative microsatellite HUMTH01 marker. This microsatellite is a

polymorphic polypyrimidine sequence localized in the first intron of the *TH* gene and is characterized by a core (TCAT)n tetranucleotide repeat iterated usually between 6 and 10 times (Polymeropoulos et al., 1991; Brinkmann et al., 1996). The perfect $(TCAT)_{10p}$ repeat is very rare (less than 1%), while an imperfect $(TCAT)_4CAT(TCAT)_5$ repeat allele, named $(TCAT)_{10i}$, is the most common allele in Caucasians (around 30%) (Puers et al., 1993). A significant association has been found between the HUMTH01 7/10i repeat genotype and bipolar disorder in a new sample of French case-controls. Moreover, the patients bearing the risk genotype were clinically characterized by having familial history of bipolar disorder and/or delusive symptoms during manic or depressive episodes (Meloni et al., 1995a). Although the *TH* gene is also a candidate gene for schizophrenia, there was no compelling evidence for linkage of the *TH* locus to schizophrenia (Byerley et al., 1993). However, the rare 10p allele of the HUMTH01 microsatellite was significantly associated with schizophrenia in two different ethnic samples from Normandy (northwestern France) and the Sousse region (eastern Tunisia) (Meloni et al., 1995b).

Several further studies inspired by these results have been inconclusive for association (Cavazzoni et al., 1996; Souery et al., 1996; Turecki et al., 1997; Burgert et al., 1998; Jonsson et al., 1998; Souery et al., 1999) or have replicated the positive association between the HUMTH01 microsatellite and both bipolar disorder (Perez de Castro et al., 1995; Lobos & Todd, 1997; Serretti et al., 1998; Serretti et al., 1998; Furlong et al., 1999; Chiba et al., 2000) and schizophrenia (Wei et al., 1995; Wei et al., 1997; Kurumaji et al., 2001).

The HUMTH01 microsatellite has also been associated with catecholamine neurotransmission by measuring catecholamine metabolite levels in lumbar cerebrospinal fluid (CSF) (Jonsson et al., 1996) or in plasma (Wei et al., 1995; Wei et al., 1997) which are an indirect index of monoamine turnover in the brain. Moreover, in a clinical study in the original Normandy sample, the schizophrenic patients bearing the 10p rare allele presented significantly lower plasma concentrations of the catecholaminergic metabolites HVA and MHPG, which are indices of central DA-ergic and NE-ergic function, respectively, as compared to patients bearing other alleles (Thibaut et al., 1997).

These results suggest a functional link between allelic variations at the HUMTH01 marker and TH activity. Indeed, the (TCAT)n motif of this microsatellite differs by only one nucleotide from the consensus AP1 sequence (TGATTCA) present in the rat and human *TH* gene (Icard Liepkalns et al., 1992), a sequence that is specifically recognized by transcription factors of the Fos and Jun proto-onco-gene families (Sassone-Corsi et al., 1988). Moreover, a less polymorphic HUMTH01 repeated sequence is conserved at its orthologous position in the first intron of the *TH* gene in several non-human primate species (Meyer et al., 1995), hinting that this motif may be an evolutionary conserved regulatory element that has expanded in the human lineage.

Therefore, the functional role of the HUMTH01 microsatellite was assessed in order to investigate the biological significance of the genetic association findings. Indeed both the (TCAT)10i and (TCAT)10p alleles enhanced transcription when placed upstream from a minimal promoter driving the expression of a luciferase reporter gene. Moreover, these repeated sequences

interacted specifically with factors of the fos/jun type and with an even higher affinity with other nuclear proteins (Meloni et al., 1998). Subsequently, ZNF191, a zinc finger protein, and HBP1, a HMG box transcription factor, were identified as the proteins specifically binding the TCAT motif. Interestingly, the specific binding of ZNF191 to the HUMTH01 sequence was correlated in a quantitative fashion to the number of TCAT repeats (Albanese et al., 2001). Moreover, *in vitro* experiments with a *TH*-reporter gene construct established that the HUMTH01 microsatellite regulates the *TH* gene expression by a quantitative silencing effect that correlates with the number of repetitions of the (TCAT) motif (Albanese et al., 2001). Thus, the HUMTH01 sequence may participate in the transcriptional regulation of the *TH* gene by modulating its expression in a quantitative fashion. Since the (TCAT)n polymorphic sequence is widespread in the genome and present in other genes, it may provide a molecular basis for the modulation of gene expression relevant to the genetics of quantitative traits.

2.2. Tryptophan Hydroxylase

Until recently, only this one gene encoding *TPH* had been described (Boularand et al., 1990; Craig et al., 1991). Since TPH catalyzes the rate-limiting step in 5-HT synthesis, this gene has been the target of a series of genetic studies for psychiatric diseases (Herault et al., 1993; Herault et al., 1994; Goldman, 1995; Bellivier et al., 1998; Furlong et al., 1998; Gelernter et al., 1998; Han et al., 1999; Kunugi et al., 1999; McQuillin et al., 1999) with a specific focus on aggressive behavior and suicidality (Nielsen et al., 1994; Abbar et al., 1995; Mann et al., 1997; Nielsen et al., 1998; Manuck et al., 1999; Rotondo et al., 1999; Vincent et al., 1999; Rujescu et al., 2003) as reviewed by Arago (Arango et al., 2003). However, the functional inactivation of the *TPH* gene in mice by two different groups has revealed that 5-HT levels were depleted in the periphery and in the pineal gland but were in the normal range in the brain stem (Cote et al., 2003; Walther et al., 2003). These results led to the detection of a second *TPH* gene, named *TPH2*, which is exclusively expressed in the brain stem of humans (Zill et al., 2004), mice (Cote et al., 2003; Zhang et al., 2004), and rats (Patel et al., 2004). The classical *TPH* gene, now called *TPH1* and whose inactivation results in a pathological cardiovascular phenotype (Cote et al., 2003), is expressed in the myenteric plexus, spleen, thymus and pineal gland. The *TPH1* and *TPH2* genes have non-overlapping expression patterns, different functions and are independently regulated, thus rendering obsolete the finding of genetic studies on *TPH1* and psychiatric diseases. *TPH2* has already been associated with major depression (Zill et al., 2004), but not with bipolar disorder or suicidality (De Luca et al., 2004).

2.3. Aromatic Amino Acid Decarboxylase

The *AAD*, or Dihydroxyphenylalanine (DOPA) Decarboxylase, gene, which encodes the enzyme involved directly in the synthesis of DA and 5-HT, has been mapped to chromosome 7p11-p13. A study for bipolar disorder on Danish

families was negative for linkage in this region (Ewald et al., 1995). Two putative functional polymorphisms have been described for the *AAD* gene: a 1-bp deletion in the promoter and a 4-bp deletion in the untranslated exon 1. Both deletions affect binding sites for known transcription factors and may thus affect *AAD* gene expression (Borglum et al., 1999). A significant association was found between the 1-bp deletion and bipolar disorder in a Danish and a British sample (Borglum et al., 1999) and between both polymorphism and early age of onset in schizophrenia (Borglum et al., 2001). However, other studies failed to reproduce the association between these markers and mood disorders in a German sample (Jahnes et al., 2002) or autism (Lauritsen et al., 2002). *AAD* is located next to the imprinted gene GRB10 which is expressed specifically from the paternal allele in foetal brains. Interestingly, a preferential paternal transmission of alleles at the 4-bp insertion/deletion was observed in family based (trios formed by the proband and both parents) association studies for ADHD (Hawi et al., 2001) and bipolar disorder (Borglum et al., 2003).

2.4. Dopamine ß Hydroxylase

The *DBH* gene is located on chromosome 9q34 (Craig et al., 1988) and encodes the enzyme that catalyses the conversion of DA to NE. Mutations in the *DBH* gene result in lack of sympathetic noradrenergic function and orthostatic hypotension (Garland et al., 2002; Deinum et al., 2004). The DBH enzyme is localised within the soluble and membrane fractions of secretory catecholamine-containing vesicles of noradrenergic and adrenergic cells. These two forms, which originate from a single mRNA with the first ATG is the only effective initiation site, arises from optional cleavage of the signal peptide. The form retaining the signal peptide is completely associated with the membrane, whereas the cleaved form is mostly soluble with only a small portion membrane-bound (Houhou et al., 1995). The soluble form of the enzyme is secreted into the circulation from nerve terminals allowing for assaying its activity in plasma or serum. DBH presents differences in enzymatic activity that are stable and appear to be genetically determined to a great extent (Stolk et al., 1982). Several polymorphisms in the gene have been implicated in these variations. A G/T polymorphism at the nucleotide 910 of the coding sequence results in a change at amino acid residue 304 between Ala (A) and Ser (S) (DBH/A and DBH/S). The resulting proteins have similar kinetic constants, but DBH/S has a homospecific activity that is about one thirteenth lower than that of human DBH/A (Ishii et al., 1991). The DBH/A allele is the most common allele in European, African and several other populations with allele frequencies greater than 0.80 in each sample and significant heterogeneity in allele frequency across population groups (Cubells et al., 1997). These allelic differences cannot alone account for the differences in the activity of DBH in blood since circulating DBH concentrations also vary considerably in the general population. Recently a novel polymorphism (-1021 C/T) in the 5' promoter region of the *DBH* gene was shown to strongly influence plasma DBH-activity, accounting for 35%-52% of its variation in different populations (Zabetian et al., 2001; Kohnke et al., 2002). A further study showed that 10

biallelic markers in a 10 Kb surrounding the -1021C/T polymorphisms were all associated with plasma DBH activity and that this association was strongly correlated with the degree of Linkage Disequilibrium between each marker and the -1021C/T polymorphism (Zabetian et al., 2003). The -1021C/T polymorphism is also associated with variation in the concentration of HVA and 5-HIAA in the CSF (Jönsson et al., 2004). Another association was found between a *DBH* TaqI polymorphism and plasma metabolites of catecholamines (Wei et al., 1998). Other polymorphic variants in the *DBH* gene are represented by a GT dinucleotide microsatellite, a single-base, 444 g/a, substitution at the 3' end of *DBH* exon 2 and a di-allelic variant, DBH5'-ins/del, located approximately 3 kb 5' to the *DBH* transcriptional start site. All these markers, which are in linkage disequilibrium, were also associated with plasma DBH activity. (Wei et al., 1997; Cubells et al., 1998; Cubells et al., 2000; Jönsson et al., 2004). Moreover, the 444 g/a marker was also associated with differences in DBH concentration in the CSF (Cubells et al., 1998; Zabetian et al., 2003).

Psychiatric genetic studies using the polymorphisms at the *DBH* gene have shown a significant association between the *DBH* TaqI polymorphism and ADHD (Daly et al., 1999). Also, albeit it did not reach statistical significance, the *DBH* GT repeat 4 allele, which is associated with high serum levels of DBH, occurred more frequently in the ADHD group than controls, (Müller Smith et al., 2003). However, other groups failed in replicating these results either with the TaqI polymorphism (Wigg et al., 2002) or with other markers (Hawi et al., 2003). A positive association was shown between the *DBH* 5'del-444a haplotype and cocaine-induced paranoia (Cubells et al., 2000) as well as non-response to antipsychotic drug treatment in schizophrenic patients (Yamamoto et al., 2003). Other studies using a *DBH* -1021 C/T variant found no positive association with schizophrenia (Jonsson et al., 2003) or unipolar major depression with psychotic features (Cubells et al., 2002). These results may indicate that the *DBH* gene is indirectly involved in schizophrenia as a modulatory factor of psychotic symptoms, severity of the disorder and therapeutic response to neuroleptic drugs.

2.5. Mono-Amino-Oxydase

MAO-A and *MAO-B* genes are situated on the X chromosome at Xp11. 23–11.4 and result from the duplication of a common ancestral gene. In humans both genes are deleted in patients with Norrie's disease, a rare X-linked recessive neurological disorder characterized by blindness, hearing loss, and mental retardation (Lan et al., 1989). A point deletion in the *MAO-A* gene was discovered in a Dutch family. This mutation resulted in a complete MAO-A inactivation and was linked to abnormally aggressive behavior in the males from this family (Brunner et al., 1993). Conversely *MAO-A* deficient mice show an increased aggressivity in males that is related to increased levels of 5-HT and NE during development and result in brain structural changes (Cases et al., 1995). A functional polymorphism located in the *MAO-A* gene promoter 1.2 kb upstream of the encoding sequence, consists of a 30 bp repeated sequence present in 3, 3.5, 4, or 5 copies. This polymorphism displays significant

variations in allele frequencies across ethnic groups and is able to affect the transcriptional activity of the *MAO-A* gene promoter (Sabol et al., 1998).

Genetic studies with this polymorphism have found that the high-activity *MAO-A* gene promoter alleles were associated with panic disorder (Deckert et al., 1999) and major depressive disorder (Schulze et al., 2000) in females, while the low activity alleles were associated with schizophrenia in males (Jonsson et al., 2003). Other studies have yielded negative results for panic disorders (Hamilton et al., 2000), schizophrenia (Syagailo et al., 2001; Fan et al., 2004) and mood disorders (Kunugi et al., 1999; Jorm et al., 2000; Furlong et al., 1999; Kirov et al., 1999; Syagailo et al., 2001; Huang et al., 2004).

However, more probant results have been found when genetic studies have taken into account an environmental component. A leading study has shown that this functional polymorphism can modulate the association between childhood maltreatment and subsequent antisocial behavior. In males, who have only one copy of the X chromosome, the *MAO-A* functional polymorphism confers either a high or a low activity genotype. The low activity *MAO-A* genotype is associated with antisocial behavior in up to 85% of a cohort of males who had been severely maltreated in their childhood but not in boys who had suffered little or no abuse. In contrast, the high activity *MAO-A* genotype has a protective effect from developing antisocial behavior in maltreated children (Caspi et al., 2002). Women have two X chromosomes and heterozygous low/high *MAO-A* activity cannot be characterized since one of the alleles is randomly inactivated. Therefore, albeit a similar trend for association between *MAO-A* genotype, antisocial behavior and child maltreatment, was present, these results were less straightforward than for males since the whole female sample cannot be analyzed correctly. However, taken together, these results show a clear influence of the *MAO-A* genotype in the behavioral effects of an environmental factor and may help in understanding the marked differences in the frequency of antisocial behavior between sexes (Caspi et al., 2002). Interestingly, in a study of healthy volunteers the functional high activity genotype was correlated with higher cerebrospinal fluid concentrations of HVA and 5-HIAA in women, while an opposite trend was observed in men (Jonsson et al., 2000).

The association between the lower expression *MAO-A* genotype and antisocial behavior consequent to childhood maltreatment has been replicated by another group (Foley et al., 2004). This risk genotype has also been associated with impulsive traits in males that have experienced early abuse (Huang et al., 2004) and with pathological gambling in males (Ibanez et al., 2000).

2.6. Catechol-O-Methyl-Transferase

The *COMT* gene, localized to chromosome 22q11.1-q11.2, encodes a soluble (S-COMT) and a membrane-bound (MB-COMT) form of the enzyme, the latter characterized by an additional 50 amino acids at the *N*-terminal (Bertocci et al., 1991; Grossman et al., 1992). The two length variants of the *COMT* are expressed from two mRNA transcripts: a long mRNA, which is able to transcribe both S-*COMT* and MB-*COMT* from two different initiation sites, and a short mRNA producing S-*COMT* only. The long mRNA and the larger

MB-*COMT* are predominant in the brain while the short mRNA and the S-*COMT* prevail in the other tissues (Tenhunen et al., 1994; Lundstrom et al., 1995).

The COMT enzymatic activity shows high, intermediate and low rates consistent with inheritance of two codominant alleles (Weinshilboum, 1978). This difference in enzyme activity is independent from protein length variations but is caused by an amino acid substitution. A G /A polymorphism in exon 4 at position 472 in the long mRNA, and 322 in the short mRNA, results in a Val to Met amino acid change at codon 158 of MB-*COMT* and codon 108 of S-*COMT*. The G (Val) allele encodes the thermostable, high activity form of the enzyme, while the A (Met) allele encodes the thermolabile, low activity variant that exhibits a 3 to 4 fold decrease in the enzymatic activity level ((Lachman et al., 1996; Lotta et al., 1995). The G (Val) and A (Met) alleles correspond also to the absence or presence, respectively, of a *Nla*III polymorphic restriction site that allows for easily genotyping the functional variations (Karayiorgou et al., 1998).

COMT is an obvious *a priori* candidate gene for neuropsychiatric disorders that involve dopaminergic or noradrenergic systems (for review see Palmatier et al., 1999) but also a strong *positional* candidate gene for schizophrenia because of its chromosomal location in the locus of the velocardiofacial syndrome (VCF). Microdeletions of 22q11 are associated with VCF which is characterized by congenital abnormalities, learning difficulties, and psychosis in up to one third of patients. Conversely, the deletion is also 80-fold more common in patients with psychosis compared to the normal population (Sugama et al., 1999). Both linkage and association studies have implied that chromosome 22q11 is a locus for schizophrenia (Pulver et al., 1994; Pulver et al., 1994; Karayiorgou et al., 1995; Karayiorgou & Gogos, 1997). The case-control association approach has consequently been used to study the role of *COMT* in schizophrenia and other psychiatric diseases, mostly using the Val108/158Met polymorphism. Positive associations have been found between *COMT* and schizophrenia (Ohmori et al., 1998; de Chaldee et al., 1999), violence in schizophrenia (Lachman et al., 1998), bipolar disorder (Li et al., 1997; Mynett-Johnson et al., 1998), unipolar disorder (Ohara et al., 1998), bipolar disorder or ADHD in VCFS patients (Lachman et al., 1996), OCD (Karayiorgou et al., 1997), drug abuse (Vandenbergh et al., 1997) and Parkinson's disease (Kunugi et al., 1997). However, other studies have excluded a major contribution of the *COMT* gene to schizophrenia (Daniels et al., 1996; Chen et al., 1997; Strous et al., 1997; Karayiorgou et al., 1998; Wei & Hemmings, 1999), bipolar disorder (Craddock et al., 1997; Gutierrez et al., 1997; Kunugi et al., 1997; Lachman et al., 1997; Geller & CookJr., 2000), ADHD or bipolar disorder in VCF syndrome patients (Lachman et al., 1996), substance abuse and violence (Lachman et al., 1998; Vandenbergh et al., 1997), as well as Parkinson's disease (Hoda et al., 1996; Syvanen et al., 1997; Xie et al., 1997).

These conflicting results have prompted a meta-analysis indicating that the *COMT* Met allele that characterizes the instable form of the enzyme with low activity phenotype, is not associated with schizophrenia (Lohmueller et al., 2003). However, a new association study conducted in a genetically

homogeneous population yielded a highly significant association between a *COMT* haplotype and schizophrenia (Shifman et al., 2003). This study is the largest case/control analysis in schizophrenia that has been reported with more than 700 patients and 4,000 control subjects. Genotyping was conducted using 12 SNPs, comprising the Val108/158Met polymorphism, across the *COMT* gene, and haplotypes with 7 of these SNPs were established in the large sample of an Israeli Ashkenazi Jewish population. This population has the advantage of presenting a founder effect that allows for reducing genetic heterogeneity thus increasing gene effect, and avoiding false-positive results due to population stratification.

The association between schizophrenia and the Val108/158Met polymorphism was moderate, but extremely high levels of statistical significance were attained when this marker was analyzed as part of a haplotype including two other noncoding SNPs that were more significantly associated with schizophrenia. Moreover, one of these polymorphisms represented a higher risk factor essentially for women than men, hinting at a possible sex-specific genetic component in schizophrenia. These results confirmed a complex association of the *COMT* locus to schizophrenia and suggested that other functional variants besides the Val108/158Met polymorphism are likely to be involved in susceptibility to schizophrenia (Shifman et al., 2003). In the impetus produced by this study a significant association was also found between bipolar disorder and the allele and haplotype in the *COMT* gene found to be associated with schizophrenia. Moreover, the relative risk, as for schizophrenia, was higher in women (Shifman et al., 2004).

In addition to these association studies, the role of *COMT* in schizophrenia and other neuropsychiatric diseases is further supported by functional genetic studies that have essentially focused on the Val108/158Met polymorphism. A large amount of experimental data suggest that heritable abnormalities of prefrontal dopamine function is a prominent feature of schizophrenia (Grace, 1991; Grace, 1993; Moore et al., 1999). *COMT* may constitute a major contributor to these abnormalities by virtue of its unique role in regulating DA-mediated prefrontal information processing, since COMT inhibitors can improve working memory in both rodents and humans (Weinberger et al., 2001). In this perspective, a study combining a genetic and a functional approach has shown that the Val allele of the Val108/158Met polymorphism that characterizes the high activity form of the *COMT* occurs at higher rates in both schizophrenics and their unaffected siblings. Moreover, patients and siblings bearing this allele performed poorly on the Wisconsin card sorting test (a neuropsychological test of frontal lobe function for working memory) and manifested inefficient brain activation as assessed by functional magnetic resonance imaging (fMRI) (Egan et al., 2001). Interestingly, amphetamine, a drug that increases DA-ergic neurotransmission, enhances the efficiency of prefrontal cortex function as assayed with fMRI during a working memory task in subjects with the high activity val/val genotype but not in subjects with the low activity met/met genotype (Mattay et al., 2003). Moreover, this polymorphism is also associated with personality traits, as assessed by the tridimensional personality questionnaire (Benjamin et al., 2000; Benjamin et al., 2000). Also, homozygosity for the

Met allele is associated, particularly in schizophrenic patients, with lower frontal P300 amplitudes which is an index of DA-ergic efficacy in reducing noise during information processing (Gallinat et al., 2003). In agreement with these findings and with the results of the association studies in Ashkenazi Jews (Shifman et al., 2004; Shifman et al., 2003), the analysis of the allele-specific expression using mRNA from human brains indicated that the haplotype implicated in schizophrenia and bipolar disorder is associated with lower expression of *COMT* mRNA (Bray et al., 2003).

These findings suggest that the *COMT* Val allele impairs prefrontal cognition and physiology and, by virtue of this effect, may condition some pathological features of schizophrenia, thus contributing, with other sequence variations at the *COMT* locus, to the increase of the risk for schizophrenia.

3. CONCLUSIONS

The hypothesis driven genetic studies on the implication in neuro-psychiatric diseases of the genes coding for the MA metabolizing enzymes have produced an impressive amount of data. Among contrasting results issued from genetic linkage or association studies using anonymous markers, a common trend has emerged related to the implication of functional polymorphisms in normal and pathological phenotypes. A composite approach that goes beyond mere genetic analysis has permitted to progress from identifying genetic variants in the MA metabolizing genes and their association with disease, to their potential impact on gene function *in vitro* and to gene expression and function in the human brain. Major improvement in this task has been achieved by the utilization of very large patient cohorts, a better definition of the phenotype investigated and the focalization of the recruitment in genetically homogeneous populations. Another fundamental improvement has been introduced by the evaluation of environmental variants in assessing the genetic components of pathological behavior as well as the coupling of genetic studies with neuroimaging techniques. The results obtained to date let us foresee that it will be possible in the near future to clearly identify several endophenotypes as measurable variants linking a genetic polymorphism to a simple biological trait. This, in turn, will help in to dissect more complex behavioral phenotypes and to elucidate the molecular bases underlying the quantitative genetic origin of complex diseases. In this context, molecular genetic studies on the genes of the MA metabolizing system may open new perspectives for understanding brain function in normal and pathological conditions.

4. AKNOWLEDGEMENTS

The authors wish to thank Susan Orsoni for useful comments in editing this manuscript.

5. REFERENCES

Abbar, M., Courtet, P., Amadeo, S., Caer, Y., Mallet, J., Baldy, M. M., Castelnau, D., & Malafosse, A., 1995, Suicidal behaviors and the tryptophan hydroxylase gene, *Arch Gen Psychiatry.* **52**: 846-849.

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., Bowers, M., van Dyck, C. H., Charney, D. S., Innis, R. B., & Laruelle, M., 1998, Increased Striatal Dopamine Transmission in Schizophrenia: Confirmation in a Second Cohort, *Am J Psychiatry.* **155**: 761-767.

Abi-Dargham, A., Rodenhiser, J., Printz, D., Zea-Ponce, Y., Gil, R., Kegeles, L. S., Weiss, R., Cooper, T. B., Mann, J. J., Van Heertum, R. L., Gorman, J. M., & Laruelle, M., 2000, Increased baseline occupancy of D2 receptors by dopamine in schizophrenia, *PNAS.* **97**: 8104-8109.

Albanese, V., Biguet, N. F., Kiefer, H., Bayard, E., Mallet, J., & Meloni, R., 2001, Quantitative effects on gene silencing by allelic variation at a tetranucleotide microsatellite, *Hum Mol Genet.* **10**: 1785-1792.

Arango, V., Huang, Y.-y., Underwood, M. D., & Mann, J. J., 2003, Genetics of the serotonergic system in suicidal behavior, *Journal of Psychiatric Research.* **37**: 375-386.

Bellivier, F., Leboyer, M., Courtet, P., Buresi, C., Beaufils, B., Samolyk, D., Allilaire, J. F., Feingold, J., Mallet, J., & Malafosse, A., 1998, Association between the tryptophan hydroxylase gene and manic- depressive illness, *Arch Gen Psychiatry.* **55**: 33-37.

Benjamin, J., Osher, Y., Kotler, M., Gritsenko, I., Nemanov, L., Belmaker, R. H., & Ebstein, R. P., 2000, Association between tridimensional personality questionnaire (TPQ) traits and three functional polymorphisms: Dopamine receptor D4 (DRD4), serotonin transporter promoter region (5-HTTLPR) and catechol O-methyltransferase (COMT), *Molecular Psychiatry.* **5**: 96-100.

Benjamin, J., Osher, Y., Lichtenberg, P., Bachner-Melman, R., Gritsenko, I., Kotler, M., Belmaker, R. H., Valsky, V., Drendel, M., & Ebstein, R. P., 2000, An interaction between the catechol O-methyltransferase and serotonin transporter promoter region polymorphisms contributes to tridimensional personality questionnaire persistence scores in normal subjects, *Neuropsychobiology.* **41**: 48-53.

Bertocci, B., Miggiano, V., Da Prada, M., Dembic, Z., Lahm, H. W., & Malherbe, P., 1991, Human catechol-O-methyltransferase: cloning and expression of the membrane-associated form, *PNAS.* **88**: 1416-1420.

Boehm, S., & Kubista, H., 2002, Fine Tuning of Sympathetic Transmitter Release via Ionotropic and Metabotropic Presynaptic Receptors, *Pharmacol Rev.* **54**: 43-99.

Borglum, A., Bruun, T., Kjeldsen, T., Ewald, H., Mors, O., Kirov, G., Russ, C., Freeman, B., Collier, D., & Kruse, T., 1999, Two novel variants in the DOPA decarboxylase gene: association with bipolar affective disorder, *Mol Psychiatry.* **4**: 545-551.

Borglum, A., Hampson, M., Kjeldsen, T., Muir, W., Murray, V., Ewald, H., Mors, O., Blackwood, D., & Kruse, T., 2001, Dopa decarboxylase genotypes may influence age at onset of schizophrenia, *Mol Psychiatry.* **6**: 712-717.

Borglum, A., Kirov, G., Craddock, N., Mors, O., Muir, W., Murray, V., McKee, I., Collier, D., Ewald, H., Owen, M., Blackwood, D., & Kruse, T., 2003, Possible parent-of-origin effect of Dopa decarboxylase in susceptibility to bipolar affective disorder, *Am J Med Genet.* **117B**: 18-22.

Boularand, S., Darmon, M. C., Ganem, Y., Launay, J. M., & Mallet, J., 1990, Complete coding sequence of human tryptophan hydroxylase, *Nucleic Acids Res.* **18**: 4257.

Bray, N., Buckland, P., Williams, N., Williams, H., Norton, N., Owen, M., & O'Donovan, M., 2003, A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain, *Am J Hum Genet.* **73**: 152-161.

Brinkmann, B., Sajantila, A., Goedde, H., Matsumoto, H., Nishi, K., & Wiegand, P., 1996, Population genetic comparisons among eight populations using allele frequency and sequence data from three microsatellite loci, *Eur J Hum Genet.* **4**: 175-182.

Brunner, H. G., Nelen, M., Breakfield, X. O., Ropers, H. H., & Van Oost, B., 1993, Abnormal Behavior Associated with a Point Mutation in the Structural Gene for Monoamine Oxidase A, *Science.* **262**: 578-580.

Burgert, E., Crocq, M. A., Bausch, E., Macher, J. P., & Morris-Rosendahl, D. J., 1998, No association between the tyrosine hydroxylase microsatellite marker HUMTH01 and schizophrenia or bipolar I disorder, *Psychiatr Genet.* **8**: 45-48.

Byerley, W., Plaetke, R., Hoff, M., Jensen, S., Holik, J., Reimherr, F., Mellon, C., Wender, P., O'Connell, P., & Leppert, M., 1992, Tyrosine hydroxylase gene not linked to manic-depression in seven of eight pedigrees, *Hum Hered.* **42**: 259-263.

Byerley, W., Plaetke, R., Hoff, M., Jensen, S., Leppert, M., Holik, J., Reimherr, F., Wender, P., Waldo, M., Myles-Worsley, M., Freedman, R., & O'Connell, P., 1993, Tyrosine hydroxylase gene not linked to schizophrenia in nine pedigrees, *Psychiatric Genet.* **3**: 29-31.

Carlsson, A., 1988, The current status of the dopamine hypothesis of schizophrenia, *Neuropsychopharmacology.* **1**: 179-186.

Cases, O., Self, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J. C., & De Maeyer, E., 1995, Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA, *Science.* **268**: 1763-1766.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., Taylor, A., & Poulton, R., 2002, Role of Genotype in the Cycle of Violence in Maltreated Children, *Science.* **297**: 851-854.

Cavazzoni, P., Alda, M., Turecki, G., Rouleau, G., Grof, E., Martin, R., Duffy, A., & Grof, P., 1996, Lithium-responsive affective disorders: no association with the tyrosine hydroxylase gene, *Psychiatry Res.* **64**: 91-96.

Chen, C. H., Lee, Y. R., Wei, F. C., Koong, F. J., Hwu, H. G., & Hsiao, K. J., 1997, Association study of NlaIII and MspI genetic polymorphisms of catechol-O-methyltransferase gene and susceptibility to schizophrenia, *Biological Psychiatry.* **41**: 985-987.

Chiba, M., Suzuki, S., Hinokio, Y., Hirai, M., Satoh, Y., Tashiro, A., Utsumi, A., Awata, T., Hongo, M., & Toyota, T., 2000, Tyrosine hydroxylase gene microsatellite polymorphism associated with insulin resistance in depressive disorder, *Metabolism.* **49**: 1145-1149.

Cote, F., Thevenot, E., Fligny, C., Fromes, Y., Darmon, M., Ripoche, M.-A., Bayard, E., Hanoun, N., Saurini, F., Lechat, P., Dandolo, L., Hamon, M., Mallet, J., & Vodjdani, G., 2003, Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function, *PNAS.* **100**: 13525-13530.

Craddock, N., Spurlock, G., McGuffin, P., Owen, M. J., Nosten-Bertrand, M., Bellivier, F., Meloni, R., Leboyer, M., Mallet, J., Mynett-Johnson, L., Murphy, V., McKeon, P., Kirov, G., Powell, J., Kunugi, H., Collier, D., Larosa, M., Nacmias, B., Sorbi, S., Schwab, S., Ackenheil, M., & Maier, W., 1997, No association between bipolar disorder and alleles at a functional polymorphism in the COMT gene, *British Journal of Psychiatry.* **170**: 526-528.

Craig, S. P., Boularand, S., Darmon, M. C., Mallet, J., & Craig, I. W., 1991, Localization of human tryptophan hydroxylase (TPH) to chromosome 11p15.3-p14 by in situ hybridization, *Cytogenet Cell Genet.* **56**: 157-159.

Craig, S. P., Buckle, V. J., Lamouroux, A., Mallet, J., & Craig, I. W., 1988, Localization of the human dopamine beta hydroxylase (DBH) gene to chromosome 9q34, *Cytogenet Cell Genet.* **48**: 48-50.

Cubells, J. F., Kobayashi, K., Nagatsu, T., Kidd, K. K., Kidd, J. R., Calafell, F., Kranzler, H. R., Ichinose, H., & Gelernter, J., 1997, Population genetics of a functional variant of the dopamine beta-hydroxylase gene (DBH), *American Journal Of Medical Genetics.* **74**: 374-379.

Cubells, J. F., Kranzler, H. R., McCance-Katz, E., Anderson, G. M., Malison, R. T., Price, L. H., & Gelernter, J., 2000, A haplotype at the DBH locus, associated with low plasma dopamine beta-hydroxylase activity, also associates with cocaine-induced paranoia, *Molecular Psychiatry.* **5**: 56-63.

Cubells, J. F., Price, L. H., Meyers, B. S., Anderson, G. M., Zabetian, C. P., Alexopoulos, G. S., Nelson, J. C., Sanacora, G., Kirwin, P., & Carpenter, L., 2002, Genotype-controlled analysis of plasma dopamine [beta]-hydroxylase activity in psychotic unipolar major depression, *Biological Psychiatry.* **51**: 358-364.

Cubells, J. F., van Kammen, D. P., Kelley, M. E., Anderson, G. M., O'Connor, D. T., Price, L. H., Malison, R., Rao, P. A., Kobayashi, K., Nagatsu, T., & Gelernter, J., 1998, Dopamine beta-hydroxylase: two polymorphisms in linkage disequilibrium at the structural gene DBH associate with biochemical phenotypic variation, *Human Genetics.* **102**: 533-540.

Daly, G., Hawi, Z., Fitzgerald, M., & Gill, M., 1999, Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children, *Mol Psychiatry.* **4**: 192-196.

Daniels, J. K., Williams, N. M., Williams, J., Jones, L. A., Cardno, A. G., Murphy, K. C., Spurlock, G., Riley, B., Scambler, P., Asherson, P., McGuffin, P., & Owen, M. J., 1996, No evidence for allelic association between schizophrenia and a polymorphism determining high or low catechol O-methyltransferase activity, *American Journal of Psychiatry.* **153**: 268-270.

de Chaldee, M., Laurent, C., Thibaut, F., Martinez, M., Samolyk, D., Petit, M., Campion, D., & Mallet, J., 1999, Linkage disequilibrium on the COMT gene in French schizophrenics and controls, *Am J Med Genet.* **88**: 452-457.

De Luca, V., Mueller, D. J., Tharmalingam, S., King, N., & Kennedy, J. L., 2004, Analysis of the novel TPH2 gene in bipolar disorder and suicidality, *Molecular Psychiatry.* **9**: 896-897.

Deckert, J., Catalano, M., Syagailo, Y., Bosi, M., Okladnova, O., Di Bella, D., Nothen, M., Maffei, P., Franke, P., Fritze, J., Maier, W., Propping, P., Beckmann, H., Bellodi, L., & Lesch, K., 1999, Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder, *Hum. Mol. Genet.* **8**: 621-624.

Deinum, J., Steenbergen-Spanjers, G. C. H., Jansen, M., Boomsma, F., Lenders, J. W. M., van Ittersum, F. J., Huck, N., van den Heuvel, L. P., & Wevers, R. A., 2004, DBH gene variants that cause low plasma dopamine {beta} hydroxylase with or without a severe orthostatic syndrome, *J Med Genet.* **41**: e38-.

Detera-Wadleigh, S., Berrettini, W., Goldin, L., Boorman, D., Anderson, S., & Gershon, E., 1987, Close linkage of c-Harvey-ras-1 and the insulin gene to affective disorder is ruled out in three North American pedigrees, *Nature.* **325**: 806-808.

Dreher, J.-C., & Burnod, Y., 2002, An integrative theory of the phasic and tonic modes of dopamine modulation in the prefrontal cortex, *Neural Networks.* **15**: 583-602.

Egan, M. F., Goldberg, T. E., Kolachana, B. S., Callicott, J. H., Mazzanti, C. M., Straub, R. E., Goldman, D., & Weinberger, D. R., 2001, Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia, *PNAS.* **98**: 6917-6922.

Egeland, J. A., Gerhard, D. S., Pauls, D. L., Sussex, J. N., Kidd, K. K., Allen, C. R., Hostetter, A. M., & Housman, D. E., 1987, Bipolar affective disorders linked to DNA markers on chromosome 11, *Nature.* **325**: 783-787.

Erickson, J. D., Schafer, M. K.-H., Bonner, T. I., Eiden, L. E., & Weihe, E., 1996, Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter, *PNAS.* **93**: 5166-5171.

Ewald, H., Mors, O., Eiberg, H., Flint, T., & Kruse, T. A., 1995, No evidence of linkage between manic depressive illness and the dopa decarboxylase gene or nearby region on chromosome 7p, *Psychiatr Genet.* **5**: 161-169.

Fan, J.-B., Yang, M.-S., Tang, J.-X., He, L., Xing, Y.-L., Shi, J.-G., Zhao, S.-M., Zhu, S.-M., Ji, L.-P., Gu, N.-F., Feng, G.-Y., & St Clair, D., 2004, Family-based association study of the functional monoamine oxidase A gene promoter polymorphism and schizophrenia, *Schizophrenia Research.* **67**: 107-109.

Foley, D. L., Eaves, L. J., Wormley, B., Silberg, J. L., Maes, H. H., Kuhn, J., & Riley, B., 2004, Childhood adversity, Monoamine Oxidase A genotype, and risk for conduct disorder, *Archives of General Psychiatry.* **61**: 738-744.

Furlong, R., Ho, L., Walsh, C., Rubinsztein, J., Jain, S., Paykel, E., Easton, D., & Rubinsztein, D., 1998, Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders, *Am J Med Genet.* **81**: 58-63.

Furlong, R. A., Ho, L., Rubinsztein, J. S., Walsh, C., Paykel, E. S., & Rubinsztein, D. C., 1999, Analysis of the monoamine oxidase A (MAOA) gene in bipolar affective disorder by association studies, meta-analyses, and sequencing of the promoter, *American Journal of Medical Genetics - Neuropsychiatric Genetics.* **88**: 398-406.

Furlong, R. A., Rubinsztein, J. S., Ho, L., Walsh, C., Coleman, T. A., Muir, W. J., Paykel, E. S., Blackwood, D. H., & Rubinsztein, D. C., 1999, Analysis and metaanalysis of two polymorphisms within the tyrosine hydroxylase gene in bipolar and unipolar affective disorders, *Am J Med Genet.* **88**: 88-94.

Gainetdinov, R. R., & Caron, M. G., 2003, Monoamine transporters: from genes to behavior, *Annual Review of Pharmacology and Toxicology.* **43**: 261-284.

Gallinat, J., Bajbouj, M., Sander, T., Schlattmann, P., Xu, K., Ferro, E. F., Goldman, D., & Winterer, G., 2003, Association of the G1947A COMT (Val108/158Met) gene polymorphism with prefrontal P300 during information processing, *Biological Psychiatry.* **54**: 40-48.

Garland, E. M., Hahn, M. K., Ketch, T. P., Keller, N. R., Kim, C.-H., Kim, K.-S., Biaggioni, I., Shannon, J. R., Blakely, R. D., & Robertson, D., 2002, Genetic Basis of Clinical Catecholamine Disorders, *Ann NY Acad Sci.* **971**: 506-514.

Gelernter, J., Kranzler, H., & Lacobelle, J., 1998, Population studies of polymorphisms at loci of neuropsychiatric interest (tryptophan hydroxylase (TPH), dopamine transporter protein (SLC6A3), D3 dopamine receptor (DRD3), apolipoprotein E (APOE), mu opioid receptor (OPRM1), and ciliary neurotrophic factor (CNTF)), *Genomics.* **52**: 289-297.

Geller, B., & CookJr., E. H., 2000, Ultradian rapid cycling in prepubertal and early adolescent bipolarity is not in transmission disequilibrium with val/met COMT alleles, *Biological Psychiatry.* **47**: 605-609.

Gerhard, D., LaBuda, M., Bland, S., Allen, C., Egeland, J., & Pauls, D., 1994, Initial report of a genome search for the affective disorder predisposition gene in the old order Amish pedigrees: chromosomes 1 and 11, *Am J Med Genet.* **54**: 398-404.

Gershon, E., Goldin, L., Badner, J., & Berrettini, W., 1996, Detection of linkage to affective disorders in the catalogued Amish pedigrees, *Am J Hum Genet.* **58**: 1381-1385.

Gill, M., Castle, D., Hunt, N., Clements, A., Sham, P., & Murray, R. M., 1991, Tyrosine hydroxylase polymorphisms and bipolar affective disorder, *J Psychiatr Res.* **25**: 179-184.

Ginns, E., Egeland, J., Allen, C., Pauls, D., Falls, K., Keith, T., & Paul, S., 1992, Update on the search for DNA markers linked to manic-depressive illness in the Old Order Amish, *J Psychiatr Res.* **26**: 305-308.

Ginns, E., Ott, J., Egeland, J., Allen, C., Fann, C., Pauls, D., Weissenbachoff, J., Carulli, J., Falls, K., Keith, T., & Paul, S., 1996, A genome-wide search for chromosomal loci linked to bipolar affective disorder in the Old Order Amish, *Nat Genet.* **12**: 431-435.

Goldman, D., 1995, Candidate genes in alcoholism, *Clin Neurosci.* **3**: 174-181.

Grace, A. A., 1991, Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia, *Neuroscience.* **41**: 1-24.

Grace, A. A., 1993, Cortical regulation of subcortical dopamine systems and its possible relevance to schizophrenia, *J Neural Transm Gen Sect.* **91**: 111-134.

Gross, C., & Hen, R., 2004, The developmental origins of anxiety, *Nat Rev Neurosci.* **5**: 545-552.

Grossman, M. H., Emanuel, B. S., & Budarf, M. L., 1992, Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1->q11.2, *Genomics.* **12**: 822-825.

Gurling, H., Smyth, C., Kalsi, G., Moloney, E., Rifkin, L., O'Neill, J., Murphy, P., Curtis, D., Petursson, H., & Brynjolfsson, J., 1995, Linkage findings in bipolar disorder, *Nat Genet.* **10**: 8-9.

Gutierrez, B., Bertranpetit, J., Guillamat, R., Valles, V., Arranz, M. J., Kerwin, R., & Fananas, L., 1997, Association analysis of the catechol O-methyltransferase gene and bipolar affective disorder, *American Journal of Psychiatry.* **154**: 113-115.

Hamilton, S., Slager, S., Heiman, G., Haghighi, F., Klein, D., Hodge, S., Weissman, M., Fyer, A., & Knowles, J., 2000, No genetic linkage or association between a functional promoter polymorphism in the monoamine oxidase-A gene and panic disorder, *Mol Psychiatry.* **5**: 465-456.

Han, L., Nielsen, D. A., Rosenthal, N. E., Jefferson, K., Kaye, W., Murphy, D., Altemus, M., Humphries, J., Cassano, G., Rotondo, A., Virkkunen, M., Linnoila, M., & Goldman, D., 1999, No coding variant of the tryptophan hydroxylase gene detected in seasonal affective disorder, obsessive-compulsive disorder, anorexia nervosa, and alcoholism, *Biol Psychiatry.* **45**: 615-619.

Hawi, Z., Foley, D., Kirley, A., McCarron, M., Fitzgerald, M., & Gill, M., 2001, Dopa decarboxylase gene polymorphisms and attention deficit hyperactivity disorder (ADHD): no evidence for association in the Irish population, *Mol Psychiatry.* **6**: 420-424.

Hawi, Z., Lowe, N., Kirley, A., Gruenhage, F., Nothen, M., Greenwood, T., Kelsoe, J., Fitzgerald, M., & Gill, M., 2003, Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci, *Mol Psychiatry.* **8**: 299-308.

Herault, J., Martineau, J., Petit, E., Perrot, A., Sauvage, D., Barthelemy, C., Mallet, J., Muh, J. P., & Lelord, G., 1994, Genetic markers in autism: association study on short arm of chromosome 11, *J Autism Dev Disord.* **24**: 233-236.

Herault, J., Perrot, A., Barthelemy, C., Buchler, M., Cherpi, C., Leboyer, M., Sauvage, D., Lelord, G., Mallet, J., & Muh, J. P., 1993, Possible association of c-Harvey-Ras-1 (HRAS-1) marker with autism, *Psychiatry Res.* **46**: 261-267.

Hoda, F., Nicholl, D., Bennett, P., Arranz, M., Aitchison, K. J., Al-Chalabi, A., Kunugi, H., Vallada, H., Leigh, P. N., Chaudhuri, K. R., & Collier, D. A., 1996, No Association between Parkinson's Disease and Low-Activity Alleles of CatecholO-Methyltransferase, *Biochemical and Biophysical Research Communications.* **228**: 780-784.

Hodgkinson, S., Sherrington, R., Gurling, H., Marchbanks, R., Reeders, S., Mallet, J., McInnis, M., Petursson, H., & Brynjolfsson, J., 1987, Molecular genetic evidence for heterogeneity in manic depression, *Nature.* **325**: 805-806.

Houhou, L., Lamouroux, A., Faucon Biguet, N., & Mallet, J., 1995, Expression of Human Dopamine-Beta-Hydroxylase in Mammalian Cells Infected by Recombinant Vaccinia Virus, *J. Biol. Chem.* **270**: 12601-12606.

Huang, Y., Cate, S., Battistuzzi, C., Oquendo, M., Brent, D., & Mann, J., 2004, An association between a functional polymorphism in the monoamine oxidase a gene promoter, impulsive traits and early abuse experiences, *Neuropsychopharmacology.* **29**: 1498-1505.

Ibanez, A., Perez de Castro, I., Fernandez-Piqueras, J., Blanco, C., & Saiz-Ruiz, J., 2000, Pathological gambling and DNA polymorphic markers at MAO-A and MAO-B genes, *Molecular Psychiatry.* **5**: 105-109.

Icard Liepkalns, C., Faucon Biguet, N., Vyas, S., Robert, J. J., Sassone Corsi, P., & Mallet, J., 1992, AP-1 complex and c-fos transcription are involved in TPA provoked and trans-synaptic inductions of the tyrosine hydroxylase gene: insights into long-term regulatory mechanisms, *J Neurosci Res.* **32**: 290-298.

Inayama, Y., Yoneda, H., Sakai, T., Ishida, T., Kobayashi, S., Nonomura, Y., Kono, Y., Koh, J., & Asaba, H., 1993, Lack of association between bipolar affective disorder and tyrosine hydroxylase DNA marker, *Am J Med Genet.* **48**: 87-89.

Ishii, A., Kobayashi, K., Kiuchi, K., & Nagatsu, T., 1991, Expression of two forms of human dopamine-beta-hydroxylase in COS cells, *Neurosci Lett.* **125**: 25-28.

Jahnes, E., Muller, D., Schulze, T., Windemuth, C., Cichon, S., Ohlraun, S., Fangerau, H., Held, T., Maier, W., Propping, P., Nothen, M., & Rietschel, M., 2002, Association study between two variants in the DOPA decarboxylase gene in bipolar and unipolar affective disorder, *Am J Med Genet.* **114**: 519-522.

Jonsson, E., Sedvall, G., Brene, S., Gustavsson, J. P., Geijer, T., Terenius, L., Crocq, M.-A., Lannfelt, L., Tylec, A., & Sokoloff, P., 1996, Dopamine-Related Genes and Their Relationships to Monoamine Metabolites in CSF, *Biological Psychiatry.* **40**: 1032-1043.

Jönsson, E. G., Bah, J., Melke, J., Jamra, R. A., Schumacher, J., Westberg, L., Ivo, R., Cichon, S., Propping, P., Nöthen, M. M., Eriksson, E., & Sedvall, G. C., 2004, Monoamine related functional gene variants and relationships to monoamine metabolite concentrations in CSF of healthy volunteers, *BMC Psychiatry.* **4**. http://www.biomedcentral.com/1471-244X/4/4

Jonsson, E. G., Geijer, T., Gyllander, A., Terenius, L., & Sedvall, G. C., 1998, Failure to replicate an association between a rare allele of a tyrosine hydroxylase gene microsatellite and schizophrenia, *Eur Arch Psychiatry Clin Neurosci.* **248**: 61-63.

Jonsson, E. G., Jamra, R. A., Schumacher, J., Flyckt, L., Edman, G., Forslund, K., Mattila-Evenden, M., Rylander, G., Asberg, M., Bjerkenstedt, L., Wiesel, F.-A., Propping, P., Cichon, S., Nothen, M. M., & Sedvall, G. C., 2003, No association between a putative functional promoter variant in the dopamine [beta]-hydroxylase gene and schizophrenia, *Psychiatric Genetics.* **13**: 175-178.

Jonsson, E. G., Norton, N., Forslund, K., Mattila-Evenden, M., Rylander, G., Asberg, M., Owen, M. J., & Sedvall, G. C., 2003, Association between a promoter variant in the monoamine oxidase A gene and schizophrenia, *Schizophrenia Research.* **61**: 31-37.

Jonsson, E. G., Norton, N., Gustavsson, J. P., Oreland, L., Owen, M. J., & Sedvall, G. C., 2000, A promoter polymorphism in the monoamine oxidase A gene and its relationships to monoamine metabolite concentrations in CSF of healthy volunteers, *Journal of Psychiatric Research.* **34**: 239-244.

Jorm, A., Henderson, A., Jacomb, P., Christensen, H., Korten, A., Rodgers, B., Tan, X., & Easteal, S., 2000, Association of a functional polymorphism of the monoamine oxidase A gene promoter with personality and psychiatric symptoms, *Psychiatr Genet.* **10**: 87-90.

Karayiorgou, M., Altemus, M., Galke, B. L., Goldman, D., Murphy, D. L., Ott, J., & Gogos, J. A., 1997, Genotype determining low catechol-O-methyltransferase activity as a risk factor for obsessive-compulsive disorder, *PNAS.* **94**: 4572-4575.

Karayiorgou, M., & Gogos, J., 1997, A turning point in schizophrenia genetics, *Neuron.* **19**: 967-979.

Karayiorgou, M., Gogos, J. A., Galke, B. L., Wolyniec, P. S., Nestadt, G., Antonarakis, S. E., Kazazian, H. H., Housman, D. E., & Pulver, A. E., 1998, Identification of sequence variants and analysis of the role of the Catechol-O-Methyl-Transferase gene in schizophrenia susceptibility, *Biological Psychiatry.* **43**: 425-431.

Karayiorgou, M., Morris, M. A., Morrow, B., Shprintzen, R. J., Goldberg, R., Borrow, J., Gos, A., Nestadt, G., Wolyniec, P. S., Lasseter, V. K., Eisen, H., Childs, B., Kazanian, H. H., Kucherlapati, R., Antonarakis, S. E., Pulver, A. E., & Housman, D. E., 1995, Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11, *PNAS.* **92**: 7612-7616.

Kawada, Y., Hattori, M., Fukuda, R., Arai, H., Inoue, R., & Nanko, S., 1995, No evidence of linkage or association between tyrosine hydroxylase gene and affective disorder, *J Affect Disord.* **34**: 89-94.

Kelsoe, J., Ginns, E., Egeland, J., Gerhard, D., Goldstein, A., Bale, S., Pauls, D., Long, R., Kidd, K., Conte, G., Housman, D., & Paul, S., 1989, Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish, *Nature.* **342**: 238-243.

Kirov, G., Norton, N., Jones, I., McCandless, F., Craddock, N., & Owen, M., 1999, A functional polymorphism in the promoter of monoamine oxidase A gene and bipolar affective disorder, *Int J Neuropsychopharmcol.* **2**: 293-298.

Kohnke, M. D., Zabetian, C. P., Anderson, G. M., Kolb, W., Gaertner, I., Buchkremer, G., Vonthein, R., Schick, S., Lutz, U., Kohnke, A. M., & Cubells, J. F., 2002, A genotype-controlled analysis of plasma dopamine [beta]-hydroxylase in healthy and alcoholic subjects: evidence for alcohol-related differences in noradrenergic function, *Biological Psychiatry.* **52**: 1151-1158.

Koob, G. F., & Nestler, E. J., 1997, The neurobiology of drug addiction, *J Neuropsychiatry Clin Neurosci.* **9**: 482-497.

Korner, J., Fritze, J., & Propping, P., 1990, RFLP alleles at the tyrosine hydroxylase locus: no association found to affective disorders, *Psychiatry Res.* **32**: 275-280.

Korner, J., Rietschel, M., Hunt, N., Castle, D., Gill, M., Nothen, M. M., Craddock, N., Daniels, J., Owen, M., Fimmers, R., Fritze, J., Möller, H.-J., & Propping, P., 1994, Association and haplotype analysis at the tyrosine hydroxylase locus in a combined German-British sample of manic depressive patients and controls., *Psychiatr Genet.* **4**: 167-175.

Kunugi, H., Ishida, S., Kato, T., Sakai, T., Tatsumi, M., Hirose, T., & Nanko, S., 1999, No evidence for an association of polymorphisms of the tryptophan hydroxylase gene with affective disorders or attempted suicide among Japanese patients, *Am J Psychiatry.* **156**: 774-776.

Kunugi, H., Ishida, S., Kato, T., Tatsumi, M., Sakai, T., Hattori, M., Hirose, T., & Nanko, S., 1999, A functional polymorphism in the promoter region of monoamine oxidase-A gene and mood disorders, *Mol Psychiatry.* **4**: 393-395.

Kunugi, H., Nanko, S., Ueki, A., Otsuka, E., Hattori, M., Hoda, F., Vallada, H. P., Arranz, M. J., & Collier, D. A., 1997, High and low activity alleles of catechol-O-methyltransferase gene: ethnic difference and possible association with Parkinson's disease, *Neuroscience Letters.* **221**: 202-204.

Kunugi, H., Vallada, H. P., Hoda, F., Kirov, G., Gill, M., Aitchison, K. J., Ball, D., Arranz, M. J., Murray, R. M., & Collier, D. A., 1997, No Evidence for an Association of Affective Disorders with High- or Low-Activity Allele of Catechol-o-Methyltransferase Gene, *Biological Psychiatry.* **42**: 282-285.

Kurumaji, A., Kuroda, T., Yamada, K., Yoshikawa, T., & Toru, M., 2001, An association of the polymorphic repeat of tetranucleotide (TCAT) in the first intron of the human tyrosine hydroxylase gene with schizophrenia in a Japanese sample, *J Neural Transm.* **108**: 489-495.

Lachman, H. M., Kelsoe, J., Moreno, L., Katz, S., & Papolos, D. F., 1997, Lack of association of catechol-O-methyltransferase (COMT) functional polymorphism in bipolar affective disorder, *Psychiatric Genetics.* **7**: 13-17.

Lachman, H. M., Morrow, B., Shprintzen, R., Veit, S., Parsia, S. S., Faedda, G., Goldberg, R., Kucherlapati, R., & Papolos, D. F., 1996, Association of codon 108/158 catechol-O-

methyltransferase gene polymorphism with the psychiatric manifestations of velo-cardio-facial syndrome, *American Journal Of Medical Genetics.* **67**: 468-472.

Lachman, H. M., Nolan, K. A., Mohr, P., Saito, T., & Volavka, J., 1998, Association between catechol O-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder, *American Journal of Psychiatry.* **155**: 835-837.

Lachman, H. M., Papolos, D. F., Saito, T., Yu, Y.-M., Szumlanski, C. L., & Weinshilboum, R. M., 1996, Human catechol-O-methyltransferase pharmacogenetics: Description of a functional polymorphism and its potential application to neuropsychiatric disorders, *Pharmacogenetics.* **6**: 243-250.

Lan, N. C., Heinzmann, C., Gal, A., Klisak, I., Orth, U., Lai, E., Grimsby, J., Sparkes, R. S., Mohandas, T., & Shih, J., 1989, Human monoamine oxidase A and B genes map to Xp 11.23 and are deleted in a patient with Norrie disease, *Genomics.* **4**: 552-559.

Lauritsen, M., Borglum, A., Betancur, C., Philippe, A., Kruse, T., Leboyer, M., & Ewald, H., 2002, Investigation of two variants in the DOPA decarboxylase gene in patients with autism, *Am J Med Genet.* **114**: 466-470.

Leboyer, M., Malafosse, A., Boularand, S., Campion, D., Gheysen, F., Samolyk, D., Henriksson, B., Denise, E., des, L. A., & Lepine, J. P., 1990, Tyrosine hydroxylase polymorphisms associated with manic-depressive illness, *Lancet.* **335**: 1219.

Li, T., Vallada, H., Curtis, D., Arranz, M., Xu, K., Cai, G., Deng, H., Liu, J., Murray, R., Liu, X., & Collier, D. A., 1997, Catechol-O-methyltransferase Val158Met polymorphism: Frequency analysis in Han Chinese subjects and allelic association of the low activity allele with bipolar affective disorder, *Pharmacogenetics.* **7**: 349-353.

Lim, L. C., Gurling, H., Curtis, D., Brynjolfsson, J., Petursson, H., & Gill, M., 1993, Linkage between tyrosine hydroxylase gene and affective disorder cannot be excluded in two of six pedigrees, *Am J Med Genet.* **48**: 223-228.

Lobos, E. A., & Todd, R. D., 1997, Cladistic analysis of disease association with tyrosine hydroxylase: application to manic-depressive disease and alcoholism, *Am J Med Genet.* **74**: 289-295.

Lohmueller, K. E., Pearce, C. L., Pike, M., Lander, E. S., & Hirschhorn, J. N., 2003, Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease, *Nature genetics.* **33**: 177-182.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., & Taskinen, J., 1995, Kinetics of human soluble and membrane-bound catechol O- methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme, *Biochemistry.* **34**: 4202-4210.

Lundstrom, K., Tenhunen, J., Tilgmann, C., Karhunen, T., Panula, P., & Ulmanen, I., 1995, Cloning, expression and structure of catechol-O-methyltransferase, *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology.* **1251**: 1-10.

Malafosse, A., Leboyer, M., d'Amato, T., Amadeo, S., Abbar, M., Campion, D., Canseil, O., Castelnau, D., Gheysen, F., Granger, B., Henrikson, B., Poirier, M. F., Sabate, O., Samolyk, D., Feingold, J., & Mallet, J., 1997, Manic depressive illness and tyrosine hydroxylase gene: linkage heterogeneity and association, *Neurobiol Dis.* **4**: 337-349.

Mallet, J., 1996, The TiPS/TINS lecture. Catecholamines: from gene regulation to neuropsychiatric disorders, *Trends Pharmacol Sci.* **17**: 129-135.

Mann, J. J., Malone, K. M., Nielsen, D. A., Goldman, D., Erdos, J., & Gelernter, J., 1997, Possible association of a polymorphism of the tryptophan hydroxylase gene with suicidal behavior in depressed patients, *Am J Psychiatry.* **154**: 1451-1453.

Manuck, S. B., Flory, J. D., Ferrell, R. E., Dent, K. M., Mann, J. J., & Muldoon, M. F., 1999, Aggression and anger-related traits associated with a polymorphism of the tryptophan hydroxylase gene, *Biol Psychiatry.* **45**: 603-614.

Masson, J., Sagne, C., Hamon, M., & El Mestikawy, S., 1999, Neurotransmitter transporters in the central nervous system, *Pharmacological Reviews.* **51**: 439-464.

Mattay, V. S., Goldberg, T. E., Fera, F., Hariri, A. R., Tessitore, A., Egan, M. F., Kolachana, B., Callicott, J. H., & Weinberger, D. R., 2003, Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine, *PNAS.* **100**: 6186-6191.

McQuillin, A., Lawrence, J., Kalsi, G., Chen, A., Gurling, H., & Curtis, D., 1999, No allelic association between bipolar affective disorder and the tryptophan hydroxylase gene, *Arch Gen Psychiatry.* **56**: 99-101.

Melichar, J. K., Daglish, M. R., & Nutt, D. J., 2001, Addiction and withdrawal – current views, *Curr Opin Pharmacol.* **1**: 84-90.

Meloni, R., Albanese, V., Ravassard, P., Treilhou, F., & Mallet, J., 1998, A tetranucleotide polymorphic microsatellite, located in the first intron of the tyrosine hydroxylase gene, acts as a transcription regulatory element in vitro, *Hum Mol Genet.* **7**: 423-428.

Meloni, R., Laurent, C., Campion, D., Ben Hadjali, B., Thibaut, F., Dollfus, S., Petit, M., Samolyk, D., Martinez, M., Poirier, M. F., & Mallet, J., 1995b, A rare allele of a microsatellite located in the tyrosine hydroxylase gene found in schizophrenic patients, *C R Acad Sci III.* **318**: 803-809.

Meloni, R., Leboyer, M., Bellivier, F., Barbe, B., Samolyk, D., Allilaire, J. F., & Mallet, J., 1995a, Association of manic-depressive illness with tyrosine hydroxylase microsatellite marker, *Lancet.* **345**: 932.

Meyer, E., Wiegand, P., Rand, S. P., Kuhlmann, D., Brack, M., & Brinkmann, B., 1995, Microsatellite polymorphisms reveal phylogenetic relationships in primates, *J Mol Evol.* **41**: 10-14.

Moore, H., West, A. R., & Grace, A. A., 1999, The regulation of forebrain dopamine transmission: relevance to the pathophysiology and psychopathology of schizophrenia, *Biological Psychiatry.* **46**: 40-55.

Müller Smith, K., Daly, M., Fischer, M., Yiannoutsos, C. T., Bauer, L., Barkley, R., & Navia, B. A., 2003, Association of the dopamine beta hydroxylase gene with attention deficit hyperactivity disorder: Genetic analysis of the Milwaukee longitudinal study, *Am J Med Genet.* **119B**: 77-85.

Mynett-Johnson, L. A., Murphy, V. E., Claffey, E., Shields, D. C., & McKeon, P., 1998, Preliminary evidence of an association between bipolar disorder in females and the catechol-O-methyltransferase gene, *Psychiatric Genetics.* **8**: 221-225.

Nagatsu, T., 2004, Progress in Monoamine Oxidase (MAO) research in relation to genetic engineering, *NeuroToxicology.* **25**: 11-20.

Nielsen, D. A., Goldman, D., Virkkunen, M., Tokola, R., Rawlings, R., & Linnoila, M., 1994, Suicidality and 5-hydroxyindoleacetic acid concentration associated with a tryptophan hydroxylase polymorphism, *Arch Gen Psychiatry.* **51**: 34-38.

Nielsen, D. A., Virkkunen, M., Lappalainen, J., Eggert, M., Brown, G. L., Long, J. C., Goldman, D., & Linnoila, M., 1998, A tryptophan hydroxylase gene marker for suicidality and alcoholism, *Arch Gen Psychiatry.* **55**: 593-602.

Ohara, K., Nagai, M., Suzuki, Y., & Ohara, K., 1998, Low activity allele of catechol-o-methyltransferase gene and Japanese unipolar depression, *NeuroReport.* **9**: 1305-1308.

Ohmori, O., Shinkai, T., Kojima, H., Terao, T., Suzuki, T., Mita, T., & Abe, K., 1998, Association study of a functional catechol-O-methyltransferase gene polymorphism in Japanese schizophrenics, *Neuroscience Letters.* **243**: 109-112.

Pakstis, A., Kidd, J., Castiglione, C., & Kidd, K., 1991, Status of the search for a major genetic locus for affective disorder in the Old Order Amish, *Hum Genet.* **87**: 475-483.

Palmatier, M. A., Kang, A. M., & Kidd, K. K., 1999, Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles, *Biological Psychiatry.* **46**: 557-567.

Patel, P. D., Pontrello, C., & Burke, S., 2004, Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland, *Biological Psychiatry.* **55**: 428-433.

Perez de Castro, I., Santos, J., Torres, P., Visedo, G., Saiz-Ruiz, J., Llinares, C., & Fernandez-Piqueras, J., 1995, A weak association between TH and DRD2 genes and bipolar affective disorder in a Spanish sample, *J Med Genet.* **32**: 131-134.

Polymeropoulos, M., Xiao, H., Rath, D., & Merril, C., 1991, Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH), *Nucleic Acids Res.* **19**: 3753.

Puers, C., Hammond, H. A., Jin, L., Caskey, T., & Schumm, J. W., 1993, Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01 $(AATG)_n$ and reassignment of alleles in population analysis by using a locus-specific allelic ladder, *Am. J. Hum. Genet.* **53**: 953-958.

Pulver, A. E., Karayiorgou, M., Lasseter, V. K., Wolyniec, P., Kasch, L., Antonarakis, S., Housman, D., Kazazian, H. H., Meyers, D., Nestadt, G., Ott, J., Liang, K.-Y., Lamacz, M., Thomas, M., Childs, B., Diehl, S. R., Wang, S., Murphy, B., Sun, C., O'Neill, F. A., Nie, L., Sham, P., Burke, J., W., D. B., Duke, F., Kipps, B. R., Bray, J., Hunt, W., Shinkwin, R., Ni Nuallain, M., Su, Y., MacLean, C. J., Walsh, D., Kendler, K. S., Gill, M., Vallada, H.,

Mant, R., Asherson, P., Collier, D., Parfitt, E., Roberts, E., Nanko, S., Walsh, C., Daniels, J., Murray, R., McGuffin, P., Owen, M., Laurent, C., Dumas, J.-B., d'Amato, T., Jay, M., Martinez, M., Campion, D., & Mallet, J., 1994, Follow-up of a report of a potential linkage for schizophrenia on chromosome 22q12-q13.1: Part 2, *Am J Med Genet.* **54**: 44-50.

Pulver, A. E., Karayiorgou, M., Wolyniec, P. S., Lasseter, V. K., Kasch, L., Nestadt, G., Antonarakis, S., Housman, D., Kazazian, H. H., Meyers, D., Ott, J., Lamacz, M., Liang, K.-Y., Hanfelt, J., Ullrich, G., De Marchi, N., Ramu, E., McHugh, P. R., Adler, L., Thomas, M., Carpenter, W. T., Manschreck, T., Gordon, C. T., Kimberland, M., Babb, R., Puck, J., & Childs, B., 1994, Sequential strategy to identify a susceptibility gene for schizophrenia: report of potential linkage on chromosome 22q12-q13.1: Part 1, *Am J Med Genet.* **54**: 36-43.

Ressler, K. J., & Nemeroff, C. B., 1999, Role of norepinephrine in the pathophysiology and treatment of mood disorders, *Biological Psychiatry.* **46**: 1219-1233.

Rotondo, A., Schuebel, K., Bergen, A., Aragon, R., Virkkunen, M., Linnoila, M., Goldman, D., & Nielsen, D., 1999, Identification of four variants in the tryptophan hydroxylase promoter and association to behavior, *Mol Psychiatry.* **4**: 360-368.

Rujescu, D., Giegling, I., Sato, T., Hartmann, A. M., & Moller, H.-J., 2003, Genetic variations in tryptophan hydroxylase in suicidal behavior: analysis and meta-analysis, *Biological Psychiatry.* **54**: 465-473.

Sabol, S., Hu, S., & Hamer, D., 1998, A functional polymorphism in the monoamine oxidase A gene promoter, *Hum Genet.* **103**: 273-279.

Sassone-Corsi, P., Lamph, W. W., Kamps, M., & Verma, I. M., 1988, fos-associated cellular p39 is related to nuclear transcription factor AP-1, *Cell.* **54**: 553-560.

Schildkraut, J. J., 1965, The catecholamine hypothesis of affective disorders: a review of supporting evidence, *Am. J. of Psychiatry.* **122**: 509-522.

Schulze, T., Muller, D., Krauss, H., Scherk, H., Ohlraun, S., Syagailo, Y., Windemuth, C., Neidt, H., Grassle, M., Papassotiropoulos, A., Heun, R., Nothen, M., Maier, W., Lesch, K., & Rietschel, M., 2000, Association between a functional polymorphism in the monoamine oxidase A gene promoter and major depressive disorder, *Am. J. Med. Genet. (Neuropsychiatr. Genet.).* **96**: 801-803.

Segawa, M., 2003, Neurophysiology of Tourette's syndrome: pathophysiological considerations, *Brain and Development.* **25**: S62-S69.

Serretti, A., Macciardi, F., Cusin, C., Verga, M., Pedrini, S., & Smeraldi, E., 1998, Tyrosine hydroxylase gene in linkage disequilibrium with mood disorders, *Mol Psychiatry.* **3**: 169-174.

Serretti, A., Macciardi, F., Verga, M., Cusin, C., Pedrini, S., & Smeraldi, E., 1998, Tyrosine hydroxylase gene associated with depressive symptomatology in mood disorder, *Am J Med Genet.* **81**: 127-130.

Shastry, B. S., 2004, Molecular genetics of attention-deficit hyperactivity disorder (ADHD): an update, *Neurochemistry International.* **44**: 469-474.

Shifman, S., Bronstein, M., Sternfeld, M., Pisanté, A., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R. D., Swartz-Vanetik, M., Knobler, H. Y., Shinar, E., Yakir, B., Zak, N. B., & Darvasi, A., 2004, COMT: A common susceptibility gene in bipolar disorder and schizophrenia, *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics.* **128B**: 61-64.

Shifman, S., Bronstein, M., Sternfeld, M., Pisante-Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R. D., Swartz-Vanetik, M., Knobler, H. Y., Shinar, E., Beckmann, J. S., Yakir, B., Risch, N., Zak, N. B., & Darvasi, A., 2003, A highly significant association between a COMT haplotype and schizophrenia, *Am. J. Hum. Genet.* **71**: 1296-1302.

Shih, J. C., Chen, K., & Ridd, M. J., 1999, Monoamine Oxidase: From Genes to Behavior, *Annual Review of Neuroscience.* **22**: 197-217.

Smyth, C., Kalsi, G., Brynjolfsson, J., O'Neill, J., Curtis, D., Rifkin, L., Moloney, E., Murphy, P., Sherrington, R., Petursson, H., & Gurling, H., 1996, Further tests for linkage of bipolar affective disorder to the tyrosine hydroxylase gene locus on chromosome 11p15 in a new series of multiplex British affective disorder pedigrees, *Am J Psychiatry.* **153**: 271-274.

Souery, D., Lipp, O., Mahieu, B., Mendelbaum, K., De Martelaer, V., Van Broeckhoven, C., & Mendlewicz, J., 1996, Association study of bipolar disorder with candidate genes

involved in catecholamine neurotransmission: DRD2, DRD3, DAT1, and TH genes, *Am J Med Genet.* **67**: 551-555.

Souery, D., Lipp, O., Rivelli, S. K., Massat, I., Serretti, A., Cavallini, C., Ackenheil, M., Adolfsson, R., Aschauer, H., Blackwood, D., Dam, H., Dikeos, D., Fuchshuber, S., Heiden, M., Jakovljevic, M., Kaneva, R., Kessing, L., Lerer, B., Lonnqvist, J., Mellerup, T., Milanova, V., Muir, W., Nylander, P. O., Oruc, L., Mendlewicz, J., & et al., 1999, Tyrosine hydroxylase polymorphism and phenotypic heterogeneity in bipolar affective disorder: a multicenter association study, *Am J Med Genet.* **88**: 527-532.

Stolk, J. M., Hurst, J. H., & Nisula, B. C., 1982, Regulation and inheritance of dopamine-beta-hydroxylase, *Behav Genet.* **12**: 37-52.

Strous, R. D., Bark, N., Woerner, M., & Lachman, H. M., 1997, Lack of Association of a Functional Catechol-O-Methyltransferase Gene Polymorphism in Schizophrenia, *Biological Psychiatry.* **41**: 493-495.

Sugama, S., Namihira, T., Matsuoka, R., Taira, N., Eto, Y., & Maekawa, K., 1999, Psychiatric inpatients and chromosome deletions within 22q11.2, *J Neurol Neurosurg Psychiatry.* **67**: 803-806.

Syagailo, Y., Stober, G., Grassle, M., Reimer, E., Knapp, M., Jungkunz, G., Okladnova, O., Meyer, J., & Lesch, K., 2001, Association analysis of the functional monoamine oxidase A gene promoter polymorphism in psychiatric disorders, *Am J Med Genet.* **105**: 168-171.

Syvanen, A.-C., Tilgmann, C., Rinne, J., & Ulmanen, I., 1997, Genetic polymorphism of catechol-O-methyltransferase (COMT): Correlation of genotype with individual variation of S-COMT activity and comparison of the allele frequencies in the normal population and Parkinsonian patients in Finland, *Pharmacogenetics.* **7**: 65-71.

Tenhunen, J., Salminen, M., Lundstrom, K., Savolainen, R., & Ulmanen, I., 1994, Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters, *European Journal of Biochemistry.* **223**: 1049-1059.

Thibaut, F., Ribeyre, J. M., Dourmap, N., Meloni, R., Laurent, C., Campion, D., Menard, J. F., Dollfus, S., Mallet, J., & Petit, M., 1997, Association of DNA polymorphism in the first intron of the tyrosine hydroxylase gene with disturbances of the catecholaminergic system in schizophrenia, *Schizophr Res.* **23**: 259-264.

Turecki, G., Rouleau, G. A., Mari, J., Joober, R., & Morgan, K., 1997, Lack of association between bipolar disorder and tyrosine hydroxylase: a meta-analysis, *Am J Med Genet.* **74**: 348-352.

Vandenbergh, D. J., Rodriguez, L. A., Miller, I. T., Uhl, G. R., & Lachman, H. M., 1997, High-activity catechol-O-methyltransferase allele is more prevalent in polysubstance abusers, *American Journal of Medical Genetics - Neuropsychiatric Genetics.* **74**: 439-442.

Vaswani, M., Linda, F. K., & Ramesh, S., 2003, Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review, *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* **27**: 85-102.

Vincent, J. B., Masellis, M., Lawrence, J., Choi, V., Gurling, H. M., Parikh, S. V., & Kennedy, J. L., 1999, Genetic association analysis of serotonin system genes in bipolar affective disorder, *Am J Psychiatry.* **156**: 136-138.

Walther, D. J., Peter, J.-U., Bashammakh, S., Hortnagl, H., Voits, M., Fink, H., & Bader, M., 2003, Synthesis of serotonin by a second tryptophan hydroxylase isoform, *Science.* **299**: 76.

Wei, J., & Hemmings, G. P., 1999, Lack of evidence for association between the COMT locus and schizophrenia, *Psychiatric Genetics.* **9**: 183-186.

Wei, J., Ramchand, C., & Hemmings, G., 1998, TaqI polymorphic sites at the human dopamine beta-hydroxylase gene possibly associated with biochemical alterations of the catecholamine pathway in schizophrenia, *Psychiatr Genet.* **8**: 19-24.

Wei, J., Ramchand, C. N., & Hemmings, G. P., 1995, Association of polymorphic VNTR region in the first intron of the human TH gene with disturbances of the catecholamine pathway in schizophrenia, *Psychiatr Genet.* **5**: 83-88.

Wei, J., Ramchand, C. N., & Hemmings, G. P., 1997, Possible association of catecholamine turnover with the polymorphic (TCAT)n repeat in the first intron of the human tyrosine hydroxylase gene, *Life Sci.* **61**: 1341-1347.

Wei, J., Ramchand, C. N., & Hemmings, G. P., 1997, Possible control of dopamine beta-hydroxylase via a codominant mechanism associated with the polymorphic (GT)n repeat at its gene locus in healthy individuals, *Human Genetics.* **99**: 52-55.

Weinberger, D. R., Egan, M. F., Bertolino, A., Callicott, J. H., Mattay, V. S., Lipska, B. K., Berman, K. F., & Goldberg, T. E., 2001, Prefrontal neurons and the genetics of schizophrenia, *Biological Psychiatry.* **50**: 825-844.

Weinshilboum, R., 1978, Human biochemical genetics of plasma dopamine-[beta]-hydroxylase and erythrocyte catechol-O-methyltransferase, *Hum Genet.* **45**: 101-112.

Wigg, K., Zai, G., Schachar, R., Tannock, R., Roberts, W., Malone, M., Kennedy, J. L., & Barr, C. L., 2002, Attention Deficit Hyperactivity Disorder and the Gene for Dopamine Beta-Hydroxylase, *Am J Psychiatry.* **159**: 1046-1048.

Wolters, E. C., & Calne, D. B., 1989, Parkinson's disease, *Cmaj.* **140**: 507-514.

Xie, T., Ho, S. L., Li, L. S. W., & Ma, O. C. K., 1997, G/A1947 polymorphism in catechol-O-methyltransferase (COMT) gene in Parkinson's disease, *Movement Disorders.* **12**: 426-427.

Yamamoto, K., Cubells, J., Gelernter, J., Benkelfat, C., Lalonde, P., Bloom, D., Lal, S., Labelle, A., Turecki, G., Rouleau, G., & Joober, R., 2003, Dopamine beta-hydroxylase (DBH) gene and schizophrenia phenotypic variability: a genetic association study, *Am J Med Genet.* **117B**: 33-38.

Zabetian, C. P., Anderson, G. M., Buxbaum, S. G., Elston, R. C., Ichinose, H., Nagatsu, T., Kim, K. S., Kim, C. H., Malison, R. T., Gelernter, J., & Cubells, J., 2001, A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus, *Am. J. Hum. Genet.* **68**: 515-522.

Zabetian, C. P., Buxbaum, S. G., Elston, R. C., Köhnke, M. D., Anderson, G. M., Gelernter, J., & Cubells, J. F., 2003, The Structure of Linkage Disequilibrium at the DBH Locus Strongly Influences the Magnitude of Association between Diallelic Markers and Plasma Dopamine -Hydroxylase Activity, *Am. J. Hum. Genet.* **72**: 1389-1400.

Zhang, X., Beaulieu, J.-M., Sotnikova, T. D., Gainetdinov, R. R., & Caron, M. G., 2004, Tryptophan Hydroxylase-2 Controls Brain Serotonin Synthesis, *Science.* **305**: 217.

Zill, P., Baghai, T. C., Zwanzger, P., Schüle, C., Eser, D., Rupprecht, R., Möller, H.-J., Bondy, B., & Ackenheil, M., SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression, *Molecular Psychiatry.* **In Press.**

Zill, P., Buttner, A., Eisenmenger, W., Bondy, B., & Ackenheil, M., Regional mRNA expression of a second tryptophan hydroxylase isoform in postmortem tissue samples of two human brains, *European Neuropsychopharmacology.* **In Press**.

12. TRANSDUCTION MECHANISMS
G Proteins

Katerina J. Damjanoska and Louis D. Van de Kar*

1. INTRODUCTION

Receptors can be classified into three large classes: ligand gated ion channels; genotropic receptors, which can act as transcription factors; and G protein-coupled receptors (GPCRs). GPCRs are membrane proteins with a unique seven-transmembrane transversing structure (hepta-helix). GTP-binding proteins (G proteins) transmit extracellular signals from cell-surface receptors to intracellular effectors such as phospholipases, adenylyl cyclases, and ion channels. G proteins are a family of trimeric proteins, consisting of α, β, and γ subunits. The α subunits of G proteins bind guanine nucleotides (GTP and GDP) with high affinity and specificity (Dessauer 1996; Neer 1995). It is this affinity for guanine nucleotides that gives them their name G proteins. Approximately 1% of the mammalian genome encodes for G-protein coupled receptors (Morris 1999), and approximately 50% of pharmaceuticals target receptors, largely GPCRs (Drews 2000). Although no drugs have been developed, so far, that specifically act on G proteins, G proteins are potential pharmaceutical targets as changes in G proteins and their associated regulatory proteins have been implicated in a number of pathological conditions.

Since the discovery of G proteins in the late 60's and early 70's of the 20th century, by Alfred G. Gilman and Martin Rodbell, research dedicated to the study of G proteins has increased dramatically. G proteins associate with a variety of receptors (See Table 1). This enables G proteins to be intracellular transducers of a variety of extracellular signals such as hormones, neurotransmitters, odorants, and

* Katerina J. Damjanoska and Louis D. Van de Kar, Ph.D., Center for Serotonin Disorders Research and Department of Pharmacology & Experimental Therapeutics, Loyola University Chicago, Stritch School of Medicine, Maywood, Illinois, 60153 U.S.A.

photons. This chapter will provide an overview of the variety of G protein subtypes and their associated proteins. As this review will focus on psychiatric disorders, we will limit our discussion to the importance of G proteins in the central nervous system.

2. TYPES OF HETEROTRIMERIC G PROTEINS

Heterotrimeric G proteins ($G_{\alpha\beta\gamma}$) are currently categorized according to the G_α subunit, historically thought to be the only active subunit of the G protein trimer. As there are four main types (classes or families) of G_α subunits, there are four main classes of heterotrimeric G proteins. The classes of G_α proteins, and hence of G protein trimers, are $G_{\alpha s}$, $G_{\alpha q}$, $G_{\alpha i}$, and $G_{\alpha 12}$. Each class of G_α proteins has subtypes (See Section 2.2). Although multiple subtypes of G_α proteins have been identified, there are not as many G_α proteins as there are G protein-coupled receptors (GPCRs). Table 1 shows the different classes of G_α proteins and some of the receptors to which they are coupled. Many GPCRs couple to the same G_α protein subtype, yet are still capable of mediating their specific cellular and physiologic effects. This overlap in signal transduction proteins can be partially explained by differential expression of proteins in cells. Yet, there are numerous cells that express more than one GPCR and multiple subtypes of G_α proteins.

One theory proposes that cellular microdomains with lipid-rich regions (lipid rafts) in the cell membrane preferentially aggregate the required and relevant proteins within close proximity of their respective receptors. G_α proteins and their effector enzymes can be localized to microdomains by their association with specific proteins of the membrane and cytoskeleton, such as tubulin (See Section 5) (Donati 2003; Huang 1997). G_α proteins can also undergo a variety of lipid modifications that may assist in targeting G_α proteins to subcellular compartments, caveolae, and lipid microdomains (See Section 5). Furthermore, not all G_α protein subtypes are sequestered to the same microdomain.[7] For instance, $G_{\alpha q}$ proteins are normally found in caveolae without being associated to $G_{\beta\gamma}$ proteins.[7] On the other hand, while $G_{\alpha i}$ and $G_{\alpha s}$ proteins can be found in caveolae, they are predominantly found in lipid rafts complexed to $G_{\beta\gamma}$ proteins (Oh 2001). The theory of cellular compartmentalization of the G_α proteins provides a plausible explanation for the speed of selection (efficiency) and selectivity of receptor-to-G protein signaling. Another emerging theory concerning receptor-to-G protein coupling is “agonist-directed trafficking of receptor stimulus” (Kenakin 1995). This theory suggests that ligands can induce different conformational changes in the receptor so that one receptor can activate multiple G_α protein-mediated signaling cascades (Kenakin 1995 ; Berg 1998).

Table 1. G_α protein families and the receptors that are coupled to them. Some of the information reported in this table for receptor-G protein coupling is obtained from *in vitro* reconstitution or studies in cell culture and are not confirmed *in vivo*. The results obtained from reconstitution or cell culture studies must be taken with caution as the protein levels and ratios of purified or transfected receptors and G proteins may exceed the physiological levels of the proteins and result in otherwise unlikely interactions. These *in vitro* studies may also lack the associated proteins critical for the association between receptors and G proteins.

G_α Protein Family	Associated Receptors	References
$G_{\alpha s}$	Adenosine (A_{2A}, A_{2B})	10, 11, 12, 13
	Adrenergic [α_{2A}, α_{2B}, α_{2C} (formerly α_2C10, α_2C2, α_2C4), β_1, β_2, β_3]	14, 15, 16, 17
	Calcitonin (CTR)	18
	Complement (C5A)	19
	Corticotropin-releasing hormone (CRH-R1, -R1α, -R2)	20, 21
	Dopamine (D1, D3, D5)	22
	Endothelin (ETAR)	23, 24
	Glucagon (GR)	25
	Gonadotropin-releasing hormone (GnRH-R)	26
	Histamine (H2)	27
	Luteinizing hormone/chorionic gonadotropin (LH/CG R)	28
	Melanocortin (MC1R, ACTHR, MC3R, MC4R, MC5R)	29
	Parathyroid hormone (PTH)	30, 31
	Prostaglandin (IP, EP2, EP4, DP, EP3B, EP3C)	32, 33
	Serotonin (5-HT4, 5-HT6, 5-HT7)	34, 35, 36
	Substance P (SPR or NK1R)	37, 38
	Thyroid Stimulating Hormone (TSH)	39
	Vasopressin (V2)	40
Gαi	Adenosine (A1 and A3)	41, 42, 43
	Adrenergic (α2A, α2B, α2C)	44, 17
	Angiotensin (AT1)	45
	Bombesin	46
	Bradykinin (B1, B2)	47, 48, 49, 50
	Calcitonin (CTR)	51, 52
	Cannabinoid (CB1, CB2)	53, 54
	Cholecystokinin (CCKB)	55

Table 1. (continued)

G_α Protein Family	Associated Receptors	References
Gαi	Compliment (C3A, C5A)	56, 57, 58
	Corticotropin-releasing hormone (CRH-R1α)	20
	Dopamine (D1, D2S, D2L, D3, D4)	22
	Endothelin (ETBR)	23, 24
	Galanin (GALR1, GALR2)	59, 60
	Glutamate (mGluR2, mGluR4)	61, 62
	Gonadotropin-releasing hormone (GnRH-R)	26
	Histamine (H3)	63
	Insulin-like growth factor (IGF IR)	64
	Luteinizing hormone/chorionic gonadotropin (LH/CG-R)	28
	Lysophosphatidic Acid (LPA)	65
	Melatonin	66, 67
	Muscarinic acetylcholine (m2 and m4)	68, 69
	Neuropeptide Y* (Y1, Y2, Y4, Y5)	70, 71
	Neurotensin* (NTS1)	72, 73, 74
	Opioid (*μ, *κ and *δ)	75, 76, 77, 78, 79
	Orphanin/Nociceptin (OFQR)	76
	Oxytocin (OTR)	80, 81
	Parathyroid hormone (PTH)	30
	Platelet-activating factor (PAF)	82
	Prostaglandin E (EP3A, EP3D, CRTH2)	83, 32, 33
	Serotonin (*5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F, 5-HT5A)	34, 84, 85, 35, 36
	Somatostatin (SRIF)	86, 87
	Substance P (SPR or NK1R)	37, 88
	Thrombin	89
	Thyroid Stimulating Hormone (TSH)	90
Gαz	Compliment (C5a)	91
	Dopamine (D2S, D2L, D3, D4, D5)	22
	Formyl peptide (fMLP)	91, 92
	Melatonin	66
	Opioid (*μ, κ and δ)	93, 94, 95, 77, 96, 78

Table 1. (continued)

G_α Protein Family	Associated Receptors	References
Gαz	Serotonin (*5-HT1A)	97
Gαq	Adenosine (A2A, A2B, A3)	98, 99, 41
	Adrenergic (α1, α2A)	100, 101
	Angiotensin (AT1)	102
	Bombesin (GRP-R, NMB-R, BRS-3)	46, 103, 104
	Bradykinin (B1, B2)	47, 48, 49, 50
	Calcitonin (CTR)	105
	Cholecystokinin (CCKA, now CCK2; CCKB, now CCK1)	106, 55, 107
	Compliment (C5A)	108
	Corticotropin-releasing hormone (CRH-R1α)	20
	Dopamine (D3)	109
	Endothelin (ETAR, ETBR)	24
	Galanin (GALR2)	59
	Glutamate (mGluR1, mGluR5)	110, 111
	Gonadotropin-releasing hormone (GnRH-R)	112, 113
Gαq	Histamine (H1, H2)	27
(continued)	Lysophosphatidic Acid (LPA)	114
	Melanocortin (MC3R)	115
	Muscarinic (m1, m5)	68, 116
	Neurokinin (NK2)	117, 118
	Neurotensin (NTS1)	73
	Orexin (types 1)	119
	Oxytocin	80
	Platelet-activating factor (PAF)	82
	Prostaglandin (TP, IP, FP, EP3D)	32, 33
	Purinoceptor (P2Y)	120
	Serotonin (5-HT2A, 5-HT2B, 5-HT2C)	34, 35, 36
	Substance P (NK1R or SPR)	37, 121
	Thrombin	122
	Thyroid Stimulating Hormone (TSH)	39
	Vasopressin (V1a, V1b)	104, 123

Table 1. (continued)

G_α Protein Family	Associated Receptors	References
Gα12	Adrenergic (α1)	124
	Bombesin (GRP-R)	46
	Endothelin (ETBR)	125
	Galanin (GALR2)	59
	Lysophosphatidic Acid (LPA)	126
	Prostaglandin (TP)	32
	Thrombin Receptor	127
	Thyroid Stimulating Hormone (TSH)	90

* Denotes receptor/G protein interactions confirmed *in vivo*. Studies performed *in vivo* consisted of 1) receptor and G_α protein colocalization depicted by immunohistochemistry, 2) identification of the G_α protein family mediating the specific response by treatment with pertussis toxin or cholera toxin, or 3) *in vivo* suppression of expression of specific G_α proteins by antisense oligodeoxynucleotides. Abbreviations: CRTH2, chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells; EP, prostaglandin E (PGE_2) receptor; IP, prostaglandin I (PGI_2) receptor; DP, prostaglandin D (PGD_2) receptor; FP, prostaglandin F ($PGF_{2\alpha}$) receptor; TP, thromboxane (TXA_2) receptor.

2.1. Regulation of G protein signaling

Receptors are coupled to the trimeric form ($\alpha\beta\gamma$) of G proteins. When a receptor agonist binds to its receptor it induces a conformational change that increases the affinity of the G_α protein for Mg^{2+}. Once Mg^{2+} is bound to the G_α protein, it stimulates the release of guanine diphosphate (GDP) from the G_α protein, and the binding of guanine triphosphate (GTP) to the G_α protein, promoting the dissociation of the $G_{\beta\gamma}$ protein dimer from the G_α protein. The GTP-bound G_α protein and the $G_{\beta\gamma}$ protein dimer are then capable of stimulating their respective effector enzymes. Hydrolysis of an inorganic phosphate from G_α-GTP converts it to G_α-GDP, decreasing its high affinity for second messenger enzymes and increasing its affinity for the $G_{\beta\gamma}$ protein dimer. The result is re-association of the G_α and $G_{\beta\gamma}$ proteins and the formation of the $G_{\alpha\beta\gamma}$ protein trimer (Fig. 1). The GDP-bound $G_{\alpha\beta\gamma}$ protein trimer can then couple to its receptors. While this scheme represents the current kinetic model of G protein signaling, the exact biochemical and structural dynamics among the $G_{\alpha\beta\gamma}$ trimer, the receptor, and the effector proteins are still under investigation (Dessauer 1996).

The activity of the G protein subunits can be regulated by accessory proteins and by post-translational modifications. Post-translational regulation of G protein subunits will be discussed in Section 5. Accessory proteins regulate the activity of G_α proteins, $G_{\beta\gamma}$ proteins, and their association with each other. Two examples of accessory proteins are the regulators of G protein signaling (RGS) proteins and

phosducin. G_α proteins have an intrinsic GTPase activity that in many cases is very slow. This intrinsic GTPase activity of G_α proteins is enhanced by RGS proteins or by some effector enzymes, such as phospholipase C_β. RGS proteins bind directly to G_α proteins, with some apparent subtype specificity, and accelerate the hydrolysis of GTP and thereby catalyze the inactivation of the G_α proteins (Fig. 1) (Berman 1998 ; Berghuis 1996 ; Berman 1996).

A second method of regulating the activity of G proteins is to prevent the formation of the $G_{\alpha\beta\gamma}$ protein trimer by inhibiting the $G_{\beta\gamma}$ protein subunits from associating with the G_α proteins (Fig.1). Phosducin is a 33-kDa protein that has been characterized in the retina and pineal gland (Reig 1990; Lee 1987) but is also expressed in various other tissues, such as the brain, liver, lung, and heart (Danner 1996). Phosducin inhibits $G_{\beta\gamma}$ proteins from reassociating with GDP-bound G_α proteins and induces translocation of the $G_{\beta\gamma}$ proteins to the cytoplasm (Tanaka 1996). This translocation of $G_{\beta\gamma}$ proteins to the cytoplasm is thought to be due to phosducin binding to the region on the $G_{\beta\gamma}$ protein dimer that is implicated in membrane binding and receptor interaction (Tanaka 1996; Lambright 1996; Sondek 1996). Phosphorylation of phosducin by protein kinase A (PKA) at serine residue 73 reduces the stability of the interaction between phosducin and the $G_{\beta\gamma}$ protein dimer, resulting in the reassociation of the $G_{\beta\gamma}$ protein dimer with the G_α protein (Gaudet 1999; Yshida 1994). *In vitro* studies have shown phosducin to inhibit β-adrenoceptor-stimulated adenylyl cyclase activity (Danner 1996). The ability of phosducin to inhibit receptor-mediated adenylyl cyclase activity can be attributed to phosducin sequestering the $G_{\beta\gamma}$ protein dimers and thereby decreasing the available pool of intracellular G protein trimers. Additional studies have shown that: 1) phosducin (IC_{50} = 100 μM) decreases $G_{\beta\gamma}$ protein-mediated activation of adenylyl cyclase and phospholipase C-β (PLC_β) and 2) phosducin (IC_{50} = 15 nM) decreases the GTPase activity of $G_{\alpha o}$ proteins in the presence of $G_{\beta\gamma}$ protein dimers, but not of isolated $G_{\alpha o}$ proteins.[140] These *in vitro* studies not only demonstrate that phosducin is capable of disrupting the $G_{\beta\gamma}$-effector interaction, but that phosducin is approximately 5 fold more effective in disrupting the $G_{\beta\gamma}$-G_α protein interaction (Bluml 1997). Phosducin is potentially a useful $G_{\beta\gamma}$ protein inhibitor. While our knowledge about phosducin is still in its infancy, it presents a possible therapeutic approach that can reduce a supersensitized signal without targeting the receptor protein.

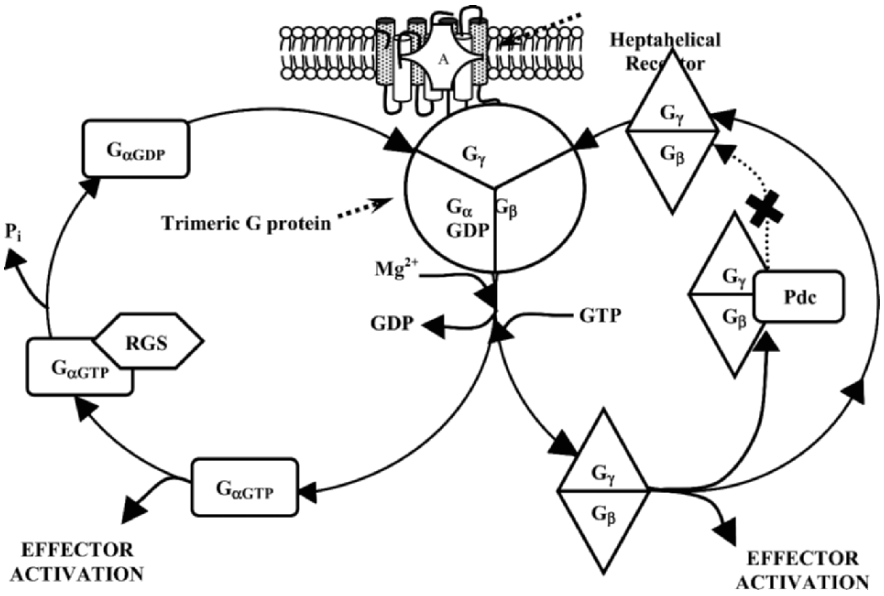


Figure 1. Regulation of G protein signaling. Abbreviations: A, agonist; GDP, guanosine diphosphate; GTP, guanosine triphosphate; Pdc, phosducin; P_i, inorganic phosphate; Mg^{2+}, magnesium ion; RGS, regulator of G protein signaling.

2.2. G_{α} protein subunits

Approximately 20% of the primary sequence of G_{α} subunits is composed of conserved amino acids. G_{α} protein subunits are categorized into 4 classes: $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$, and $G_{\alpha 12}$. These 4 classes or families of G_{α} protein subunits are based on sequence similarities aside from the conserved amino acids present in all G_{α} protein subunits (Hepler 1992; Wilkie 1992). Each G protein family is named based on the prototypical G_{α} protein family member. Most of the current research focuses on the function of G_{α} proteins at the intracellular portion of the cell membrane and its role in GPCR signaling. Although G_{α} proteins have been localized in the Golgi complex (Nagahama 2002; Ercolani 1990), endoplasmic reticulum (Audigier 1988), and endosomal structures (Colombo 1992) the function of G_{α} proteins in these subcellular compartments is not entirely understood. It is possible that localization to these subcellular regions may enable G_{α} protein subunits to influence the formation and transport of secretory vesicles (Leyte 1992; Stow 1991; Tooze 1990), affect intra-compartmental protein transport (Schwaninger 1992), and regulate Golgi structure (Nagahama 2002).

The $G_{\alpha s}$ protein family consists of $G_{\alpha s}$ and $G_{\alpha olf}$ proteins, with long ($G_{\alpha s}$-L) and short ($G_{\alpha s}$-S) splice variants of the $G_{\alpha s}$ protein (Kozasa 1988; Robishaw 1986). Both proteins activate adenylyl cyclase and are adenosine 5'-diphosphate (ADP)-ribosylated by cholera toxin (*Vibrio cholera*) on an arginine residue (Gilman 1989). This covalent, post-translational modification inhibits the intrinsic GTPase activity of the $G_{\alpha s}$ protein family, which hinders the inactivation of the proteins in $G_{\alpha s}$ family. $G_{\alpha s}$ proteins are widely distributed in various tissues, including the brain (Dessauer 1996). $G_{\alpha olf}$ proteins are associated only with the olfactory areas of the brain. In the gastrointestinal system, defects in the termination of $G_{\alpha s}$ protein activity are attributed to the etiology of the diarrhea induced by *Vibrio cholerae*. Other pathologies associated with defective termination of $G_{\alpha s}$ protein activity include acromegaly, McCune-Albright syndrome, and some thyroid adenomas (Farfel 1999). Pseudohypoparathyroidism type I is caused by an inactivation or genetic loss of $G_{\alpha s}$ proteins.

The $G_{\alpha i}$ protein family consists of $G_{\alpha i\text{-}1}$, $G_{\alpha i\text{-}2}$, $G_{\alpha i\text{-}3}$, $G_{\alpha z}$, $G_{\alpha o\text{-}1}$, $G_{\alpha o\text{-}2}$, and $G_{\alpha t}$ (retinal specific) proteins (Dessauer 1996). The $G_{\alpha i}$ protein family inhibits adenylyl cyclase activity and also opens K^+ ion channels. The retina-specific $G_{\alpha t}$ protein is the only member that activates cGMP phosphodiesterase. All the members of the $G_{\alpha i}$ protein family, except for $G_{\alpha z}$ proteins, are ADP-ribosylated by pertussis toxin (*Bortadella pertusis*) on a conserved COOH-terminal cysteine residue (Ui 1990). After this covalent modification occurs, the proteins of the $G_{\alpha i}$ family are uncoupled from their receptors and their intracellular signaling is inactivated. Many investigators have used pertussis toxin in order to determine whether proteins of the $G_{\alpha i}$ family play a role in specific receptor-mediated intracellular signaling cascades. All the members of $G_{\alpha i}$ protein family are expressed in a variety of tissues, including the brain. Defects in the termination of $G_{\alpha i}$ protein activity are implicated in the etiology of some adenomas of the adrenal cortex and endocrine tumors of the ovary (Farfel 1999). ADP-ribosylation of $G_{\alpha i}$ proteins by *Bordetella pertussis* hinders the activation of $G_{\alpha i}$ proteins and causes whooping cough. A point mutation genetically inactivates $G_{\alpha t}$ proteins in affected individuals of dominantly inherited congenital stationary night blindness (Dryja 1996).

The $G_{\alpha q}$ family of proteins consists of $G_{\alpha q}$, $G_{\alpha 11}$, $G_{\alpha 14}$, $G_{\alpha 15}$, and $G_{\alpha 16}$ proteins. Phospholipase C (PLC) is activated by all members of the $G_{\alpha q}$ protein family. Proteins of the $G_{\alpha q}$ family do not undergo ADP-ribosylation by either cholera toxin or pertussis toxin. $G_{\alpha 11}$ and $G_{\alpha q}$ proteins are widely distributed in various tissues, including the brain.[155] $G_{\alpha 15}$ and $G_{\alpha 16}$ proteins are specifically found in myeloid and lymphoid tissue while $G_{\alpha 14}$ proteins are found in a variety of tissues but not in brain (Dessauer 1996). While none of the proteins of the $G_{\alpha q}$ family have been associated with the etiology of a disease, a change in levels of $G_{\alpha q/11}$ proteins was observed in brain areas affected by Alzheimer's disease[158] and by antidepressant treatment (Lesch 1992).

Finally, the family of $G_{\alpha 12}$ proteins consists of $G_{\alpha 12}$ and $G_{\alpha 13}$ proteins. Both are widely distributed in various tissues, including the brain (Dessauer 1996). $G_{\alpha 12}$ and $G_{\alpha 13}$ proteins were suggested to regulate the activity of Na^+/Cl^- antiporters (Offermanns 1996), activate small G proteins (Rho) (Mao 1998), and activate mitogen-activated protein kinases, such as c-Jun kinases (Jho 1997; Collins 1996; Voyno-Yasenetskaya 1996; Prasad 1995). While the $G_{\alpha 12}$ protein family has not yet been implicated in any disorders, the $G_{\alpha 12/13}$ genes are considered to be proto-oncogenes. Out of all four G protein families, activated mutant forms of $G_{\alpha 12}$ and $G_{\alpha 13}$ proteins had the highest transformation efficiency in NIH3T3 cells (Offermanns 1994). Furthermore, a screen of human tumor cell lines has shown an increased expression of $G_{\alpha 12}$ and $G_{\alpha 13}$ proteins in a number of breast, colon, and prostate adeno-carcinoma-derived cell lines (Xu 1993).

2.3. $G_{\beta\gamma}$ protein subunits

Seven isoforms of G_β proteins ($G_{\beta 1}$, $G_{\beta 2}$, $G_{\beta 3}$, $G_{\beta 3S}$, $G_{\beta 4}$, $G_{\beta 5}$, $G_{\beta 5L}$)[168, 169] and 11 isoforms of G_γ proteins ($G_{\gamma 1}$, $G_{\gamma 2}$, $G_{\gamma 3}$, $G_{\gamma 4}$, $G_{\gamma 5}$, $G_{\gamma 7}$, $G_{\gamma 8}$, $G_{\gamma 10}$, $G_{\gamma 11}$, $G_{\gamma 12}$, $G_{\gamma C}$) have been documented (Downes 1999; Gautam 1998). Two of the isoforms of the G_β proteins are splice variants: $G_{\beta 3S}$ protein (short form of $G_{\beta 3}$ protein) and $G_{\beta 5L}$ protein (long form of $G_{\beta 5}$ protein). All the members of the G_β protein family, except for the $G_{\beta 5}$ and $G_{\beta 5L}$ proteins, have a great degree of homology in their amino acid sequence (Gautam 1998). The G_γ protein family has more diversity in their primary sequence than the G_β protein family. This allows the G_γ protein subtypes to be grouped into 4 subfamilies based on sequence similarity. Group I includes $G_{\gamma 1}$, $G_{\gamma C}$, $G_{\gamma 11}$; Group II includes $G_{\gamma 2}$, $G_{\gamma 3}$, $G_{\gamma 4}$; Group III includes $G_{\gamma 7}$, $G_{\gamma 12}$; and Group IV includes $G_{\gamma 5}$, $G_{\gamma 8}$, $G_{\gamma 10}$. Group I G_γ proteins are predominantly expressed in the retina and Group II G_γ proteins are abundant in the nervous system.

G_β and G_γ proteins form a tight association with each other and are usually found as a dimer. With 7 isoforms of G_β protein, 11 isoforms of G_γ protein, and over a dozen isoforms of G_α protein, there are numerous combinations of G protein trimers that can be formed in the cell. The issue of which G_α, G_β, and G_γ isoforms associate with one another with high selectivity has been addressed using transfected cell assay systems (Zhou 2000; Pronin 1992), yeast two-hybrid systems (Yan 1996), and immunoprecipitation of proteins from tissue preparations (Asano 1999). This selectivity along with differential distribution of the subunits in tissues (Brunk 1999; Betty 1998) is one possible mechanism by which so many receptors use the same G protein subunits to elicit a variety of specific cellular and physiological responses.

$G_{\beta\gamma}$ protein dimers can regulate effector enzymes and ion channels. Their importance in signal transduction was initially unknown but is now coming to the forefront of research. $G_{\beta\gamma}$ subunits are capable of regulating enzyme activity, such as activation of phosholipase A_2 (PLA_2) (Jelsema 1987), phospholipase C (PLC)

(Rebecchi 2000 ; Barr 2000), and phospho-inositide (PI) 3-kinase (Stephens 1997); inhibition of adenylyl cyclase type I, activation of adenylyl cyclase type II and IV; (Taussig 1993; Tang 1991 and 1992) activation of K^+ channels (Clapham 1989); and inhibition of Ca^{2+} channels by $G_{\beta 1}$ and $G_{\beta 3}$ proteins (Shekter 1997). Although some patients with hypertension have been shown to contain a gain of function mutation in their $G_{\beta 3}$ gene (Siffert 1998), there is still very little information regarding disorders associated with a change in the function of G_β or G_γ proteins.

3. RGS PROTEINS

Regulators of G protein signaling (RGS) proteins are GTPase activating proteins (GAPs) that enhance the intrinsic GTPase activity of G proteins (Hollinger 2002), but RGS proteins can regulate G protein activity by mechanisms other than their GAP activity. RGS proteins can also bind to actived G_α proteins to prevent effector activation, independent of their GAP activity (Carman 1999; Hepler 1997). In addition, the GoLoco motif, found in RGS12 and RGS14 proteins, allows RGS proteins to bind to $G_{\alpha i}$-GDP proteins (Kimple 2001 and 2002). The ability of certain RGS proteins to bind to inactived G_α proteins provides them with guanine nucleotide (GDP) dissociation inhibitor (GDI) activity in addition to their GAP activity. Phosphorylation of RGS14 increases its GDI activity without affecting its GAP activity (Hollinger 2003). Hence, RGS proteins can catalyze the inactivation of G_α proteins, prolong the period of inactivation of G_α proteins, and also prevent G_α proteins from activating downstream effectors. Furthermore, RGS proteins can inhibit effector activation mediated by $G_{\beta\gamma}$ proteins. For instance, RGS3 inhibits $G_{\beta\gamma}$-mediated inositol phosphate production and MAPK activation (Shi 2001). Alterations in RGS proteins have been implicated in anxiety, aggression, and schizophrenia (Mirnics 2001 ; Oliveira-dos-Santos 2000).

Table 2. RGS proteins and their associated G_α protein families.

RGS Family	RGS Protein	Associated G_α Family	References
R4	RGS1	$G_{\alpha i}$	205
	RGS2 †	$G_{\alpha q}$, $G_{\alpha i}$	206, 207
	RGS3 †	$G_{\alpha i}$, $G_{\alpha q}$	208
	RGS4 †	$G_{\alpha q}$, $G_{\alpha i}$	188, 130, 209
	RGS5 †	$G_{\alpha i}$	210, 211, 212
	RGS8 †	$G_{\alpha i}$	213, 214
	RGS13	$G_{\alpha i}$, $G_{\alpha q}$	201
	RGS16 †	$G_{\alpha i}$	215, 211
	RGS18	$G_{\alpha i}$, $G_{\alpha q}$	216, 217
R7	RGS6 †	$G_{\alpha o}$ ‡	218, 212
	RGS7 †	$G_{\alpha i}$, $G_{\alpha q}$	213, 219, 220, 212

	RGS9 †	$G_{\alpha t}$ ‡, $G_{\alpha i}$	221, 219, 222
	RGS11 †	$G_{\alpha o}$ ‡	223
R12	RGS10 †	$G_{\alpha i}$	224
	RGS12 †	$G_{\alpha i}$, $G_{\alpha 12}$	161, 225
	RGS14 †	$G_{\alpha 12}$, $G_{\alpha i}$	191, 189, 226
RA	Axin †	ND	227
	Conductin †	ND	228
RL	P115RhoGEF †	$G_{\alpha 12}$	229, 230, 161
	RhoGEF	ND	231
	LscRhoGEF	ND	232
	D-AKAP2	ND	233
	GRK2	$G_{\alpha q}$	234
	RGSPX1	$G_{\alpha s}$ ‡	198
RZ	RGSZ1 †	$G_{\alpha z}$ ‡	235, 236
	RGSZ2	$G_{\alpha z}$ ‡	143
	RET-RGS1	$G_{\alpha t}$ ‡	237
	GAIP †	$G_{\alpha i}$, $G_{\alpha q}$	188, 238, 130
?	RGS15 †	ND	212

† Denotes RGS proteins that can undergo alternative splicing[239].

‡ Denotes the specific G_α protein of a G_α protein family with which the RGS protein interacts. Most RGS proteins associate with multiple members of a G_α protein family, but there are some RGS proteins that act as GAPs for only certain members of a G_α protein family.

Abbreviations: D- AKAP, dual specificity A kinase anchoring protein; GAIP, G_α interacting protein; GRK, G protein receptor kinase; ND, associated G_α protein is not yet determined; ? = RGS family not known.

RGS proteins are widely distributed in the brain. Although RGS proteins are considered soluble hydrophobic proteins, they are found in the cytosol and are also associated with the cellular membrane (Bernstein 2000; Srinivasa 1998). RGS protein subtypes are categorized into 6 families (R4, R7, R12, RZ, RL and RA) based on their domain or sequence similarities (Table 2) (Hollinger 2002; Ross 2000; Zheng 1999). All RGS proteins have GAP activity except for axin and conductin (also known as axin-like or axil), which are in the RA family of RGS proteins. The RA family is included in the classification of RGS proteins because of their sequence similarity to RGS proteins, even though they have not been shown to have GAP activity (Gold 1997). Both axin and conductin are scaffold proteins in the Wingless-type (Wnt) frizzled protein receptor pathway. The Wnt signaling pathway is present in various species, from invertebrates to mammals, and is important in development, cellular proliferation, and cellular differention.

Most G proteins have multiple RGS proteins that can enhance their GTPase activity (Table 2). $G_{\alpha s}$ proteins are the exception. Except for RGSPX1, no other wild-type RGS proteins have GAP activity for $G_{\alpha s}$ proteins (Zheng 1999). RGS16 and RGS4 proteins can act as GAPs for $G_{\alpha s}$ proteins only after a point mutation

(A229S) is incorporated into the $G_{\alpha s}$ protein (Natochin 1998). Yet, by attenuating the activation of the effector enzyme other RGS proteins, such as RGS2 and RGS13, can regulate $G_{\alpha s}$ activity indirectly (Kehrl 2002; Johnson 2002; Sinnarajah 2001.

There is a greater variety of RGS proteins than there are G_{α} proteins. As depicted in Table 2, the GTPase activity of many G proteins is regulated by multiple RGS proteins. The presence of this natural redundancy may be to ensure proper control of intracellular signaling under a variety of cellular or physiological conditions. On the other hand, cell- or region-specific expression of subtypes of RGS proteins and G_{α} proteins may limit the possible interactions and may be one way that specificity is achieved between RGS proteins and G_{α} proteins (Gold 1997).

4. TRANSCRIPTIONAL REGULATION OF RECEPTOR SIGNALING

Transcriptional regulation usually signifies a change in the amount of protein produced by the cell's transcriptional machinery. This is typically measured as a change in the level of mRNA and/or a change in the level of protein. A change in the levels of mRNA does not indisputably result in a change in the levels of protein as other post-transcriptional factors, such as protein degradation and stability of the mRNA must be considered. Both transcriptional and post-transcriptional regulation will be discussed in this section.

4.1. Transcriptional regulation of receptor signaling

Transcriptional regulation of receptor signaling can encompass a downregulation of receptors, G proteins, RGS proteins, or second messenger enzymes and other effectors. Downregulation is defined as a decrease in the amount of protein expressed or a decrease in the density of receptors labeled with an antagonist.

A reduction in the density of membrane receptors, while all other signaling components remain unchanged, will decrease signal transduction across the membrane. Hence, in a dose-response experiment, the maximal response (E_{max}) will be reduced. An increase in the density of membrane receptors may cause a larger cellular and physiologic response if the expressed receptors are functionally coupled to their intracellular signaling components (increased E_{max}). A reduction in the amount of G proteins will hinder the external signal from being fully amplified between the receptor and second messenger system. In a dose-response experiment, this will result in an increase of the dose of drug needed to produce 50% of the maximal response (ED_{50}). A surplus of G proteins may allow for a more efficient transfer of the extracellular signal (increased ED_{50}). While many treatments have been shown to alter receptor expression, chronic treatment with antidepressant drugs (clorgyline, desipramine, fluoxetine, imipramine) also produces treatment- and

region-specific changes in protein levels and in mRNA levels of $G_{\alpha s}$, $G_{\alpha i}$, and $G_{\alpha q}$ proteins in rat brain (Lesch 1992). This could be one mechanism of action of certain antidepressant agents. Therefore, either changes in levels of receptor proteins or changes in levels of G_{α} proteins or both are possible mechanisms to alter signal transduction.

A reduction in RGS proteins would lead to a decrease in the velocity of the GTPase activity of the G_{α} proteins. The G protein would activate second messenger enzymes for a longer time and thus would evoke a greater physiologic response (increased E_{max}). On the other hand, an increase in the amount of RGS protein would enhance the GTPase activity of G_{α} proteins and lead to a smaller physiologic response (decreased E_{max}). A decrease in the levels of RGS4 proteins was identified in prefrontal cortex of schizophrenic patients (Mirnics 2001). It is not known whether this change in levels of RGS4 proteins is due to a genetic factor or due to a specific adaptation to the disease. This decrease in RGS4 proteins may allow for a greater cellular response and, subsequently, greater neuronal signaling in the prefrontal cortex. The alteration in levels of RGS4 proteins may provide insight in the regional/cellular etiology of schizophrenia.

4.2. Post-transcriptional regulation of receptor signaling

Post-transcriptional regulation of signaling can occur at the level of degredation of proteins or post-translational modifications (See Section 5) that change a protein's ability to function. Increased degredation without a compensatory increase in transcription and translation of a specific protein will eventually lead to a decrease in the total amount of the protein within the cell or in the cellular membrane. The opposite is also likely. An increase in transcription/translation without a compensatory increase of degradation will lead to an increase in total levels of the protein. While this type of regulation can occur for receptors, G proteins, RGS proteins, and effector proteins, there are not many examples of a change in the half-lives of proteins changing due to a disorder or its treatment. The agonist-induced decrease in serotonin 2A (5-HT_{2A}) receptor density is preceeded by a decrease in 5-HT_{2A} receptor mRNA (Anji 2001). It is possible that protein degredation may only be an immediate response to decreased protein expression before a more stable change in mRNA can occur.

5. POST-TRANSLATIONAL REGULATION OF SIGNALING

Post-translational modifications of proteins by an attachment of a phosphate side-chain (phosphorylation) or the addition of a fatty acid side-chain can be integral to the proper function of the protein and its cellular localization. Phosphorylation of G proteins can change the interaction between G proteins and other proteins, such as

receptors and RGS proteins, or change the activity of the G protein. A common example of phosphorylation changing protein function is kinase-mediated internalization of receptors and desensitization of receptor signaling (Lohse 1992). This section will focus on phosphorylation and the most commonly described lipid modifications (myristoylation, palmitoylation and prenylation) of G proteins and their associated proteins.

Palmitoylation of G_α proteins and prenylation of G_γ proteins are important lipid modifications that assist in the association of the G protein subunits with the receptor and the bilayer lipid membrane (Fig. 2). No lipid modifications have been identified for G_β proteins, although this is not to say that none exist. The exact mechanism as to how lipid modification targets the G protein to the bilayer membrane is unknown. The simplest explanation is that the lipid inserts directly into the hydrophobic bilayer membrane and thereby anchors the G protein to the cell membrane. *In vitro* assays measuring the affinity of various acylated peptides for lipid membranes showed that myristoylation and farnesylation do not have sufficient energy to dock proteins to the membrane on their own (Silvius 1994; Peitzsch 1993). Only palmitate and geranylgeranyl isoprenoids have sufficient energy to stably anchor a protein to a lipid membrane. Thus, while some lipid modifications of proteins are sufficient by themselves to target some proteins to the cell membrane, other proteins require additional factors, such as protein-protein interactions. Some lipid modifications may target the G protein to an integral membrane protein rather than the membrane.

Protein-protein interactions can assist in recruiting the G protein or RGS protein to the membrane by association with membrane bound proteins (such as phosphodiesterase MIR16) (Zheng 2000), intracellular scaffolding proteins (14-3-3 proteins) (Benzing 2000), cytoskeletal proteins (tubulin) (Donati 2003), coatomer proteins on secretory membranes (β-COP) (Sullivan 2000), and binding partners such as GIPC (GAIP-interacting protein, C terminus) (Booth 2002; Lou 2001; De Vries 1998). Axin and conductin, RGS proteins of the RA family, do not have GAP activity but can serve as a scaffolding proteins for G proteins by linking the G protein to its downstream effectors (Hollinger 2002). The $G_{\beta\gamma}$ proteins are also thought to assist in recruiting the G_α protein to the cell membrane by acting as membrane-bound docking proteins. This was demonstrated by coexpressed $G_{\beta\gamma}$ and G_α proteins increasing the amount of functional, membrane-bound G_α protein (Linder 1993). Thus, some lipid modifications of G_α proteins do not directly anchor the G_α protein to the cell membrane but may anchor G_α proteins to $G_{\beta\gamma}$ proteins or other membrane-bound proteins.

5.1. Prenylation

Prenylation is the attachment of a 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid to cysteine residues of proteins. All G_γ proteins are geranylgeranylated on their C-terminal, except for the G_γ protein subunits ($G_{\gamma 1}$ and

$G_{\gamma 11}$) of the retinal trimeric G protein, which are farnesylated (Gautam 1998; Yamane 1990; Mumby 1990; Fukada 1990). The exact reason for the different type of prenylation for the retinal G_γ protein is unkown. Generally, prenylation of the G_γ protein is necessary for its localization to the membrane (Muntz 1992; Simonds 1991) but is not necessary for the formation of the $G_{\beta\gamma}$ dimer (Iniguez-Lluhi 1992). Even though the $G_{\beta\gamma}$ dimer can be formed with an unprenylated G_γ protein, the $G_{\beta\gamma}$ dimer does not have high affinity interactions with G_α proteins or adenylyl cyclase (Fig. 2). This suggests that prenylation of G_γ proteins is important to cellular signaling by affecting both cellular localization and protein-protein interactions.

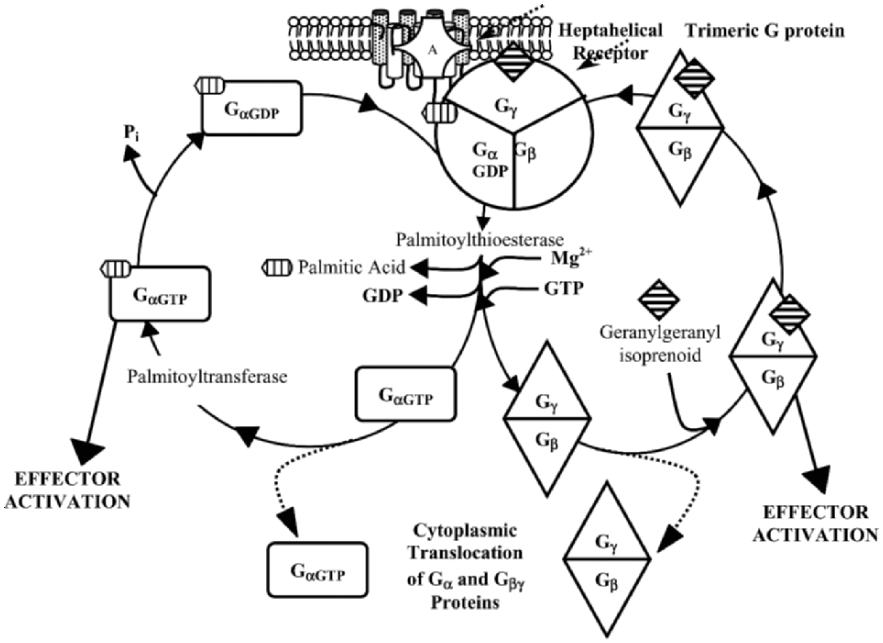


Figure 2. Importance of palmitoylation of G_α protein and prenylation of G_γ protein to the efficiency of signaling. Abbreviations: A, agonist; GDP, guanosine diphosphate; GTP, guanosine triphosphate; P_i, inorganic phosphate; Mg^{2+}, magnesium ion.

5.2. Myristoylation

Myristoylation is the attachment of a 14-carbon myristate fatty acid to a N-terminal glycine of G_α proteins. The attachment of this lipid was once thought to be critical for membrane anchoring of G_α proteins, such as $G_{\alpha z}$ (Hallak 1994). Yet, the expression of myristoylated, but not palmitoylated, forms of $G_{\alpha o}$ and $G_{\alpha i}$ proteins in cells led to an increased proportion of G_α proteins in the soluble cellular fraction (Mumby 1994; Galbiati 1994; Grassie 1994; Degtayarev 1994). This demonstrated that myristoylation is not critical for membrane anchoring of all subtypes of G_α proteins. It was hypothesized that myristoylation of certain G_α proteins, such as $G_{\alpha i}$ proteins, may be necessary before those G_α proteins could be palmitoylated (Galbiati 1994). This hypothesis does not hold true for all G_α proteins as some G_α proteins do not undergo myristoylation but are palmitoylated, such as $G_{\alpha s}$ and $G_{\alpha q}$ proteins (Mumby 1994). Therefore, palmitoylation may be more important than myristoylation for membrane localization of certain G_α proteins. Thus, the importance of myristoylation is not universal and is dependent on the type of G_α protein.

Myristoylation can effect the ability of certain G_α proteins to stimulate downstream effectors, independent of membrane association of the G_α protein. While non-myristoylated $G_{\alpha z}$ proteins still retained some of their ability to inhibit adenylyl cyclase activity (Wilson 1995), myristoylation of $G_{\alpha i}$ proteins is required for the inhibition of adenylyl cyclase in an *in vitro*, cell-free system (Taussig 1993). Thus, while myristoylation is not required for the membrane localization of $G_{\alpha i}$ proteins, it is necessary to proper interaction between $G_{\alpha i}$ proteins and their effector enzyme. Hence, myristoylation is important for membrane localization and G_α protein-effector protein interactions for certain G_α proteins.

5.3. Palmitoylation

Because of its importance in protein localization to the membrane, palmitoylation is important for proper receptor-mediated signaling (Qanbar 2003). Palmitoylation is the covalent attachment of palmitate (16-carbon fatty acid) to any cysteine residue. No consensus sequence is required for palmitoylation and it is a reversible process (Mumby 1997). All G_α proteins, except for $G_{\alpha t}$ proteins, are palmitoylated (Wedegaertner 1995). Palmitoylation of G_α proteins is important for the localization of the G_α protein to lipid membranes (Fig. 2). *In vitro* assays have shown that palmitoylated $G_{\alpha o}$ subunits associate more strongly with membrane fractions than their non-palmitoylated counterparts.[266] Mutations of the palmitoylation sites of G_α proteins have yielded an increased amount of mislocalized or soluble G_α proteins (Qanbar 2003; Fishburn 2000). Membrane-bound proteins or kinases may also assist in sequestering G_α proteins to the lipid membrane. Membrane-bound palmitoyltransferases (MIR16) (Zheng 2000) or $G_{\beta\gamma}$ proteins

(Hughes 2001), acting as a temporary membrane-bound docking proteins, may recruit a non-palmitoylated G_α protein to the cell membrane. Once it is palmitoylated, the G_α protein can associate with the cell membrane directly. Other lipid modifications, such as myristoylation, or protein interactions of the G_α protein with receptors or $G_{\beta\gamma}$ proteins may localize the G_α protein after its activation.

Activation of $G_{\alpha s}$, $G_{\alpha i}$, and $G_{\alpha q}$ proteins by β-adrenoceptors (Mumby 1994; Degtyarev 1993), cholera toxin, or serotonin receptors (Chen 2000; Bhamre 1998) in cell culture or in tissue preparations resulted in an increase in palmitate labeling of the G_α proteins. This increase in palmitate labeling could either be due to an increase in the incorporation of palmitate into G_α proteins or rather an increase in the turnover of the palmitoylation-depalmitoylation cycle represented by the attachment of palmitate into G_α proteins. Pulse-chase methods and half-life measurements in cell culture studies have supported the latter hypothesis (Mumby 1994; Wedegaertner 1994). The exact mechanisms of how and when the G_α proteins become palmitoylated and depalmitoylated are still not known. Based on our current knowledge (Fig. 2), stimulation of receptors causes a depalmitoylation of G_α proteins, but the signal that triggers the repalmitoylation of the G_α proteins is not known. While palmitoylation is an important component to proper membrane localization, it is not clear how the activation-dependent depalmitoylation of G_α proteins fits into proper signaling. Perhaps depalmitoylation and dissociaton from the membrane are required before the G_α proteins can undergo transformation into their GTP-bound form. On the other hand, immunohistochemical evidence in transfected HEK-293 cells showed that α_{2A}-adrenergic receptor-mediated and AlF_4-mediated activation of $G_{\alpha q}$ proteins did not change the cellular localization of the $G_{\alpha q}$ proteins (Hughes 2001). These $G_{\alpha q}$ proteins were still associated to the cellular membrane. This suggests that activation of G_α proteins may not necessarily lead to depalmitoylation and cellular relocation of the G_α proteins. Thus, the current model of palmitoylation/depalmitoylation of G_α proteins (Fig. 2) will most likely undergo some fine tuning in the near future. Although many of the regulatory mechanisms need to be elucidated, the regulation of palmitoylation and depalmitoylation of G_α proteins may provide us with novel pharmacological targets to control G_α protein-mediated signaling.

RGS proteins, as well as G proteins, can be palmitoylated (RGS4, RGS7, RGS10, RGS16, and GAIP) (Osterhout 2003; Hollinger 2002). This lipid modification has been shown to be important for the GAP activity of RGS4, RGS10, and RGS16, and membrane association of RGS4, RGS7, and GAIP (Hollinger 2002). Thus, G protein signaling can be altered by palmitoylation of G_α proteins and/or RGS proteins.

Palmitoylation of receptors also affects signaling (Qanbar 2003). Mutations on the palmitoylation sites of β_2-adenoceptors (Moffett 1993; O'Dowd 1989), endothelin A (ET_A) receptors (Doi 1999), m2 muscarinic receptors (Hayashi 1997),

and somatostatin receptors (Hukovic 1998) resulted in the uncoupling of the receptors from their G proteins, thereby decreasing receptor-mediated enzyme activity. Interestingly, palmitoylation of the receptor can have differential effects on the G proteins that bind to it. The unpalmitoylated form of the human ET_A receptor is less effective in stimulating $G_{\alpha i}$ and $G_{\alpha q}$ proteins but it stimulates $G_{\alpha o}$ proteins to the same degree as palmitoylated ET_A receptors (Doi 1999). Site-directed mutagenesis of a palmitoyolated cysteine (C341G) on the β_2-adrenergic receptors led to the receptor being highly phosphorylated and decreased receptor coupling to $G_{\alpha s}$ proteins (Moffett 1993). Thus, post-translational lipid modifications can contribute to the specificity of a receptor for a particular G protein, its degree of interaction, and also influence other post-translational modifications.

5.4. Phosphorylation

Phosphorylation of GPCRs occurs mainly in consensus sequences in the second and third intracellular loops of these receptors. However, other intracellular portions of the receptors also can be phosphorylated. The phosphorylation of receptors can lead to the inactivation of the receptor and a reduction in signaling due to internalization and/or a disruption of the interaction between the receptor and its trimeric G protein. Phosphorylation of receptor proteins by kinases has been associated with receptor desensitization due to β-arrestin-dependent or -independent internalization and inactivation of adrenergic and serotonergic receptors (Gray 2001; Lohse 1992). Phosphorylation of the gastrin-releasing peptide receptor, in the absence of arrestins can reduce receptor-mediated activation of G_α proteins by approximately 80% (Kroog 1999). This same study showed that the desensitization observed was due to a decrease in the catalysis of guanine nucleotide exchange rather than a change in the affinity of the receptor for trimeric G proteins. Hence, phosphorylation of receptors can alter intracellular signaling by internalizing the receptor, hindering receptor-G_α protein interaction, or altering guanine nucleotide exchange.

Phosphorylation of G protein subunits (Chen 2001) directly can decrease the association between the trimeric G protein and receptor or between the G_α protein and effector enzyme leading to a decrease in G protein-mediated signaling. Phosphorylation of $G_{\alpha z}$ proteins hinders them from reassociating with $G_{\beta\gamma}$ proteins (Fields 1995) and decreases the susceptibility of the $G_{\alpha z}$ protein to the GAP activity of RGSZ1 (Glick 1998). The phosphorylation of G_γ proteins, causes them to associate more with G_α proteins than with effector enzymes, thereby decreasing G_γ protein-mediated signaling.[281] Phosphorylation does not necessarily have to affect G protein signaling in a negative manner. Phosphorylated forms of $G_{\alpha q/11}$ proteins are more efficacious in stimulating phospholipase C_β (PLC_β) activity (Liu 1996). For receptors that activate kinases, this may be one mechanism by which they can amplify their own signal by enhancing $G_{\alpha q/11}$ protein activity (Umemori 1997).

Phosphorylation of RGS proteins can also affect G protein signaling (Hollinger 2002). Phosphoryl-ation of RGS proteins functions to directly alter GAP activity, membrane localization, and association with 14-3-3 scaffolding proteins.[186] Phosphorylation is necessary for the interaction of RGS3 and RGS7 with the scaffolding protein 14-3-3 (Benzing 2000 and 2002; Niu 2002). These same studies showed that interacting with 14-3-3 proteins decreased the ability of RGS3 proteins to interact with G_α proteins and decreased the GAP activity of RGS7 proteins (Niu 2002; Benzing 2000). After phosphorylation, GAP activity is attenuated for RGS2 (Cunningham 2001) and RGS16 (Chen 2001) proteins but is enhanced for GAIP proteins (Ogier-Denis 2000). Phosphorylation of RGS proteins can affect the activity of RGS proteins by mechanisms other than directly affecting GAP activity. For example, the RGS proteins associate with the 14-3-3 protein instead of G_α proteins, thereby increasing the duration that the G_α proteins can remain in their active form (G_α-GTP). In addition, nuclear translocation of RGS10 occurs after phosphorylation and is associated with a reduction in the ability of RGS10 to regulate G_α protein activation (Burgon 2001). Thus, phosphorylation of RGS proteins can modulate the activity of RGS, G_α, and $G_{\beta\gamma}$ proteins by a variety of mechanisms.

6. G PROTEIN-ASSOCIATED CNS DISEASES

This section will highlight psyciatric disorders associated with changes in G proteins, RGS proteins, and some GPCRs. Since each disorder will be covered in its own chapter within this book, we will only focus on changes of G proteins and related proteins or signaling cascades associated with the disease state or with its treatment. Many of the studies measure mRNA or protein levels in post-mortem tissue while others use animal models to investigate the effect of pharmacological treatments on receptor signaling and protein levels. Although we have mentioned a few studies utilizing knock-out animals, we will not provide a comprehensive overview of all the studies utilizing transgenic and knock-out animals. These genetic manipulations in whole animals occur prior to birth and, if the alteration is not fatal, may lead to complementary developmental changes, such as compensation by another protein or signaling pathway. Animals with inducible knock-out genes, where the elimination of a specific protein occurs after the animal matures, are a novel attempt to avoid the issue of developmental compensation in genetically altered animals.

6.1. Anxiety and neuroticism

Anxiety disorders encompass generalized anxiety disorder (GAD), panic disorder, social phobia, posttraumatic stress disorder, and obsessive-compulsive disorder (OCD) (Nutt 1996). Anxiety is considered to be an inappropriate fear

response or a fear response at an inappropriate time and is associated with improper signaling from the central nucleus of the amygdala (Ninan 1999). An estimated 19 million American adults suffer from some form of anxiety (NIMH 2002). Anxiety disorders are traditionally treated with benzodiazepines, although serotonin 1A (5-HT_{1A}) receptor agonists (buspirone) and selective serotonin reuptake inhibitors (SSRIs) are efficacious without the risks of abuse, dependence, and withdrawal effects that are associated with benzodiazepines. A better understanding of the role of the gamma amino butyric acid (GABA) system in anxiety and in the mechanism of action of benzodiazepines has led to the development of GABA receptor antagonists that target specific subunits of $GABA_A$ receptors to regulate GABA-ergic transmission. Glutamate, corticotrophin releasing factor/hormone (CRF or CRH) and substance P are other neurotransmitters that are abnormally regulated in anxiety disorders. Antagonists of CRF and substance P receptors are currently under investigation for the treatment of anxiety (Goman 2003).

Subchronic treatment of rats with buspirone, a 5-HT_{1A} receptor partial agonist, decreases $G_{\alpha i1}$ and $G_{\alpha i2}$ protein levels in the cerebellum (Dwivedi 1997). On the other hand, alprazolam, a benzodiazapine, and metachlorophenylpiperazine (m-CPP), an anxiogenic drug, did not change the levels of $G_{\alpha s}$, $G_{\alpha i}$, or $G_{\alpha q/11}$ proteins in a variety of brain regions. Alterations in levels of G_α proteins could be treatment-specific. Thus, the importance of G_α proteins in the etiology or treatment of anxiety is still not known. The importance of RGS2 proteins in anxiety and aggression is implicated by knockout mice (rgs-/-) displaying increased anxiety and decreased male aggression (Oliveira-dos-Santos 2000).

6.2. Autism and autistic disorders

Autism is a childhood behavioral and neurological disorder with onset prior to three years of age. The main features of autism spectrum disorders (ASD) are deficits in language, social, and emotional functioning, with significantly variable secondary symptoms of aggression, self-injurious behavior and impulsivity. Autism now affects 62-67 per 10,000 births, with boys being affected four times more frequently than girls (Bertrand 2001; Chakrabarti 2001).

While the exact cause of autism is unknown, an abnormal functioning of neurotransmitter receptors may be one mechanism involved. The theory of vaccine-related autism is actually related to the pertussis toxin found in the diphtheria, pertussis, and tetanus (DPT) vaccine. The toxin in the vaccine uncouples G_α proteins from retinoid receptors in the brain, thereby functionally uncoupling receptors from their intracellular signaling proteins (Megson 2000). A pre-existing family history of defects in G_α proteins (night blindness, pseudohypoparathyroidism, and thyroid or pituitary adenoma) increases the risk of autism due to the vaccine.

6.3. Bipolar Disorder

Bipolar (manic-depressive) disorder is characterized by cycling episodes of depression and mania with a lifetime prevalence of 1.2% (Weissman 1988). The postmortem frontal cortex tissue of bipolar victims shows enhanced receptor-to-G protein coupling along with increased trimeric states of the G proteins (Friedman 1996). This study shows an increased expression of $G_{\alpha s}$ proteins without changes in levels of other G proteins, but all G proteins show a higher propensity to be in their trimeric state. The higher fraction of trimeric G proteins may indicate a supersensitization of G protein-mediated signaling in patients with bipolar disorder.

Lithium is a common antimanic agent. In agreement with the possibility that bipolar disorder may be partially due to an increase in $G_{\alpha s}$ protein levels, bipolar patients treated with lithium showed a decrease in $G_{\alpha s}$ protein levels in the occipital cortex (Dowlatshahi 1999). Lithium treatment also decreases ADP-ribosylation of $G_{\alpha i}$ proteins (Watanabe) and $G_{\alpha i}$ protein expression in cerebral cortex (Colin 1991) while increasing adenylyl cyclase expression in the cerebral cortex. Lithium has also been shown to decrease levels of $G_{\alpha i1/2}$ in rat cortex and hippocampus after subchronic treatment (Dwivedi 1997). Lithium's ability to change the levels of adenylyl cyclase, $G_{\alpha i}$ and $G_{\alpha s}$ proteins, both of which regulate adenylyl cyclase activity, indicates that bipolar disorder may be due to an altered signaling of adenylyl cyclase pathways.

More recent studies suggest that lithium, valproic acid, and carbamazepine decrease the levels of inositol in the brain (Harwood 2003; Wolfson 2000; Dixon 1997; Berridge 1982). Inositol is the precursor to phosphatidyl inositol bisphosphate (PIP_2) which is cleaved by activated PLC to generate inositol trisphosphate (IP_3) and diacylglycerol (DAG). Thus, the signaling cascade is desensitized by decreasing the amount of the required precursor (inositol) and not by modifying the function of either enzyme or G protein. The observation that lithium decreases inositol levels in critical brain areas gave rise to the most widely accepted hypothesis for bipolar disorder the inositol depletion hypothesis. One major criticism of the hypothesis is that the decrease in inositol levels does not necessarily correlate to the therapeutic onset of lithium treatment, although it has not been demonstrated that the therapeutic onset of lithium is independent of a decrease in inositol levels (Moore 1999).

6.4. Depression

Depression is an affective (mood) disorder characterized by anhedonia that affects approximately 9.5% of American adults annually (NIMH 2002). A decrease in the synaptic levels of monoamines (serotonin, norepinephrine, epinephrine) in the CNS may be an underlying cause of several mood disorders, including depression and anxiety (Delgado 200). The serotonin system has been of particular interest in antidepressant treatment after the success of selective serotonin reuptake inhibitors

(SSRIs), such as fluoxetine (Prozac®). Although fluoxetine increases the synaptic levels of serotonin (5-HT) within a few days after administration of the drug, the symptoms of depression are not alleviated for another 2-3 weeks. This delay indicates that a mere increase in levels of synaptic monoamine is not sufficient to provide antidepressant effects. Thus, neuroadaptive changes occurring within the intracellular signaling cascade may mediate the antidepressant effects of SSRIs (Manji 1999; Li 1996; Raap 1999).

In comparison to the number of studies that have examined the role of receptors in depression, there are fewer studies that have investigated changes in G proteins or RGS proteins in depression or its therapy (Donati 2003). PET scans in brains of depressed patients have generally shown decreased 5-HT_{1A} receptor densities and increased 5-HT_{2A} receptor densities (Dhaenen 2001). Abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis are associated with depression as an attenuated release of ACTH to CRH administration is seen in depressed patients (Holsboer 2000; Heuser 1994). Clinical studies have shown that HPA activity normalizes after chronic antidepressant treatment and is associated with an improved prognosis (Inder 2001; Sonino 1996; Barden 1995). Neuroendocrine measures have shown decreased 5-HT_{1A} receptor-mediated secretion of ACTH and cortisol in depressed patients (Meltzer 1994 and 1995; Pitchot 1995; Lesch 1990). Treatment with chronic fluoxetine desensitizes postsynaptic 5-HT_{1A} receptors in the hypothalamic paraventricular nucleus in rats, a phenomenon associated with decreased levels of $G_{\alpha i}$, $G_{\alpha o}$, and $G_{\alpha z}$ proteins (Raap 1999; Li 1997). Human studies also indicate that treatment with SSRIs induces a desensitization of hypothalamic post-synaptic 5-HT_{1A} receptors (Lerer 1999; Berlin 1998; Sargent 1997). However, this desensitization may be a unique effect of SSRis because the monoamine oxidase inhibitor phenelzine increases levels of $G_{\alpha i1/2}$ in the cortex and hippocampus of rats after subchronic treatment (Dwivedi 1997). The tricyclic antidepressants do not seem to affect G proteins as subchronic treatment with desipramine does not alter levels of $G_{\alpha s}$, $G_{\alpha i1/2}$, or $G_{\alpha q/11}$ proteins in the brain. Hence, the effects of SSRIs on post-synaptic 5-HT_{1A} receptors in hypothalamus and amygdala (Bosker 2001) may represent their unique therapeutic effects on a variety of other mood disorders (anxiety, eating disorders, OCD, premenstrual syndrome). On the other hand, it is likely that several different neurochemical disorders underlie the symptoms of depression. Thus, different anti-depressants can be useful to treat depression because they may alter different neurochemical abnormalities. Consistent with this possibility is the fact that approximately 30% of depressed patients do not respond to the first monotherapy with an antidepressant.

Densities of β-adrenoceptors have been shown to increase, decrease, and not change in brains of depressed victims (De Paermentier 1990 and 1911). These different effects of antidepressant treatments on densities of β-adrenoceptors may be due to differences in the brain area studied, psychiatric diagnosis, and previous pharmacological treatment of the suicide victims. On the other hand, an increase in

the high-affinity state of α_{2A}-adrenoceptors without a change in the density of α_{2A}-adrenoceptor was observed in tissue samples of frontal cortex, hypothalamus, and locus coeruleus obtained from suicide victims with major depression (Callado 1998). The increase in the density of the high-affinity state of α_{2A}-adrenoceptors suggests a change in the receptor-to-G protein coupling, which may be indicative of a change that occurs within the intracellular portion of the lipid bilayer. Coincidently, increased levels of $G_{\alpha s}$ proteins have been reported in the cerebral cortex of subjects with major depressive disorder (Pacheco 1996). Hence, it is possible that the increase in the high-affinity state of α_{2A}-adrenoceptors in individuals affected with major depression is due to an increase in the respective G_{α} protein, specifically $G_{\alpha s}$ protein.

6.5. Neurodegenerative diseases

Neuronal loss is the pathological trademark of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Most research in neurodegeneration focuses on theories based on changes in amyloid, tau, or synuclein proteins. The amyloid and tau theories state that β-amyloid (Aβ) proteins and tau aggregate into β-amyloid plaques and neurofibrillary tangles, respectively; both being hallmarks of Alzheimer pathology. While both of these events are thought to be key in neuronal destruction, other research has shown neurotrophic growth factors (Siegel 2000), estrogen (Gandy 2003), cholesterol (Puglielli 2003), and lipoproteins (Neely 2002) also to have an effect on neurodegenerative diseases. We will focus on the research that specifies the involvement of G proteins and their associated proteins.

6.5.1. Alzheimer's disease

Alzheimer's disease is the leading cause of dementia and affects about 4.5 million Americans (Alzheimer's Association, Statistics 2003). Alzheimer's disease is associated with a disruption in the coupling of $G_{\alpha q}$ proteins to their effector enzyme phospholipase C, which may lead to a reduction in glutamate, histamine, and serotonin receptor-mediated phosphoinositide hydrolysis (Cowburn 1996). This disruption occurs without changes in the levels of $G_{\alpha q}$ proteins in various brain regions of affected individuals. However, another study has shown a decrease in total levels of $G_{\alpha q/11}$ and RGS4 proteins, without a change in the membrane levels of either protein, in parietal cortex obtained post-mortem from patients with Alzheimer's disease (Muma 2003). $G_{\alpha s}$ protein-mediated adenylyl cyclase activity, but not forskolin-stimulated adenylyl cyclase activity, is reduced in various brain areas from patients with Alzheimer's disease (Cowburn 1996). Interestingly, $G_{\alpha i}$ mediated intracellular signaling does not change in brains of Alzheimer's patients. Thus, while not all the results seem to be consistant, Alzheimer's disease does appear to effect the signal transduction of both $G_{\alpha q}$ and $G_{\alpha s}$ proteins.

6.5.2. Parkinson's disease

Parkinson's disease is characterized by abnormalities in extrapyramidal motor function. The pathology of Parkinson's disease is the degeneration of dopaminergic neurons in the midbrain substantia nigra (Brooks 2003). These neurons project to the striatum, composed of the caudate nucleus and the putamen. It is the loss of striatal dopaminerigic innervation that leads to a decrease in the dopamine content of the striatum. While the decrease in striatal dopamine explains the abnormalities in extrapyramidal motor functions, a complete knowledge of the molecular adaptations that follow the dopaminergic denervation of the striatum is lacking. There have been a few studies that have addressed this issue. Western blot analyses show an increase in RGS9 protein levels in the caudate and putamen (or dorsal striatum) of patients with Parkinson's disease (Tekumalla 2001). This indicates a striatal adaptation that occurs in Parkinson's disease and may provide a target for future pharmacological agents.

6.6. Schizophrenia

Schizophrenia is a psychiatric disorder characterized by positive symptoms (visual and auditory hallucinations, delusions) and negative symptoms (apathy and lack of motivation). The original (typical) antipsychotic drugs are dopamine (D_2) receptor antagonists. Atypical antipsychotic agents that are antagonists of serotonin (5-HT) receptors, with weak antagonism of D_2 receptors, have been more effective than typical antipsychotic drugs in the treatment of the positive symptoms of schizophrenia and cause less extrapyramidal side-effects than typical antipsychotic drugs. An upregulation of D_2 receptors (Kapur 1996) and 5-HT_{1A} receptors (Bantick 2001) and a downregulation of 5-HT_{2A} receptors (Hernandez 2000) have been documented in brains of schizophrenic patients post-mortem. A decrease in levels of $G_{\alpha i}$, $G_{\alpha o}$, and $G_{\alpha q}$ proteins, but not of $G_{\alpha s}$ or G_{β} proteins, was detected in the superior temporal cortex of schizophrenic patients (Yang 1998). Paradoxically, an upregulation of $G_{\alpha i}$ protein-coupled receptors, such as D_2 and 5-HT_{1A} receptors, was observed while a decrease in $G_{\alpha i}$ proteins was observed in another study. These data may lead us to hypothesize that D_2 and 5-HT_{1A} receptors are upregulated to compensate for a decrease in levels of $G_{\alpha i}$ proteins. In addition to changes in levels of G_{α} proteins, the regulation of G_{α} proteins might also be altered in schizophrenia. Gene microarray analysis showed a 50-84% decrease in expression of RGS4 protein in cortical areas of subjects with schizophrenia.[203] RGS4 has GAP activity for both $G_{\alpha i}$ and $G_{\alpha q}$ proteins. Since a decrease in cortical levels of $G_{\alpha i}$ and $G_{\alpha q}$ proteins was reported, a decrease in RGS4 protein levels would be one possible compensatory mechanism to counter-balance the decrease in levels of G_{α} proteins. These thoughts are just speculation as it is not know which proteins change first and which are

compensations. Furthermore, because of the difficulty of finding brain tissue from untreated schizophrenic patients, it is not entirely clear that the changes in the schizophrenic brain are due to the disorder or an adaptive change induced by treatment with antipsychotic drugs. Nevertheless, these studies add support to the idea that schizophrenia may be mediated by abnormal intracellular signaling due to alterations in G proteins in various brain areas.

7. CONCLUSION

The importance of G proteins and their associated signaling cascades in the etiology of neuropsychiatric disorders is receiving increasingly extensive attention. G proteins and their associated signaling proteins provide intracellular regulation and modulation of extracellular signals, which can be utilized to develop novel pharmacological agents to treat psychiatric disorders. Such medications may not have the specificity of drugs that affect specific receptors. However, medications that address post-receptor signaling proteins may be useful in disorders involving multiple receptor systems, particularly those that utilize common second messenger systems (for example, the treatment of bipolar disorder).

8. REFERENCES

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N., and Nakanishi, S., 1992, Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca2+ signal transduction, *J. Biol. Chem.* **267**(19), 13361-13368.

Allgeier, A., Offermanns, S., Van Sande, J., Spicher, K., Schultz, G., and Dumont, J. E., 1994, The human thyrotropin receptor activates G-proteins Gs and Gq/11, *J. Biol. Chem.* **269**(19), 13733-13735.

Alzheimer's Association, Statistics: About Alzheimer's Disease, (2003), http://www.alz.org/AboutAD/Statistics.htm

Amatruda, T. T., III, Gerard, N. P., Gerard, C., and Simon, M. I., 1993, Specific interactions of chemoattractant factor receptors with G-proteins, *J. Biol. Chem.* **268**(14), 10139-10144.

Anji, A., Hanley, N. R. S., Kumari, M., and Hensler, J. G., 2001, The role of protein kinase C in the regulation of serotonin-2A receptor expression, *Journal of Neurochemistry* **77**(2), 589-597.

Aragay, A. M.,Collins, L. R., Post, G. R., Watson, A. J., Feramisco, J. R., Brown, J. H., and Simon, M. I., 1995, G12 requirement for thrombin-stimulated gene expression and DNA synthesis in 1321N1 astrocytoma cells, *J. Biol. Chem.* **270**(34), 20073-20077.

Aramori, I., and Nakanishi, S., 1992, Coupling of two endothelin receptor subtypes to differing signal transduction in transfected Chinese hamster ovary cells, *J. Biol. Chem.* **267**(18), 12468-12474.

Asano, T., Morishita, R., Ueda, H., and Kato, K., 1999, Selective association of G protein beta(4) with gamma(5) and gamma(12) subunits in bovine tissues, *J. Biol. Chem.* **274**(30), 21425-21429.

Audigier, Y., Nigam, S. K., and Blobel, G., 1988, Identification of a G protein in rough endoplasmic reticulum of canine pancreas, *J. Biol. Chem.* **263**(31), 16352-16357.

Austin, C. E., Faussner, A., Robinson, H. E., Chakravarty, S., Kyle, D. J., Bathon, J. M., and Proud, D., 1997, Stable expression of the human kinin B1 receptor in Chinese hamster ovary cells. Characterization of ligand binding and effector pathways, *J. Biol. Chem.* **272**(17), 11420-11425.

Baffy, G., Yang, L., Raj, S., Manning, D. R., and Williamson, J. R., 1994, G protein coupling to the thrombin receptor in Chinese hamster lung fibroblasts, *J. Biol. Chem.* **269**(11), 8483-8487.

Bantick, R. A., Deakin, J. F. W., and Grasby, P. M., 2001, The 5-HT_{1A} receptor in schizophrenia: a promising target for novel atypical neuroleptics?, *Journal of Psychopharmacology* **15**(1), 37-46.

Barden, N., Reul, J. M. H. M., and Holsboer, F., 1995, Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system, *Trends Neurosci.* **18**, 6-11.

Barr, A. J., Ali, H., Haribabu, B., Snyderman, R., and Smrcka, A. V., 2000, Identification of a region at the N-terminus of phospholipase C-β3 that interacts with G protein βgamma subunits, *Biochemistry* **39**(7), 1800-1806.

Barrett, R. W., Steffey, M. E., and Wolfram, C. A., 1989, Type-A cholecystokinin receptors in CHP212 neuroblastoma cells: evidence for association with G protein and activation of phosphoinositide hydrolysis, *Mol. Pharmacol.* **35**(4), 394-400.

Bayewitch, M., Avidor-Reiss, T., Levy, R., Barg, J., Mechoulam, R., and Vogel, Z., 1995, The peripheral cannabinoid receptor: adenylate cyclase inhibition and G protein coupling, *FEBS Lett.* **375** (1-2), 143-147.

Behrens, J., Jerchow, B. A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D., and Birchmeier, W., 1998, Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta, *Science* **280**(5363), 596-599.

Benzing, T., Kottgen, M., Johnson, M., Schermer, B., Zentgraf, H., Walz, G., and Kim, E., 2002, Interaction of 14-3-3 Protein with Regulator of G Protein Signaling 7 Is Dynamically Regulated by Tumor Necrosis Factor-alpha, *J. Biol. Chem.* **277**(36), 32954-32962.

Benzing, T., Yaffe, M. B., Arnould, T., Sellin, L., Schermer, B., Schilling, B., Schreiber, R., Kunzelmann, K., Leparc, G. G., Kim, E., and Walz, G., 2000, 14-3-3 Interacts with regulator of G protein signaling proteins and modulates their activity, *J. Biol. Chem.* **275**(36), 28167-28172.

Berg, K. A., Maayani, S., Goldfarb, J., Scaramellini, C., Leff, P., and Clarke, W. P., 1998, Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: Evidence for agonist-directed trafficking of receptor stimulus, *Mol. Pharmacol.* **54**, 94-104.

Berghuis, A. M., Lee, E., Raw, A. S., Gilman, A. G., and Sprang, S. R., 1996, Structure of the GDP-Pi complex of Gly203 $G_{i\alpha1}$: A mimic of the ternary product complex of Gα-catalyzed GTP hydrolysis, *Structure* **4**, 1277-1290.

Berlin, I., Warot, D., Legout, V., Guillemant, S., Schöllnhammer, G., and Puech, A. J., 1998, Blunted 5-HT_{1A}-receptor agonist-induced corticotropin and cortisol responses after long-term ipsapirone and fluoxetine administration to healthy subjects, *Clin. Pharmacol. Ther.* **63**, 428-436.

Berman, D. M., and Gilman, A. G., 1998, Mammalian RGS proteins: Barbarians at the gate, *J. Biol. Chem.* **273**, 1269-1272.

Berman, D. M., Kozasa, T., and Gilman, A. G., 1996, The GTPase-activating protein RGS4 stabilizes the transition state for nucleotide hydrolysis, *J. Biol. Chem.* **271**, 27209-27212.

Berman, D. M., Wilkie, T. M., and Gilman, A. G., 1996, GAIP and RGS4 are GTPAse-activating proteins for the G_i subfamily of G protein α subunits, *Cell* **86**, 445-452.

Bernstein, L. S., Grillo, A. A., Loranger, S. S., and Linder, M. E., 2000, RGS4 binds to membranes through an amphipathic α-helix, *J. Biol. Chem.* **275**(24), 18520-18526.

Berridge, M. J., Downes, C. P., and Hanley, M. R., 1982, Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands, *Biochemical Journal* **206**(3), 587-595.

Berstein, G., Blank, J. L., Smrcka, A. V., Higashijima, T., Sternweis, P. C., Exton, J. H., and Ross, E. M., 1992, Reconstitution of agonist-stimulated phosphatidylinositol 4,5-bisphosphate hydrolysis using purified m1 muscarinic receptor, Gq/11, and phospholipase C-beta 1, *J. Biol. Chem.* **267**, 8081-8088.

Bertrand, J., Mars, A., Boyle, C., Bove, F., Yeargin-Allsopp, M., and Decoufle, P., 2001, Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation, *Pediatrics* **108**(5), 1155-1161.

Betty, M., Harnish, S. W., Rhodes, K. J., and Cockett, M. I., 1998, Distribution of heterotrimeric G-protein beta and gamma subunits in the rat brain, *Neuroscience* **85**(2), 475-486.

Bhamre, S., Wang, H. Y., and Friedman, E., 1998, Serotonin-mediated palmitoylation and depalmitoylation of G *alpha* proteins in rat brain cortical membranes, *J. Pharmacol. Exp. Ther.* **286**, 1482-1489.

Birnbaumer, L., 1992, Receptor-to-effector signaling through G proteins: roles for beta gamma dimers as well as alpha subunits. [Review] [37 refs], *Cell* **71**(7), 1069-1072.

Bluml, K., Schnepp, W., Schroder, S., Beyermann, M., Macias, M., Oschkinat, H., and Lohse, M. J., 1997, A small region in phosducin inhibits G-protein betagamma-subunit function, *EMBO Journal* **16**(16), 4908-4915.

Booth, R. A., Cummings, C., Tiberi, M., and Liu, X. J., 2002, GIPC participates in G protein signaling downstream of insulin-like growth factor 1 receptor, *J. Biol. Chem.* **277**(8), 6719-6725.

Bosker, F. J., Cremers, T. I. F. H., Jongsma, M. E., Westerink, B. H. C., Wikström, V. H., and Den Boer, J. A., 2001, Acute and chronic effects of citalopram on postsynaptic 5-hydroxytryptamine$_{1A}$ receptor-mediated feedback: a microdialysis study in the amygdala, *Journal of Neurochemistry* **76**(6), 1645-1653.

Brooks, D. J., 2003, PET studies on the function of dopamine in health and Parkinson's disease, *Ann. N. Y. Acad. Sci.* **991**, 22-35.

Brunk, I., Pahner, I., Maier, U., Jenner, B., Veh, R. W., Nürnberg, B., and Ahnert-Hilger, G., 1999, Differential distribution of G-protein β-subunits in brain: An immunocytochemical analysis, *Eur. J. Cell Biol.* **78**(5), 311-322.

Buhl, A. M., Johnson, N. L., Dhanasekaran, N., and Johnson, G. L., 1995, G alpha 12 and G alpha 13 stimulate Rho-dependent stress fiber formation and focal adhesion assembly, *J. Biol. Chem.* **270**(42), 24631-24634.

Burgon, P. G., Lee, W. L., Nixon, A. B., Peralta, E. G., and Casey, P. J., 2001, Phosphorylation and nuclear translocation of a regulator of G protein signaling (RGS10), *J. Biol. Chem.* **276**(35), 32828-32834.

Callado, L. F., Meana, J. J., Grijalba, B., Pazos, A., Sastre, M., and Garcia-Sevilla, J. A., 1998, Selective increase of alpha2A-adrenoceptor agonist binding sites in brains of depressed suicide victims, *J. Neurochem.* **70**(3), 1114-1123.

Carlson, L. L., Weaver, D. R., and Reppert, S. M., 1989, Melatonin signal transduction in hamster brain: inhibition of adenylyl cyclase by a pertussis toxin-sensitive G protein, *Endocrinology* **125**, 2670-2676.

Carman, C. V., Parent, J. L., Day, P. W., Pronin, A. N., Sternweis, P. M., Wedegaertner, P., B., Gilman, A. G., Benovic, J. L. and Kozasa, T. 1999, Selective regulation of G$\alpha_{q/11}$ by an RGS domain in the G protein-coupled receptor kinase, GRK2, *J. Biol. Chem.* **274**(48), 34483-34492.

Cavicchini, E., Spampinato, S., and Ferri, S., 1990, Gi proteins and calcium in dynorphin-induced hypothermia and behaviour, *Eur. J. Pharmacol.* **180**, 357-360.

Chabre, O., Conklin, B. R., Brandon, S., Bourne, H. R., and Limbird, L. E., 1994, Coupling of the alpha 2A-adrenergic receptor to multiple G- proteins. A simple approach for estimating receptor-G -protein coupling efficiency in a transient expression system, *J. Biol. Chem.* **269**, 5730-5734.

Chakrabarti, S., and Fombonne, E., 2001, Pervasive developmental disorders in preschool children.[comment], *JAMA* **285**(24), 3093-3099.

Chakraborty, M., Chatterjee, D., Kellokumpu, S., Rasmussen, H., and Baron, R., 1991, Cell cycle-dependent coupling of the calcitonin receptor to different G proteins, *Science* **251**(4997), 1078-1082.

Chen, C., Zheng, B., Han, J., and Lin, S. C., 1997, Characterization of a novel mammalian RGS protein that binds to Galpha proteins and inhibits pheromone signaling in yeast, *J. Biol. Chem.* **272**(13), 8679-8685.

Chen, C. A., and Manning, D. R., 2000, Regulation of galpha i palmitoylation by activation of the 5- hydroxytryptamine-1A receptor, *J. Biol. Chem.* **275**(31), 23516-23522.

Chen, C. A., and Manning, D. R., 2001, Regulation of G proteins by covalent modification, *Oncogene* **20**(13), 1643-1652.

Chen, C., Wang, H., Fong, C. W., and Lin, S. C., 2001, Multiple phosphorylation sites in RGS16 differentially modulate its GAP activity, *FEBS Letters* **504**(1-2), 16-22.

Chen, F. M., Bilezikjian, L. M., Perrin, M. H., Rivier, J., and Vale, W., 1986, Corticotropin releasing factor receptor-mediated stimulation of adenylate cyclase activity in the rat brain, *Brain Res.* **381**(1), 49-57.

Chen, Y., Shyu, J. F., 1998, A. Santhanagopal, D. Inoue, J. P. David, S. J. Dixon, W. C. Horne, and R. Baron, The calcitonin receptor stimulates Shc tyrosine phosphorylation and Erk1/2 activation. Involvement of Gi, protein kinase C, and calcium, *J. Biol. Chem.* **273**(31), 19809-19816.

Cho, H., Kozasa, T., Takekoshi, K., De Gunzburg, J., and Kehrl, J. H., 2000, RGS14, a GTPase-activating protein for Gialpha, attenuates Gialpha- and G13alpha-mediated signaling pathways, *Molecular Pharmacology* **58**(3), 569-576.

Clapham, D. E., and Kim, D., 1989, G protein activation mechanisms of the cardiac K+ channel, iK.ACh, *Soc. Gen. Physiol Ser.* **44**, 55-68.

Colin, S. F., Chang, H. C., Mollner, S., Pfeuffer, T., Reed, R. R., Duman, R. S., and Nestler, E. J., 1991, Chronic lithium regulates the expression of adenylate cyclase and Gi-protein alpha subunit in rat cerebral cortex, *Proceedings of the National Academy of Sciences of the United States of America* **88**(23), 10634-10637.

Collins, L. R., Minden, A., Karin, M., and Brown, J. H., 1996, Galpha12 stimulates c-Jun NH2-terminal kinase through the small G proteins Ras and Rac, *J. Biol. Chem.* **271**(29), 17349-17353.

Colombo, M. I., Mayorga, L. S., Casey, P. J., and Stahl, P. D., 1992, Evidence of a role for heterotrimeric GTP-binding proteins in endosome fusion, *Science* **255**(5052), 1695-1697.

Corvol, J. C., Studler, J. M., Schonn, J. S., Girault, J. A., and Herve, D., 2001, Galpha(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum, *J. Neurochem.* **76**(5), 1585-1588 .

Cowburn, R. F., Fowler, C. J, and O'Neill, C, 1996, Neurotransmitter receptor/G-protein mediated signal transduction in Alzheimer's disease brain, *Neurodegeneration.* **5**(4), 483-488.

Cunningham, M. L., Waldo, G. L., Hollinger, S., Hepler, J. R., and Harden, T. K., 2001, Protein kinase C phosphorylates RGS2 and modulates its capacity for negative regulation of $G\alpha_{11}$ signaling, *J. Biol. Chem.* **276**(8), 5438-5444.

Danner, S., and Lohse, M. J., 1996, Phosducin is a ubiquitous G-protein regulator, *Proceedings of the National Academy of Sciences of the United States of America* **93**(19), 10145-10150.

De Paermentier, F., Cheetham, S. C., Crompton, M. R., Katona, C. E., and Horton, R. W., 1990, Brain β-adrenoceptor binding sites in antidepressant-free depressed suicide victims, *Brain Res.* **525**, 71-77.

De Paermentier, F., Cheetham, S. C., Crompton, M. R., Katona, C. L., and Horton, R. W., 1991, Brain beta-adrenoceptor binding sites in depressed suicide victims: effects of antidepressant treatment, *Psychopharmacology* **105**(2), 283-288.

De Vries, L., Lou, X. J., Zhao, G., Zheng, B., and Farquhar, M. G., 1998, GIPC, a PDZ domain containing protein, interacts specifically with the C terminus of RGS-GAIP, *Proc. Natl. Acad. Sci. USA* **95**, 12340-12345.

De Vries, L., Elenko, E., Hubler, L., Jones, T. L. Z., and Farquhar, M. G., 1996, GAIP is membrane-anchored by palmitoylation and interacts with the activated (GTP-bound) form of $G\alpha_i$ subunits, *Proc. Natl. Acad. Sci. USA* **93**, 15203-15208.

De Vries, L., Zheng, B., Fischer, T., Elenko, E., and Farquhar, M. G., 2000, The regulator of G protein signaling family, *Annu. Rev. Pharmacol. Toxicol.* **40**, 235-271.

De Weerd, W. F., and Leeb-Lundberg, L. M., 1997, Bradykinin sequesters B2 bradykinin receptors and the receptor-coupled Galpha subunits Galphaq and Galphai in caveolae in DDT1 MF-2 smooth muscle cells, *J. Biol. Chem.* **272**(28), 17858-17866.

Degtyarev, M. Y., Spiegel, A. M., and Jones, T. L., 1993, Increased palmitoylation of the Gs protein alpha subunit after activation by the beta-adrenergic receptor or cholera toxin, *J. Biol. Chem.* **268**(32), 23769-23772.

Degtyarev, M. Y., Spiegel, A. M., and Jones, T. L., 1994, Palmitoylation of a G protein alpha i subunit requires membrane localization not myristoylation, *J. Biol. Chem.* **269**, 30898-30903.

Delgado, P. L., 2000, Depression: the case for a monoamine deficiency, *J. Clin. Psychiatry* **61** (Suppl 6), 7-11.

Dessauer, C. W., Posner, B. A., and Gilman, A. G., 1996, Visualizing signal transduction: receptors, G-proteins, and adenylate cyclases, *Clin. Sci. (Lond)* **91**(5), 527-537.

Dessauer, C. W., Posner, B. A., and Gilman, A. G., 1996, Visualizing signal transduction: receptors, G-proteins, and adenylate cyclases [published erratum appears in Clin Sci (Colch) 1997 Feb;92(2):223], *Clinical Science.* **91**, 527-537.

Dhaenen, H., 2001, Imaging the serotonergic system in depression. [Review] [36 refs], *European Archives of Psychiatry & Clinical Neuroscience* **251** (Suppl 2), II76-II80.

Dixon, J. F., and Hokin, L. E., 1997, The antibipolar drug valproate mimics lithium in stimulating glutamate release and inositol 1,4,5-trisphosphate accumulation in brain cortex slices but not accumulation of inositol monophosphates and bisphosphates, *Proc. Natl. Acad. Sci. U. S. A* **94**(9), 4757-4760.

Doi, T., Sugimoto, H., Arimoto, I., Hiroaki, Y., and Fujiyoshi, Y., 1999, Interactions of endothelin receptor subtypes A and B with Gi, Go, and Gq in reconstituted phospholipid vesicles, *Biochemistry* **38**(10), 3090-3099.

Donati, R. J., and Rasenick, M. M., 2003, G protein signaling and the molecular basis of antidepressant action. [Review] [102 refs], *Life Sciences.* **73**(1), 1-17.

Dowlatshahi, D., MacQueen, G. M., Wang, J. F., Reiach, J. S., and Young, L. T., 1999, G Protein-coupled cyclic AMP signaling in postmortem brain of subjects with mood disorders: effects of diagnosis, suicide, and treatment at the time of death, *Journal of Neurochemistry* **73**(3), 1121-1126.

Downes, G. B., Gilbert, D. J., Copeland, N. G., Gautam, N., and Jenkins, N. A., 1999, Chromosomal mapping of five mouse G protein gamma subunits, *Genomics* **57**(1), 173-176.

Drews, J., 2000, Drug discovery: a historical perspective.[comment], *Science.* **287**(5460), 1960-1964.

Druey, K. M., Blumer, K. J., Kang, V. H., and Kehrl, J. H., 1996, Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family, *Nature* **379**, 742-746.

Dryja, T. P., Hahn, L. B., Reboul, T., and Arnaud, B., 1996, Missense mutation in the gene encoding the alpha subunit of rod transducin in the Nougaret form of congenital stationary night blindness, *Nature Genet.* **13**(3), 358-360.

Dwivedi, Y., and Pandey, G. N., 1997, Effects of subchronic administration of antidepressants and anxiolytics on levels of the α subunits of G proteins in the rat brain, *J. Neural Transm.* **104**, 747-760.

Eason, M. G., Kurose, H., Holt, B. D., Raymond, J. R., and Liggett, S. B., 1992, Simultaneous coupling of alpha 2-adrenergic receptors to two G-proteins with opposing effects. Subtype-selective coupling of alpha 2C10, alpha 2C4, and alpha 2C2 adrenergic receptors to Gi and Gs, *J. Biol. Chem.* **267**(22), 15795-15801.

Edgerton, M. D., Chabert, C., Chollet, A., and Arkinstall, S., 1994, Palmitoylation but not the extreme amino-terminus of Gq alpha is required for coupling to the NK2 receptor, *FEBS Letters* **354**, 195-199.

Endou, M., Poli, E., and Levi, R., 1994, Histamine H3-receptor signaling in the heart: possible involvement of Gi/Go proteins and N-type Ca++ channels, *JPET* **269**(1), 221-229.

Ercolani, L., Stow, J. L., Boyle, J. F., Holtzman, E. J., Lin, H., Grove, J. R., and Ausiello, D. A., 1990, Membrane localization of the pertussis toxin-sensitive G-protein subunits alpha i-2 and alpha i-3 and expression of a metallothionein-alpha i-2 fusion gene in LLC-PK1 cells.[erratum appears in Proc Natl Acad Sci U S A 1990 Sep;87(18):7344], *Proceedings of the National Academy of Sciences of the United States of America* **87**(12), 4635-4639.

Farfel, Z., Bourne, H. R., and Iiri, T., 1999, The expanding spectrum of G protein diseases, *N. Engl. J. Med.* **340**(13), 1012-1020.

Faurobert, E., and Hurley, J. B., 1997, The core domain of a new retina specific RGS protein stimulates the GTPase activity of transducin *in vitro*, *Proc. Natl. Acad. Sci. USA* **94**, 2945-2950.

Feoktistov, I., and Biaggioni, I., 1997, Adenosine A2B receptors. [Review] [226 refs], *Pharmacological Reviews* **49**(4), 381-402.

Fields, T. A., and Casey, P. J., 1995, Phosphorylation of Gz alpha by protein kinase C blocks interaction with the beta gamma complex, *J. Biol. Chem.* **270**, 23119-23125.

Fishburn, C. S., Pollitt, S. K., and Bourne, H. R., 2000, Localization of a peripheral membrane protein: Gbetagamma targets Galpha(Z), *Proc. Natl. Acad. Sci. U. S. A* **97**(3), 1085-1090.

Freissmuth, M., Schutz, W., and Linder, M. E., 1991, Interactions of the bovine brain A1-adenosine receptor with recombinant G protein alpha-subunits. Selectivity for rGi alpha-3, *J. Biol. Chem.* **266**(27), 17778-17783.

Friedman, E., and Wang, H.-Y., 1996, Receptor-mediated activation of G proteins is increased in postmortem brains of bipolar affective disorder subjects, *J. Neurochem.* **67**, 1145-1152.

Fu, M. J., Lin, K. S., Chan, J. Y., and Chan, S. H., 1992, Participation of pertussis toxin-sensitive GTP-binding regulatory proteins in the suppression of baroreceptor reflex by neurotensin in the rat, *Regulatory Peptides* **37**, 167-180.

Fukada, Y., Takao, T., Ohguro, H., Yoshizawa, T, Akino, T., and Shimonishi, Y., 1990, Farnesylated gamma-subunit of photoreceptor G protein indispensable for GTP-binding, *Nature* **346**(6285), 658-660.

Gagnon, A. W., Murray, D., and Leadley, R. J., 2002, Cloning and characterization of a novel regulator of G protein signalling in human platelets, *Cellular Signalling* **14**(7), 595-606.

Gailly, P., Najimi, M., and Hermans, E., 2000, Evidence for the dual coupling of the rat neurotensin receptor with pertussis toxin-sensitive and insensitive G-proteins, *FEBS Letters* **483**(2-3), 109-113.

Galbiati, F., Guzzi, F., Magee, A. I., Milligan, G., and Parenti, M., 1994, N-terminal fatty acylation of the alpha-subunit of the G-protein Gi1: only the myristoylated protein is a substrate for palmitoylation, *Biochemical Journal* **303**, 697-700.

Gandy, S., 2003, Estrogen and neurodegeneration. [Review] [41 refs], *Neurochemical Research* **28**(7), 1003-1008.

Garcia, M., Sakamoto, K., Shigekawa, M., Nakanishi, S., and Ito, S., 1994, Multiple mechanisms of arachidonic acid release in Chinese hamster ovary cells transfected with cDNA of substance P receptor, *Biochem. Pharmacol.* **48**(9), 1735-1741.

Gaudet, R., Savage, J. R., McLaughlin, J. N., Willardson, B. M., and Sigler, P. B., 1999, A molecular mechanism for the phosphorylation-dependent regulation of heterotrimeric G proteins by phosducin, *Mol. Cell* **3**(5), 649-660.

Gautam, N., Downes, G. B., Yan, K., and Kisselev, O., 1998, The G-protein betagamma complex. [Review] [135 refs], *Cellular Signalling* **10**(7), 447-455.

Gilman, A. G., 1989, The Albert Lasker Medical Awards. G proteins and regulation of adenylyl cyclase, *JAMA* **262**(13), 1819-1825.

Glick, J. L., Meigs, T. E., Miron, A., and Casey, P. J., 1998, RGSZ1, a G_z-selective regulator of G protein signaling whose action is sensitive to the phosphorylation state of $G_z\alpha$, *J. Biol. Chem.* **273**, 26008-26013.

Gold, S. J., Ni, Y. G., Dohlman, H. G., and Nestler, E. J., 1997, Regulators of G-protein signaling (RGS) Proteins: region-specific expression of nine subtypes in rat brain, *J. Neurosci.* **17**, 8024-8037.

Gorman, J. M., 2003, New molecular targets for antianxiety interventions. [Review] [45 refs], *Journal of Clinical Psychiatry* **64** (Suppl 3), 28-35.

Grammatopoulos, D. K., Randeva, H. S., Levine, M. A., Kanellopoulou, K. A., and Hillhouse, E. W., 2001, Rat cerebral cortex corticotropin-releasing hormone receptors: evidence for receptor coupling to multiple G-proteins, *Journal of Neurochemistry* **76**(2), 509-519.

Granneman, J. G., Zhai, Y., Zhu, Z., Bannon, M. J., Burchett, S. A., Schmidt, C. J., Andrade, R., and Cooper, J., 1998, Molecular characterization of human and rat RGS 9L, a novel splice variant enriched in dopamine target regions, and chromosomal localization of the RGS 9 gene, *Mol. Pharmacol.* **54**(4), 687-694.

Grassie, M. A., McCallum, J. F., Guzzi, F., Magee, A. I., Milligan, G., and Parenti, M., 1994, The palmitoylation status of the G-protein G(o)1 alpha regulates its activity of interaction with the plasma membrane, *Biochem. J.* **302** (Pt 3), 913-920.

Gray, J. A., and Roth, B. L., 2001, Paradoxical trafficking and regulation of $5HT_{2A}$ receptors by agonists and antagonists, *Brain Research Bulletin* **56**(5), 441-451.

Hallak, H., Brass, L. F., and Manning, D. R., 1994, Failure to myristoylate the alpha subunit of Gz is correlated with an inhibition of palmitoylation and membrane attachment, but has no effect on phosphorylation by protein kinase C, *J. Biol. Chem.* **269**, 4571-4576.

Hanley, N. R., and Van de Kar, L. D., 2003, Serotonin and the neuroendocrine regulation of the hypothalamic–pituitary-adrenal axis in health and disease, *Vitam. Horm.* **66**, 189-255.

Hart, M. J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W. D., Gilman, A. G., Sternweis, P. C., and Bollag, G., 1998, Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13, *Science* **280**(5372), 2112-2114.

Harwood, A. J., and Agam, G., 2003, Search for a common mechanism of mood stabilizers, *Biochem. Pharmacol.* **66**(2), 179-189.

Hawes, B. E., Fried, S., Yao, X., Weig, B., and Graziano, M. P., 1998, Nociceptin (ORL-1) and mu-opioid receptors mediate mitogen-activated protein kinase activation in CHO cells through a Gi-coupled signaling pathway: evidence for distinct mechanisms of agonist-mediated desensitization, *J. Neurochem.* **71**(3), 1024-1033.

Hayashi, M. K., and Haga, T., 1997, Palmitoylation of muscarinic acetylcholine receptor m2 subtypes: reduction in their ability to activate G proteins by mutation of a putative palmitoylation site, cysteine 457, in the carboxyl-terminal tail, *Archives of Biochemistry & Biophysics* **340**(2), 376-382.

He, W., Cowan, C. W., and Wensel, T. G., 1998, RGS9, a GTPase accelerator for phototransduction, *Neuron* **20**, 95-102.

Hendry, I. A., Kelleher, K. L., Bartlett, S. E., Leck, K. J., Reynolds, A. J., Heydon, K., Mellick, A., Megirian, D., and Matthaei, K. I., 2000, Hypertolerance to morphine in $G_{z\alpha}$-deficient mice, *Brain Research* **870**(1-2), 10-19.

Hepler, J. R., and Gilman, A. G., 1992, G proteins, *Trends Biochem. Sci.* **17**(10), 383-387.

Hepler, J. R., Berman, D. M., Gilman, A. G., and Kozasa, T., 1997, RGS4 and GAIP are GTPase-activating proteins for $G_{q\alpha}$ and block activation of phospholipase Cβ by τ-thio-GTP-$G_{q\alpha}$, *Proc. Natl. Acad. Sci.* **94**, 428-432.

Hernandez, I., and Sokolov, B. P., 2000, Abnormalities in 5-HT2A receptor mRNA expression in frontal cortex of chronic elderly schizophrenics with varying histories of neuroleptic treatment, *J. Neurosci. Res.* **59**(2), 218-225.

Heuser, I., Yassouridis, A., and Holsboer, F., 1994, The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders, *J. Psychiatr. Res.* **28**(4), 341-356.

Heximer, S. P., Watson, N., Linder, M. E., Blumer, K. J., and Hepler, J. R., 1997, RGS2/G0S8 is a selective inhibitor of Gqα function, *Proc. Natl. Acad. Sci. USA* **94**, 14389-14393.

Hollinger, S., and Hepler, J. R., 2002, Cellular regulation of RGS proteins: Modulators and integrators of G protein signaling, *Pharmacological Reviews* **54**(3), 527-559.

Hollinger, S., Ramineni, S., and Hepler, J. R., 2003, Phosphorylation of RGS14 by protein kinase A potentiates its activity toward G alpha i, *Biochemistry* **42**(3), 811-819.

Holsboer, F., 2000, The corticosteroid receptor hypothesis of depression, *Neuropsychopharmacology* **23**(5), 477-501.

Houamed, K. M., Kuijper, J. L., Gilbert, T. L., Haldeman, B. A., O'Hara, P. J., Mulvihill, E. R., Almers, W., and Hagen, F. S., 1991, Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain, *Science* **252**(5010), 1318-1321.

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylecharane, E. J., Saxena, P. R., and Humphrey, P. P. A., 1994, VII. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), *Pharmacol. Rev.* **46**, 157-204.

Hoyer, D., and Martin, G., 1997, 5-HT receptor classification and nomenclature: Towards a harmonization with the human genome, *Neuropharmacology* **36**, 419-428.

Hsieh, K. P., and Martin, T. F., 1992, Thyrotropin-releasing hormone and gonadotropin-releasing hormone receptors activate phospholipase C by coupling to the guanosine triphosphate-binding proteins Gq and G11, *Molecular Endocrinology* **6**, 1673-1681.

Huang, C. F., Hepler, J. R., Chen, L. T., Gilman, A. G., Anderson, R. G. W., and Mumby, S. M., 1997, Organization of G proteins and adenylyl cyclase at the plasma membrane, *Mol. Biol. Cell* **8**, 2365-2378.

Huang, L. J. S., Durick, K., Weiner, J. A., Chun, J., and Taylor, S. S., 1997, D-AKAP2, a novel protein kinase A anchoring protein with a putative RGS domain, *Proc. Natl. Acad. Sci. USA* **94**, 11184-11189.

Hughes, T. E., Zhang, H., Logothetis, D. E., and Berlot, C. H., 2001, Visualization of a functional Galpha q-green fluorescent protein fusion in living cells. Association with the plasma membrane is disrupted by mutational activation and by elimination of palmitoylation sites, but not by activation mediated by receptors or AlF_4^-, *J. Biol. Chem.* **276**(6), 4227-4235.

Hukovic, N., Panetta, R., Kumar, U., Rocheville, M., and Patel, Y. C., 1998, The cytoplasmic tail of the human somatostatin receptor type 5 is crucial for interaction with adenylyl cyclase and in mediating desensitization and internalization, *J. Biol. Chem.* **273**(33), 21416-21422.

Hung, D. T., Wong, Y. H., Vu, T. K., and Coughlin, S. R., 1992, The cloned platelet thrombin receptor couples to at least two distinct effectors to stimulate phosphoinositide hydrolysis and inhibit adenylyl cyclase, *J. Biol. Chem.* **267**(29), 20831-20834.

Hunt, T. W., Fields, T. A., Casey, P. J., and Peralta, E. G., 1996, RGS10 is a selective activator of Gαi GTPase activity, *Nature* **383**, 175-177.

Inder, W. J., Prickett, T. C. R., Mulder, R. T., Donald, R. A., and Joyce, P. R., 2001, Reduction in basal afternoon plasma ACTH during early treatment of depression with fluoxetine, *Psychopharmacology* **156**(1), 73-78.

Ingi, T., Krumins, A. M., Chidiac, P., Brothers, G. M., Chung, S., Snow, B. E., Barnes, C. A., Lanahan, A A., Siderovski, D. P., Ross, E. M., Gilman, A. G., and Worley, P. F., 1998, Dynamic regulation of RGS2 suggests a novel mechanism in G-protein signaling and neuronal plasticity, *J. Neurosci.* **18**, 7178-7188.

Iniguez-Lluhi, J. A., Simon, M. I., Robishaw, J. D., and Gilman, A. G., 1992, G protein beta gamma subunits synthesized in Sf9 cells. Functional characterization and the significance of prenylation of gamma, *J. Biol. Chem.* **267**(32), 23409-23417.

Jelinek, L. J., Lok, S., Rosenberg, G. B., Smith, R. A., Grant, Biggs, S., Bensch, P. A., Kuijper, J. L., Sheppard, P. O., and Sprecher, C. A., 1993, Expression cloning and signaling properties of the rat glucagon receptor, *Science* **259**(5101), 1614-1616.

Jelsema, C. L., and Axelrod, J., 1987, Stimulation of phospholipase A2 activity in bovine rod outer segments by the beta gamma subunits of transducin and its inhibition by the alpha subunit, *Proceedings of the National Academy of Sciences of the United States of America* **84**(11), 3623-3627.

Jho, E. H., Davis, R. J., and Malbon, C. C., 1997, c-Jun amino-terminal kinase is regulated by Galpha12/Galpha13 and obligate for differentiation of P19 embryonal carcinoma cells by retinoic acid, *J. Biol. Chem.* **272**(39), 24468-24474.

Jian, X., Sainz, E., Clark, W. A., Jensen, R. T., Battey, J. F., and Northup, J. K., 1999, The bombesin receptor subtypes have distinct G protein specificities, *J. Biol. Chem.* **274**(17), 11573-11581.

Jockers, R., Linder, M. E., Hohenegger, M., Nanoff, C., Bertin, B., Strosberg, A. D., Marullo, S., and Freissmuth, M., 1994, Species difference in the G protein selectivity of the human and bovine A1-adenosine receptor, *J. Biol. Chem.* **269**, 32077-32084.

Johnson, E. N., and Druey, K. M., 2002, Functional characterization of the G protein regulator RGS13, *J. Biol. Chem.* **277**(19), 16768-16774.

Kapur, S., and Remington, G., 1996, Serotonin-dopamine interaction and its relevance to schizophrenia, *Am. J. Psychiatry* **153**, 466-476.

Kawanabe, Y., Hashimoto, N., and Masaki, T., 2002, Characterization of G proteins involved in activation of nonselective cation channels by endothelin(B) receptor, *British Journal of Pharmacology* **136**(7), 1015-1022.

Kehrl, J. H., and Sinnarajah, S., 2002, RGS2: a multifunctional regulator of G-protein signaling. [Review] [26 refs], *International Journal of Biochemistry & Cell Biology* **34**(5), 432-438.

Kenakin, T., 1995, Agonist-receptor efficacy. II. Agonist trafficking of receptor signals, *Trends Pharmacol. Sci.* **16**(7), 232-238.

Kilts, J. D., Gerhardt, M. A., Richardson, M. D., Sreeram, G., Mackensen, G. B., Grocott, H. P., White, W. D., Davis, R. D., Newman, M. F., Reves, J. G., Schwinn, D. A., and Kwatra, M. M., 2000, Beta(2)-adrenergic and several other G protein-coupled receptors in human atrial membranes activate both G(s) and G(i).[comment], *Circulation Research* **87**(8), 705-709.

Kimple, R. J., De Vries, L., Tronchere, H., Behe, C. I., Morris, R. A., Gist, F. M., and Siderovski, D. P., 2001, RGS12 and RGS14 GoLoco motifs are G alpha(i) interaction sites with guanine nucleotide dissociation inhibitor Activity, *J. Biol. Chem.* **276**(31), 29275-29281.

Kimple, R. J., Kimple, M. E., Betts, L., Sondek, J., and Siderovski, D. P., 2002, Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits, *Nature* **416**(6883), 878-881.

Kishida, S., Yamamoto, H., Ikeda, S., Kishida, M., Sakamoto, I., Koyama, S., and Kikuchi, A., 1998, Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin, *J. Biol. Chem.* **273**(18), 10823-10826.

Koch, B. D., Dorflinger, L. J., and Schonbrunn, A., 1985, Pertussis toxin blocks both cyclic AMP-mediated and cyclic AMP-independent actions of somatostatin. Evidence for coupling of Ni to decreases in intracellular free calcium, *J. Biol. Chem.* **260**(24), 13138-13145.

Koelle, M. R., and Horvitz, H. R., 1996, EGL-10 regulates G protein signaling in the C. elegans nervous system and shares a conserved domain with many mammalian proteins, *Cell* **84**, 115-125.

Konda, Y., Gantz, I., DelValle, J., Shimoto, Y., Miwa, H., and Yamada, T., 1994, Interaction of dual intracellular signaling pathways activated by the melanocortin-3 receptor, *J. Biol. Chem.* **269**(18), 13162-13166.

Kozasa, T., Itoh, H., Tsukamoto, T., and Kaziro, Y., 1988, Isolation and characterization of the human Gs alpha gene, *Proceedings of the National Academy of Sciences of the United States of America* **85**(7), 2081-2085.

Kozasa, T., Jiang, X., Hart, M. J., Sternweis, P. M., Singer, W. D., Gilman, A. G., Bollag, G., and Sternweis, P. C., 1998, p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13, *Science* **280**(5372), 2109-2111.

Kroog, G. S., Jian, X. Y., Chen, L. Northup, J. K., and Battey, J. F., 1999, Phosphorylation uncouples the gastrin-releasing peptide receptor from G_q, *J. Biol. Chem.* **274**(51), 36700-36706.

Krsmanovic, L.Z., Mores, N., Navarro, C. E., Arora, K. K., and Catt, K. J., 2003, An agonist-induced switch in G protein coupling of the gonadotropin-releasing hormone receptor regulates pulsatile neuropeptide secretion, *Proceedings of the National Academy of Sciences of the United States of America* **100**(5), 2969-2974.

Kull, B., Svenningsson, P., and Fredholm, B. B., 2000, Adenosine A(2A) receptors are colocalized with and activate g(olf) in rat striatum, *Molecular Pharmacology* **58**(4), 771-777.

Kwatra, M. M., Schwinn, D. A., Schreurs, J., Blank, J. L., Kim, C. M., Benovic, J. L., Krause, J. E., Caron, M. G., and Lefkowitz, R. J., 1993, The substance P receptor, which couples to Gq/11, is a substrate of beta-adrenergic receptor kinase 1 and 2, *J. Biol. Chem.* **268**(13), 9161-9164.

Lai, H. W., Minami, M., Satoh, M., and Wong, Y. H., 1995, Gz coupling to the rat kappa-opioid receptor, *FEBS Lett.* **360**(1), 97-99.

Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B., 1996, The 2.0 A crystal structure of a heterotrimeric G protein.[comment], *Nature* **379**(6563), 311-319.

Laszlo, F. A., Laszlo, F., Jr., and De Wied, D., 1991, Pharmacology and clinical perspectives of vasopressin antagonists. [Review] [498 refs], *Pharmacological Reviews* **43**(1), 73-108.

Laszlo, F. A., Laszlo, Jr. F., and De Wied, D., 1991, Pharmacology and clinical perspectives of vasopressin antagonists, *Pharmacol. Rev.* **43**(1), 73-108.

Laugwitz, K. L., Allgeier, A., Offermanns, S., Spicher, K., Van Sande, J., Dumont, J. E., and Schultz, G., 1996, The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families, *Proc. Natl. Acad. Sci. U. S. A* **93**(1), 116-120.

Lee, C. H., Shin, I. C., Kang, J. S., Koh, H. C., Ha, J. H., and Min, C. K., 1998, Differential coupling of G alpha q family of G-protein to muscarinic M1 receptor and neurokinin-2 receptor, *Arch. Pharm. Res.* **21**(4), 423-428.

Lee, R. H., Lieberman, B. S., and Lolley, R. N., 1987, A novel complex from bovine visual cells of a 33,000-dalton phosphoprotein with beta- and gamma-transducin: purification and subunit structure, *Biochemistry* **26**(13), 3983-3990.

Leopoldt, D., Harteneck, C., and Nurnberg, B., 1997, G proteins endogenously expressed in Sf 9 cells: interactions with mammalian histamine receptors, *Naunyn Schmiedebergs Arch. Pharmacol.* **356**(2), 216-224.

Lerer, B., Gelfin, Y., Gorfine, M., Allolio, B., Lesch, K. P., and Newman, M. E., 1999, 5-HT_{1A} receptor function in normal subjects on clinical doses of fluoxetine: Blunted temperature and hormone responses to ipsapirone challenge, *Neuropsychopharmacology* **20**(6), 628-639.

Lesch, K. P., and Manji, H. K., 1992, Signal-transduction G proteins and antidepressant drugs: evidence for modulation of α subunit gene expression in rat brain, *Biol. Psychiatry* **32**, 549-579.

Lesch, K. P., Mayer, M., Disselkamp-Tietze, J., Hoh, A., Wiesmann, M., Osterheider, M., and Schulte, H. M., 1990, 5-HT_{1A} receptor responsivity in unipolar depression. Evaluation of ipsapirone-induced ACTH and cortisol secretion in patients and controls, *Biol. Psychiatry* **28**, 620-628.

Leyte, A., Barr, F. A., Kehlenbach, R. H., and Huttner, W. B., 1992, Multiple trimeric G-proteins on the trans-Golgi network exert stimulatory and inhibitory effects on secretory vesicle formation, *EMBO Journal* **11**(13), 4795-4804.

Li, Q., Muma, N. A., and Van de Kar, L. D., 1996, Chronic fluoxetine induces a gradual desensitization of 5-HT_{1A} receptors: reductions in hypothalamic and midbrain G_i and G_o proteins and in neuroendocrine responses to a 5-HT_{1A} agonist, *J. Pharmacol. Exp. Ther.* **279**, 1035-1042.

Li, Q., Muma, N. A., Battaglia, G., and Van de Kar, L. D., 1997, A desensitization of hypothalamic 5-HT_{1A} receptors by repeated injections of paroxetine: reduction in the levels of G_i and G_o proteins and neuroendocrine responses, but not in the density of 5-HT_{1A} receptors, *J. Pharmacol. Exp. Ther.* **282**, 1581-1590.

Liao, J. K., and Homcy, C. J., 1993, The G proteins of the G alpha i and G alpha q family couple the bradykinin receptor to the release of endothelium-derived relaxing factor, *J. Clin. Invest* **92**(5), 2168-2172.

Liebmann, C., Offermanns, S., Spicher, K., Hinsch, K. D., Schnittler, M., Morgat, J. L., Reissmann, S., Schultz, G., and Rosenthal, W., 1990, A high-affinity bradykinin receptor in membranes from rat myometrium is coupled to pertussis toxin-sensitive G-proteins of the Gi family, *Biochemical & Biophysical Research Communications* **167**(3), 910-917.

Lin, C. H., Huang, Y. C., Tsai, J. J., and Gean, P. W., 2001., Modulation of voltage-dependent calcium currents by serotonin in acutely isolated rat amygdala neurons, *Synapse* **41**(4), 351-359.

Linden, J., Thai, T., Figler, H., Jin, X., and Robeva, A. S., 1999, Characterization of human A(2B) adenosine receptors: radioligand binding, western blotting, and coupling to G(q) in human embryonic kidney 293 cells and HMC-1 mast cells, *Mol. Pharmacol.* **56**(4), 705-713.

Linder, M. E., Middleton, P., Hepler, J. R., Taussig, R., Gilman, A. G., and Mumby, S. M., 1993, Lipid modifications of G proteins: alpha subunits are palmitoylated, *Proceedings of the National Academy of Sciences of the United States of America* **90**, 3675-3679.

Liu, W. W., Mattingly, R. R., and Garrison, J. C., 1996, Transformation of Rat-1 fibroblasts with the v-src oncogene increases the tyrosine phosphorylation state and activity of the alpha subunit of Gq/G11, *Proceedings of the National Academy of Sciences of the United States of America* **93**(16), 8258-8263.

Lohse, M. J., Andexinger, S., Pitcher, J., Trukawinski, S., Codina, J., Faure, J. P., Caron, M. G., and Lefkowitz, R. J., 1992, Receptor-specific desensitization with purified proteins. Kinase dependence and receptor specificity of beta-arrestin and arrestin in the beta 2-adrenergic receptor and rhodopsin systems, *J. Biol. Chem.* **267**(12), 8558-8564.

Lou, X., Yano, H., Lee, F., Chao, M. V., and Farquhar, M. G., 2001, GIPC and GAIP form a complex with TrkA: a putative link between G protein and receptor tyrosine kinase pathways, *Molecular Biology of the Cell* **12**(3), 615-627.

Luttrell, L. M., Van Biesen, T., Hawes, B. E., Koch, W. J., Touhara, K., and Lefkowitz, R. J., 1995, G beta gamma subunits mediate mitogen-activated protein kinase activation by the tyrosine kinase insulin-like growth factor 1 receptor, *J. Biol. Chem.* **270**(28), 16495-16498.

MacKinnon, A. C., Waters, C., Jodrell, D., Haslett, C., and Sethi, T., 2001, Bombesin and substance P analogues differentially regulate G-protein coupling to the bombesin receptor. Direct evidence for biased agonism, *J. Biol. Chem.* **276**(30), 28083-28091.

Manji, H. K., Bebchuk, J. M., Moore, G. J., Glitz, D., Hasanat, K. A., and Chen, G., 1999, Modulation of CNS signal transduction pathways and gene expression by mood-stabilizing agents: therapeutic implications, *J. Clin. Psychiatry* **60** (Suppl 2), 27-39.

Mao, J., Yuan, H., Xie, W., and Wu, D., 1998, Guanine nucleotide exchange factor GEF115 specifically mediates activation of Rho and serum response factor by the G protein alpha subunit Galpha13, *Proceedings of the National Academy of Sciences of the United States of America* **95**(22), 12973-12976.

Martín-Ruiz, R., and Ugedo, L., 2001, Electrophysiological evidence for postsynaptic 5-HT_{1A} receptor control of dorsal raphe 5-HT neurones, *Neuropharmacology* **41**(1), 72-78.

Maruyama, Y., Nishida, M., Sugimoto, Y., Tanabe, S., Turner, J. H., Kozasa, T., Wada, T., Nagao, T., and Kurose, H., 2002, Galpha(12/13) mediates alpha(1)-adrenergic receptor-induced cardiac hypertrophy, *Circulation Research* **91**(10), 961-969.

May, L. G., and Gay, C. V., 1997, Multiple G-protein involvement in parathyroid hormone regulation of acid production by osteoclasts, *J. Cell. Biochem.* **64**(1), 161-170 (1997).

Megson, M. N., 2000, Is autism a G-alpha protein defect reversible with natural vitamin A?, *Med. Hypotheses* **54**(6), 979-983.

Meltzer, H. Y., and Maes, M., 1994, Effects of buspirone on plasma prolactin and cortisol levels in major depressed and normal subjects, *Biol. Psychiatry* **35**, 316-323.

Meltzer, H. Y., and Maes, M., 1995, Effects of ipsapirone on plasma cortisol and body temperature in major depression, *Biol. Psychiatry* **38**, 450-457.

Michel, M. C., Beck-Sickinger, A., Cox, H., Doods, H. N., Herzog, H., Larhammar, D., Quirion, R., Schwartz, T., and Westfall, T., 1998, XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. [Review] [79 refs], *Pharmacological Reviews* **50**(1), 143-150.

Mirnics, K., Middleton, F. A., Stanwood, G. D., Lewis, D. A., and Levitt, P., 2001, Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia, *Mol. Psychiatry* **6**(3), 293-301.

Mizuno, K., Kanda, Y., Kuroki, Y., and Watanabe, Y., 2000,The stimulation of beta(3)-adrenoceptor causes phosphorylation of extracellular signal-regulated kinases 1 and 2 through a G(s)- but not G(i)-dependent pathway in 3T3-L1 adipocytes, *European Journal of Pharmacology* **404** (1-2), 63-68.

Moffett, S., Mouillac, B., Bonin, H., and Bouvier, M., 1993, Altered phosphorylation and desensitization patterns of a human beta 2-adrenergic receptor lacking the palmitoylated Cys341, *EMBO J.* **12**(1), 349-356.

Monk, P. N., Pease, J. E., and Barker, M. D., 1994, C5a stimulus-secretion coupling in rat basophilic leukaemia (RBL-2H3) cells transfected with the human C5a receptor is mediated by pertussis and cholera toxin-sensitive G proteins, *Biochemistry & Molecular Biology International* **32**(1), 13-20.

Moolenaar, W. H., 1995, Lysophosphatidic acid, a multifunctional phospholipid messenger. [Review] [55 refs], *J. Biol. Chem.* **270**(22), 12949-12952.

Moon, H. E., Cavalli, A., Bahia, D. S., Hoffmann, M., Massotte, D., and Milligan, G., 2001, The human delta opioid receptor activates G(i1)alpha more efficiently than G(o1)alpha, *Journal of Neurochemistry* **76**(6), 1805-1813.

Moore, G. J., Bebchuk, J. M., Parrish, J. K., Faulk, M. W., Arfken, C. L., Strahl-Bevacqua, J., and Manji, H. K., 1999, Temporal dissociation between lithium-induced changes in frontal lobe myo-inositol and clinical response in manic-depressive illness, *Am. J. Psychiatry* **156**(12), 1902-1908.

Moratz, C., Kang, V. H., Druey, K. M., Shi, C. S., Scheschonka, A., Murphy, P. M., Kozasa, T., and Kehrl, J. H., 2000, Regulator of G protein signaling 1 (RGS1) markedly impairs Gi alpha signaling responses of B lymphocytes, *Journal of Immunology* **164**(4), 1829-1838.

Morris, A. J., and Malbon, C. C, 1999, Physiological regulation of G protein-linked signaling, *Physiol. Rev.* **79**(4), 1373-1430.

Muma, N. A., Mariyappa, R., Williams, K., and Lee, J. M., 2003, Differences in regional and subcellular localization of $G_{q/11}$ and RGS4 protein levels in Alzheimer's disease: Correlation with muscarinic M1 receptor binding parameters, *Synapse* **47**(1), 58-65.

Mumby, S. M., 1997, Reversible palmitoylation of signaling proteins, *Curr. Opin. Cell Biol.* **9**, 148-154.

Mumby, S. M., Casey, P. J., Gilman, A. G., Gutowski, S., and Sternweis, P. C., 1990, G protein gamma subunits contain a 20-carbon isoprenoid, *Proc. Natl. Acad. Sci. USA* **87**, 5873-5877.

Mumby, S. M., Kleuss, C., and Gilman, A. G., 1994, Receptor regulation of G-protein palmitoylation, *Proceedings of the National Academy of Sciences of the United States of America* **91**, 2800-2804.

Muntz, K. H., Sternweis, P. C., Gilman, A. G., and Mumby, S. M., 1992, Influence of gamma subunit prenylation on association of guanine nucleotide-binding regulatory proteins with membranes, *Molecular Biology of the Cell* **3**(1), 49-61.

Murthy, K. S., and Makhlouf, G. M., 1998, Coexpression of ligand-gated P2X and G protein-coupled P2Y receptors in smooth muscle. Preferential activation of P2Y receptors coupled to phospholipase C (PLC)-beta1 via Galphaq/11 and to PLC-beta3 via Gbetagammai3, *J. Biol. Chem.* **273**(8), 4695-4704.

Nagahama, M., Usui, S., Shinohara, T., Yamaguchi, T., Tani, K., and Tagaya, M., 20202, Inactivation of Galpha(z) causes disassembly of the Golgi apparatus, *J. Cell Sci.* **115**(Pt 23), 4483-4493.

Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N., Miyajima, N., Tanaka, A., Kotani, H., Nomura, N., and Ohara, O., 1997, Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro, *DNA Research* **4**(2), 141-150.

Najimi, M., Gailly, P., Maloteaux, J. M., and Hermans, E., 2002, Distinct regions of C-terminus of the high affinity neurotensin receptor mediate the functional coupling with pertussis toxin sensitive and insensitive G-proteins, *FEBS Letters* **512**(1-3), 329-333.

Namba, T., Sugimoto, Y., Negishi, M., Irie, A., Ushikubi, F., Kakizuka, A., Ito, S., Ichikawa, A., and Narumiya, S., 1993, Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP3 determines G-protein specificity [comment], *Nature* **365**(6442), 166-170.

Narvaez, J. A., Aguirre, J. A., d. P. Van, I., and Fuxe, K., 1992, Intracerebroventricularly administered pertussis toxin blocks the central vasopressor action of neuropeptide Y(13-36) in the awake unrestrained male rat, *Neuroscience Letters* **140**(2), 273-276.

Natochin, M., and Artemyev, N. O., 1998, A single mutation Asp^{229} Ser confers upon $G_s\alpha$ the ability to interact with regulators of G protein signaling, *Biochemistry* **37**, 13776-13780.

Neely, M. D., and Montine, T. J., 2002, CSF lipoproteins and Alzheimer's disease. [Review] [148 refs], *Journal of Nutrition, Health & Aging* **6**(6), 383-391.

Neer, E. J., 1995, Heterotrimeric G proteins: organizers of transmembrane signals, *Cell.* **80**(2), 249-257.

Negishi, M., Harazono, A., Sugimoto, Y., Hazato, A., Kurozumi, S., and Ichikawa, A., 1995, Selective coupling of prostaglandin E receptor EP3D to multiple G proteins depending on interaction of the carboxylic acid of agonist and arginine residue of seventh transmembrane domain, *Biochemical & Biophysical Research Communications* **212**(2), 279-285.

Newman-Tancredi, A., Cussac, D., Audinot, V., Pasteau, V., Gavaudan, S., and Millan, M. J., 1999, G protein activation by human dopamine D3 receptors in high-expressing Chinese hamster ovary cells: A guanosine-5'-O-(3-[35S]thio)- triphosphate binding and antibody study, *Mol. Pharmacol.* **55**(3), 564-574.

Nilsson, G., Johnell, M., Hammer, C. H., Tiffany, H. L., Nilsson, K., Metcalfe, D. D., 1996, A. Siegbahn, and P. M. Murphy, C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway, *J. Immunol.* **157**(4), 1693-1698.

NIMH, Anxiety disorders, NIH Publication No. 02-3879, (2002); http://www.nimh.nih.gov/anxiety.

NIMH, Depression, NIH Publication No. 02-3561 (2002), http://www.nimh.nih.gov/publicat/depression.htm

Ninan, P. T., 1999, The functional anatomy, neurochemistry, and pharmacology of anxiety, *J. Clin. Psychiatry* **60** (Suppl 22), 12-17.

Niu, J. X., Scheschonka, A., Druey, K. M., Davis, A., Reed, E., Kolenko, V., Bodnar, R., Voyno-Yasenetskaya, T., Du, X. P., Kehrl, J., and Dulin, N. O., 2002, RGS3 interacts with 14-3-3 via the N-terminal region distinct from the RGS (regulator of G-protein signalling) domain, *Biochemical Journal* **365**(3), 677-684.

Norgauer, J., Dobos, G., Kownatzki, E., Dahinden, C., Burger, R., Kupper, R., and Gierschik, P., 1993, Complement fragment C3a stimulates Ca2+ influx in neutrophils via a pertussis-toxin-sensitive G protein, *European Journal of Biochemistry* **217**(1), 289-294.

Nutt, D. J., 1996, The psychopharmacology of anxiety, *Br. J. Hosp. Med.* **55**(4), 187-191.

Obayashi, K., Horie, M., Xie, L. H., Tsuchiya, K., Kubota, A., Ishida, H., and Sasayama, S., 1997, Angiotensin II inhibits protein kinase A-dependent chloride conductance in heart via pertussis toxin-sensitive G proteins, *Circulation* **95**(1), 197-204.

O'Dowd, B. F., Hnatowich, M., Caron, M. G., Lefkowitz, R. J., and Bouvier, M., 1989, Palmitoylation of the human beta 2-adrenergic receptor. Mutation of Cys341 in the carboxyl tail leads to an uncoupled nonpalmitoylated form of the receptor, *J. Biol. Chem.* **264**(13), 7564-7569.

Offermanns, S., and Simon, M. I., 1996, Organization of transmembrane signalling by heterotrimeric G proteins, *Cancer Surv.* **27**, 177-198.

Offermanns, S., and Schultz, G., 1994, What are the functions of the pertussis toxin-insensitive G proteins G12, G13 and Gz?, *Mol. Cell Endocrinol.* **100**(1-2), 71-74.

Offermanns, S., and Simon, M. I., 1995, G alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C, *J. Biol. Chem.* **270**(25), 15175-15180.

Offermanns, S., Heiler, E., Spicher, K., and Schultz, G., 1994, G_q and G_{11} are concurrently activated by bombesin and vasopressin in Swiss 3T3 cells, *FEBS Lett.* **349**, 201-204.

Offermanns, S., Iida-Klein, A., Segre, G. V., and Simon, M. I., 1996, G alpha q family members couple parathyroid hormone (PTH)/PTH-related peptide and calcitonin receptors to phospholipase C in COS-7 cells, *Mol. Endocrinol.* **10**(5), 566-574.

Offermanns, S., Wieland, T., Homann, D., Sandmann, J., Bombien, E., Spicher, K., Schultz, G., and Jakobs, K. H., 1994, Transfected muscarinic acetylcholine receptors selectively couple to Gi-type G proteins and Gq/11, *Mol. Pharmacol.* **45**(5), 890-898.

Ogier-Denis, E., Pattingre, S., El Benna, J., and Codogno, P., 2000, Erk1/2-dependent phosphorylation of Galpha-interacting protein stimulates its GTPase accelerating activity and autophagy in human colon cancer cells, *J. Biol. Chem.* **275**(50), 39090-39095.

Oh, P., and Schnitzer, J. E., 2001, Segregation of heterotrimeric G proteins in cell surface microdomains. G(q) binds caveolin to concentrate in caveolae, whereas G(i) and G(s) target lipid rafts by default, *Molecular Biology of the Cell* **12**(3), 685-698.

Olah, M. E., 1997, Identification of A2a adenosine receptor domains involved in selective coupling to Gs. Analysis of chimeric A1/A2a adenosine receptors, *J. Biol. Chem.* **272**(1), 337-344.

Oliveira-dos-Santos, A. J., Matsumoto, G., Snow, B. E., Bai, D. L., Houston, F. P., Whishaw, I. Q., Mariathasan, S., Sasaki, T., Wakeham, A., Ohashi, P. S., Roder, J. C., Barnes, C. A., Siderovski, D. P., and Penninger, J. M., 2000, Regulation of T cell activation, anxiety, and male aggression by RGS2, *Proceedings of the National Academy of Sciences of the United States of America* **97**(22), 12272-12277.

Orcel, P., Tajima, H., Murayama, Y., Fujita, T., Krane, S. M., Ogata, E. , Goldring, S. R., and Nishimoto, I., 2000, Multiple domains interacting with Gs in the porcine calcitonin receptor, *Molecular Endocrinology* **14**(1), 170-182.

Osterhout, J. L., Waheed, A. A., Hiol, A., Ward, R. J., Davey, P. C., Nini, L., Wang, J., Milligan, G., Jones, T. L., and Druey, K. M., 2003, Palmitoylation Regulates Regulator of G-protein Signaling (RGS) 16 Function: II. Palmitoylation of a cysteine residue in the RGS box is critical for RGS16 GTPase accelerating activity and regulation of Gi-coupled signaling, *J. Biol. Chem.* **278**(21), 19309-19316.

Pacheco, M. A., Stockmeier, C., Meltzer, H. Y., Overholser, J. C., Dilley, G. E., and Jope, R. S., 1996, Alterations in phosphoinositide signaling and G-protein levels in depressed suicide brain, *Brain Research* **723**(1-2), 37-45.

Palmer, T. M., Gettys, T. W., and Stiles, G. L., 1995, Differential interaction with and regulation of multiple G-proteins by the rat A3 adenosine receptor, *J. Biol. Chem.* **270**(28), 16895-16902.

Parker, E. M., Kameyama, K., Higashijima, T., and Ross, E. M., 1991, Reconstitutively active G protein-coupled receptors purified from baculovirus-infected insect cells, *J. Biol. Chem.* **266**(1), 519-527.

Peitzsch, R. M., and McLaughlin, S., 1993, Binding of acylated peptides and fatty acids to phospholipid vesicles: pertinence to myristoylated proteins, *Biochemistry* **32**(39), 10436-10443.

Pitchot, W., Ansseau, M., Gonzalez, M. A., Lembreghts, M., Hansenne, M., Wauthy, J., Reel, C., Jammaer, R., Papart, P., Sulon, J. and ., 1995, The flesinoxan 5-HT1A receptor challenge in major depression and suicidal behavior, *Pharmacopsychiatry* **28** (Suppl 2), 91-92.

Pommier, B., Da Nascimento, S., Dumont, S., Bellier, B., Million, E., Garbay, C, Roques, B. P., and Noble, F., 1999, The cholecystokininB receptor is coupled to two effector pathways through pertussis toxin-sensitive and -insensitive G proteins, *J. Neurochem.* **73**, 281-288.

Pommier, B., Marie-Claire, C., Da Nascimento, S., Wang, H. L., Roques, B. P., and Noble, F., 2003, Further evidence that the CCK2 receptor is coupled to two transduction pathways using site-directed mutagenesis, *Journal of Neurochemistry* **85**(2), 454-461.

Posner, B. A., Gilman, A. G., and. Harris, B., 1999, Regulators of g protein signaling 6 and 7-Purification of complexes with Gβ5 and assessment of their effects on G protein-mediated signaling pathways, *J. Biol. Chem.* **274**(43), 31087-31093.

Prasad, M. V., Dermott, J. M., Heasley L. E., Johnson G. L., and Dhanasekaran, N., 1995, Activation of Jun kinase/stress-activated protein kinase by GTPase-deficient mutants of G alpha 12 and G alpha 13, *J. Biol. Chem.* **270**(31), 18655-18659.

Prather, P. L., Martin, N. A., Breivogel, C. S., and Childers, S. R., 2000, Activation of cannabinoid receptors in rat brain by WIN 55212-2 produces coupling to multiple G protein alpha-subunits with different potencies, *Mol. Pharmacol.* **57**(5), 1000-1010.

Pronin, A. N., and Gautam, N., 1992, Interaction between G-protein beta and gamma subunit types is selective, *Proceedings of the National Academy of Sciences of the United States of America* **89**(13), 6220-6224.

Puglielli, L., Tanzi, R. E., and Kovacs, D. M., 2003, Alzheimer's disease: the cholesterol connection. [Review] [70 refs], *Nature Neuroscience* **6**(4), 345-351.

Qanbar, R., and Bouvier, M., 2003, Role of palmitoylation/depalmitoylation reactions in G-protein-coupled receptor function. [Review] [480 refs], *Pharmacology & Therapeutics* **97**(1), 1-33.

Raap, D. K., Evans, S., Garcia, F., Li, Q., Muma, N. A., Wolf, W. A., Battaglia, G., and Van de Kar, L. D., 1999, Daily injections of fluoxetine induce dose-dependent desensitization of hypothalamic 5-HT_{1A} receptors: reductions in neuroendocrine responses to 8-OH-DPAT and in levels of G_z and G_i proteins, *J. Pharmacol. Exp. Ther.* **288**, 98-106.

Rajagopalan-Gupta, R. M., Rasenick, M. M., and Hunzicker-Dunn, M., 1997, Luteinizing hormone/choriogonado-tropin- dependent, cholera toxin-catalyzed adenosine 5'-diphosphate (ADP)-ribosylation of the long and short forms of Gs alpha and pertussis toxin-catalyzed ADP-ribosylation of Gi alpha*, *Molecular Endocrinology* **11**(5), 538-549.

Rana, B. K., 2003, New insights into G-protein-coupled receptor signaling from the melanocortin receptor system, *Molecular Pharmacology* **64**(1), 1-4.

Rebecchi, M. J., and Pentyala, S. N., 2000, Structure, function, and control of phosphoinositide-specific phospholipase C. [Review] [422 refs], *Physiol. Rev.* **80**(4), 1291-1335.

Reig, J. A., Yu, L., and Klein, D. C., 1990, Pineal transduction. Adrenergic cyclic AMP-dependent phosphorylation of cytoplasmic 33-kDa protein (MEKA) which binds beta gamma-complex of transducin, *J. Biol. Chem.* **265**(10), 5816-5824.

Robishaw, J. D., Smigel, M. D., and Gilman, A. G., 1986, Molecular basis for two forms of the G protein that stimulates adenylate cyclase, *J. Biol. Chem.* **261**(21), 9587-9590.

Ross, E. M., and Wilkie, T. M., 2000, GTPase-activating proteins for heterotrimeric G proteins: Regulators of G protein signaling (RGS) and RGS-like proteins, *Annual Review of Biochemistry* **69**, 795-827.

Roush, E. D., and Kwatra, M. M., 1998, Human substance P receptor expressed in Chinese hamster ovary cells directly activates G(alpha q/11), G(alpha s), G(alpha o), *FEBS Letters* **428**(3), 291-294.

Saitoh, O., Kubo, Y., Odagiri, M., Ichikawa, M., Yamagata, K., and Sekine, T., 1999, RGS7 and RGS8 differentially accelerate G protein-mediated modulation of K^+ currents, *J. Biol. Chem.* **274**(14), 9899-9904.

Saitoh, O., Kubo, Y., Miyatani, Y., Asano, T., and Nakata, H., 1997, RGS8 accelerates G-protein-mediated modulation of K^+ currents, *Nature* **390**, 525-529.

Samson, W. K., and Taylor, M. M., 2001, Hypocretin/orexin suppresses corticotroph responsiveness in vitro, *Am. J. Physiol Regul. Integr. Comp Physiol* **281**(4), R1140-R1145.

Sanchez-Blazquez, P., Garcia-Espana, A., and Garzon, J., 1995, In vivo injection of antisense oligodeoxynucleotides to G alpha subunits and supraspinal analgesia evoked by mu and delta opioid agonists, *J. Pharmacol. Exp. Ther.* **275**, 1590-1596.

Sargent, P., Williamson, D. J., Pearson, G., Odontiadis, J., and Cowen, P. J., 1997, Effect of paroxetine and nefazodone on 5-HT1A receptor sensitivity, *Psychopharmacology (Berl)* **132**, 296-302.

Sawaki, K., Baum, B. J., and Ambudkar, I. S., 1995, Alpha 1-adrenergic and m3-muscarinic receptor stimulation of phosphatidylinositol 4,5-bisphosphate-specific phospholipase C are independently mediated by G alpha q/11 in rat parotid gland membranes, *Archives of Biochemistry & Biophysics* **316**(1), 535-540.

Sawyer, N., Cauchon, E., Chateauneuf, A., Cruz, R. P., Nicholson, D. W., Metters, K. M., O'Neill, G. P., and Gervais, F. G., 2002, Molecular pharmacology of the human prostaglandin D2 receptor, CRTH2, *British Journal of Pharmacology* **137**(8), 1163-1172.

Schneider, H., Feyen, J. H., and Seuwen, K., 1994, A C-terminally truncated human parathyroid hormone receptor is functional and activates multiple G proteins, *FEBS Lett.* **351**(2), 281-285.

Schwaninger, R., Plutner, H., Bokoch, G. M., and Balch, W. E., 1992, Multiple GTP-binding proteins regulate vesicular transport from the ER to Golgi membranes, *J. Cell Biol.* **119**(5), 1077-1096.

Seki, N., Sugano, S., Suzuki, Y., Nakagawara, A. Ohira, M., Muramatsu, M., Saito, T., and Hori, T., 1998, Isolation, tissue expression, and chromosomal assignment of human RGS5, a novel G-protein signaling regulator gene, *Journal of Human Genetics* **43**(3), 202-205.

Serres, F., Li, Q., Garcia, F., Raap, D. K., Battaglia, G., Muma, N. A., and Van de Kar, L. D., 2000, Evidence that G_z proteins couple to hypothalamic 5-HT_{1A} receptors in vivo, *J. Neurosci.* **20**, 3095-3103.

Shah, B. H., and Milligan, G., 1994, The gonadotrophin-releasing hormone receptor of alpha T3-1 pituitary cells regulates cellular levels of both of the phosphoinositidase C-linked G proteins, Gq alpha and G11 alpha, equally, *Molecular Pharmacology* **46**, 1-7.

Shekter, L. R., Taussig, R., Gillard, S. E., and Miller, R. J., 1997, Regulation of human neuronal calcium channels by G protein betagamma subunits expressed in human embryonic kidney 293 cells, *Mol. Pharmacol.* **52**(2), 282-291.

Shi, C. S., Lee, S. B., Sinnarajah, S., Dessauer, C. W., Rhee, S. G., and Kehrl, J. H., 2001, Regulator of G-protein signaling 3 (RGS3) inhibits $G\beta_1gamma_2$-induced inositol phosphate production, mitogen-activated protein kinase activation, and Akt activation, *J. Biol. Chem.* **276**(26 PT 2), 24293-24300.

Shi, L. C., Wang, H. Y., Horwitz, J., and Friedman, E., 1996, Guanine nucleotide regulatory proteins, Gq and Gi1/2, mediate platelet-activating factor-stimulated phosphoinositide metabolism in immortalized hippocampal cells, *J. Neurochem.* **67**(4), 1478-1484.

Shuey, D. J., Betty, M., Jones, P. G., Khawaja, X. Z., and Cockett, M. I., 1998, RGS7 attenuates signal transduction through the $G_{\alpha q}$ family of heterotrimeric G proteins in mammalian cells, *J. Neurochem.* **70**, 1964-1972.

Sidhu, A., and Niznik, H. B., 2000, Coupling of dopamine receptor subtypes to multiple and diverse G proteins, *Int. J. Dev. Neurosci.* **18**(7), 669-677.

Siegel, G. J., and Chauhan, N. B., 2000, Neurotrophic factors in Alzheimer's and Parkinson's disease brain. [Review] [243 refs], *Brain Research - Brain Research Reviews* **33**(2-3), 199-227.

Siffert, W., Rosskopf, D., Siffert, G., Busch, S., Moritz, A., Erbel, R., Sharma, A. M., Ritz, E., Wichmann, H. E., Jakobs, K. H., and Horsthemke, B., 1998, Association of a human G-protein beta3 subunit variant with hypertension.[comment], *Nature Genet.* **18**(1), 45-48.

Silvius, J. R., and L'Heureux, F., 1994, Fluorimetric evaluation of the affinities of isoprenylated peptides for lipid bilayers, *Biochemistry* **33**(10), 3014-3022.

Simonds, W. F., Butrynski, J. E., Gautam, N., Unson, C. G., and Spiegel, A. M., 1991, G-protein beta gamma dimers. Membrane targeting requires subunit coexpression and intact gamma C-A-A-X domain, *J. Biol. Chem.* **266**(9), 5363-5366.

Sinnarajah, S., Dessauer, C. W., Srikumar, D., Chen, J., Yuen, J., Yilma, S., Dennis, J. C., Morrison, E. E., Vodyanoy, V., and Kehrl, J. H., 2001, RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III, *Nature* **409**(6823), 1051-1055.

Skiba, N. P., Yang, C. S., Huang, T., Bae, H., and Hamm, H. E., 1999, The alpha-helical domain of Galphat determines specific interaction with regulator of G protein signaling 9, *J. Biol. Chem.* **274**(13), 8770-8778.

Snow, B. E., Antonio, L., Suggs, S. Gutstein, H. B., and Siderovski, D. P., 1997, Molecular cloning and expression analysis of rat Rgs12 and Rgs14, *Biochem. Biophys. Res. Commun.* **233**, 770-777.

Snow, B. E., Antonio, L., Suggs, S., and Siderovski, D. P., 1998, Cloning of a retinally abundant regulator of G-protein signaling (*RGS-r/RGS16*): genomic structure and chromosomal localization of the human gene, *Gene* **206**, 247-253.

Snow, B. E., Krumins, A. M., Brothers, G. M., Lee, S. F., Wall, M. A., Chung, S., Mangion, J., Arya, S., Gilman, A. G., and Siderovski, D. P., 1998, A G protein gamma subunit-like domain shared between RGS11 and other RGS proteins specifies binding to $G_{\beta 5}$ subunits, *Proc. Natl. Acad. Sci. USA* **95**, 13307-13312.

Sondek, J., Bohm, A., Lambright, D. G., Hamm, H. E., and Sigler, P. B., 1996, Crystal structure of a G-protein beta gamma dimer at 2.1A resolution.[comment][erratum appears in Nature 1996 Feb 29;379(6568):847], *Nature* **379**(6563), 369-374.

Sonino, N., and Fava, G. A., 1996, Serotonin, Cushing's disease, and depression, *Psychother. Psychosom.* **65**, 63-65.

Srinivasa, S. P., Bernstein, L. S., Blumer, K. J., and Linder, M. E., 1998, Plasma membrane localization is required for RGS4 function in *Saccharomyces cerevisiae*, *Proc. Natl. Acad. Sci. USA* **95**, 5584-5589.

Stephens, L. R., Eguinoa, A., Erdjument-Bromage, H., Lui, M., Cooke, F., Coadwell, J., Smrcka, A. S., Thelen, M., Cadwallader, K., Tempst, P., and Hawkins, P. T., 1997, The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101, *Cell* **89**(1), 105-114.

Sterne-Marr, R., Tesmer, J. J., Day, P. W., Stracquatanio, R. P., Cilente, J. A., O'Connor, K. E., Pronin, A. N., Benovic, J. L., and Wedegaertner, P. B., 2003, G protein-coupled receptor Kinase 2/G alpha q/11 interaction. A novel surface on a regulator of G protein signaling homology domain for binding G alpha subunits, *J. Biol. Chem.* **278**(8), 6050-6058.

Stow, J. L., De Almeida, J. B., Narula, N., Holtzman, E. J., Ercolani, L., and Ausiello, D. A., 1991, A heterotrimeric G protein, G alpha i-3, on Golgi membranes regulates the secretion of a heparan sulfate proteoglycan in LLC-PK1 epithelial cells, *J. Cell Biol.* **114**(6), 1113-1124.

Strakova, Z., and Soloff, M. S., 1997, Coupling of oxytocin receptor to G proteins in rat myometrium during labor: Gi receptor interaction, *Am. J. Physiol* **272**(5 Pt 1), E870-E876.

Strakova, Z., Copland, J. A., Lolait, S. J., and Soloff, M. S., 1998, ERK2 mediates oxytocin-stimulated PGE2 synthesis, *Am. J. Physiol* **274**(4 Pt 1), E634-E641.

Sullivan, B. M., Harrison-Lavoie, K. J., Marshansky, V., Lin, H. Y., Kehrl, J. H., Ausiello, D. A., Brown, D., and Druey, K. M., 2000, RGS4 and RGS2 bind coatomer and inhibit COPI association with Golgi membranes and intracellular transport, *Molecular Biology of the Cell* **11**(9), 3155-3168.

Takagi, Y., Ninomiya, H., Sakamoto, A., Miwa, S., and Masaki, T., 1995, Structural basis of G protein specificity of human endothelin receptors. A study with endothelinA/B chimeras, *J. Biol. Chem.* **270**(17), 10072-10078.

Tanabe, Y., Masu, M., Ishii, T., Shigemoto, R., and Nakanishi, S., 1992, A family of metabotropic glutamate receptors, *Neuron* **8**(1), 169-179.

Tanaka, H., Kuo, C. H., Matsuda, T., Fukada, Y., Hayashi, F., Ding, Y., Irie, Y., and Miki, N., 1996, MEKA/phosducin attenuates hydrophobicity of transducin beta gamma subunits without binding to farnesyl moiety, *Biochem. Biophys. Res. Commun.* **223**(3), 587-591.

Tang, W. J., and Gilman, A. G., 1991, Type-specific regulation of adenylyl cyclase by G protein beta gamma subunits, *Science* **254**(5037), 1500-1503.

Tang, W. J., Iniguez-Lluhi, J. A., Mumby, S., and Gilman, A. G., 1992, Regulation of mammalian adenylyl cyclases by G-protein alpha and beta gamma subunits, *Cold Spring Harb. Symp. Quant. Biol.* **57**, 135-144.

Taussig, R., Iniguez-Lluhi, J. A., and Gilman, A. G., 1993, Inhibition of adenylyl cyclase by Gi alpha, *Science* **261**, 218-221.

Tekumalla, P. K., Calon, F., Rahman, Z., Birdi, S., Rajput, A. H., Hornykiewicz, O., Di Paolo, T., Bedard, P. J., and Nestler, E. J., 2001, Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease, *Biol. Psychiatry* **50**(10), 813-816.

Thekkumkara, T. J., and Linas, S. L., 2003, Evidence for involvement of 3'-untranslated region in determining angiotensin II receptor coupling specificity to G-protein, *Biochemical Journal* **370**(Pt 2), 631-639.

Thomsen, C., Kristensen, P., Mulvihill, E., Haldeman, B., and Suzdak, P. D., 1992, L-2-amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to adenylate cyclase.[erratum appears in Eur J Pharmacol 1993 Jan 15;244(2):187], *European Journal of Pharmacology* **227**(3), 361-362.

Tooze, S. A., Weiss, U., and Huttner, W. B., 1990, Requirement for GTP hydrolysis in the formation of secretory vesicles, *Nature* **347**(6289), 207-208.

Tso, P. H., and Wong, Y. H., 2000, Gz can mediate the acute actions of μ- and kappa-opioids but is not involved in opioid-induced adenylyl cyclase supersensitization, *J. Pharmacol. Exp. Ther.* **295**(1), 168-176.

Tso, P. H., Yung, L. Y., and Wong, Y. H., 2000, Regulation of adenylyl cyclase, ERK1/2, and CREB by G_z following acute and chronic activation of the δ-opioid receptor, *Journal of Neurochemistry* **74**(4), 1685-1693.

Tsu, R. C., Allen, R. A., and Wong, Y. H., 1995, Stimulation of type II adenylyl cyclase by chemoattractant formyl peptide and C5a receptors, *Mol. Pharmacol.* **47**(4), 835-841.

Tsu, R. C., Chan, J. S., and Wong, Y. H., 1995, Regulation of multiple effectors by the cloned delta-opioid receptor: stimulation of phospholipase C and type II adenylyl cyclase, *J. Neurochem.* **64**, 2700-2707.

Tsu, R. C., Lai, H. W., Allen, R. A., and Wong, Y. H., 1995, Differential coupling of the formyl peptide receptor to adenylate cyclase and phospholipase C by the pertussis toxin-insensitive Gz protein [published erratum appears in Biochem J 1995 Nov 1;311(Pt 3):1039], *Biochemical Journal* **309**(Pt 1), 331-339.

Ui, M., and Katada, T., 1990, Bacterial toxins as probe for receptor-Gi coupling, *Adv. Second Messenger Phosphoprotein Res.* **24**, 63-69.

Umemori, H., Inoue, T., Kume, S., Sekiyama, N., Nagao, M., Itoh, H., Nakanishi, S., Mikoshiba, K., and Yamamoto, T., 1997, Activation of the G protein Gq/11 through tyrosine phosphorylation of the α subunit, *Science* **276**, 1878-1881.

Van Corven, E. J., Hordijk, P. L., Medema, R. H., Bos, J. L., 1993, and W. H. Moolenaar, Pertussis toxin-sensitive activation of p21ras by G protein-coupled receptor agonists in fibroblasts, *Proceedings of the National Academy of Sciences of the United States of America* **90**(4), 1257-1261.

Vanek, M., Hawkins, L. D., and Gusovsky, F., 1994, Coupling of the C5a receptor to Gi in U-937 cells and in cells transfected with C5a receptor cDNA, *Molecular Pharmacology* **46**(5), 832-839.

Voyno-Yasenetskaya, T. A., Faure, M. P., Ahn, N. G., and Bourne, H. R., 1996, Galpha12 and Galpha13 regulate extracellular signal-regulated kinase and c-Jun kinase pathways by different mechanisms in COS-7 cells, *J. Biol. Chem.* **271**(35), 21081-21087.

Wade, S. M., Lim, W. K., Lan, K. L., Chung, D. A., Nanamori, M., and Neubig, R. R., 1999, G(i) activator region of alpha(2A)-adrenergic receptors: distinct basic residues mediate G(i) versus G(s) activation, *Molecular Pharmacology* **56**(5), 1005-1013.

Wang, J., Ducret, A., Tu, Y. P., Kozasa, T., Aebersold, R., and Ross, E. M., 1998, RGSZ1, a G_z-selective RGS protein in brain - Structure, membrane association, regulation by $G\alpha_z$ phosphorylation, and relationship to a G_z GTPase-activating protein subfamily, *J. Biol. Chem.* **273**, 26014-26025.

Wang, S., Hashemi, T., Fried, S., Clemmons, A. L., and Hawes, B. E., 1998, Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes, *Biochemistry* **37**(19), 6711-6717.

Watanabe, Y., Morita, H., Imaizumi, T., Takeda, M., Hariguchi, S., Nishimura, T., and Yoshida, H., 1990, Changes of ADP-ribosylation of GTP-binding protein by pertussis toxin in human platelets during long-term treatment of manic depression with lithium carbonate, *Clin. Exp. Pharmacol. Physiol* **17**(11), 809-812.

Watson, N., Linder, M. E., Druey, K. M., Kehrl, J. H., and Blumer, K. J., 1996, RGS family members: GTPase-activating proteins for heterotrimeric G-protein α-subunits, *Nature* **383**, 172-175.

Wedegaertner, P. B., and Bourne, H. R., 1994, Activation and depalmitoylation of Gs alpha, *Cell* **77**(7), 1063-1070.

Wedegaertner, P. B., Wilson, P. T., and Bourne, H. R., 1995, Lipid modifications of trimeric G proteins, *J. Biol. Chem.* **270**, 503-506.

Weissman, M. M., Warner, V., John, K., Prusoff, B. A., Merikangas, K. R., Wickramaratne, P., and Gammon, G. D., 1988, Delusional depression and bipolar spectrum: evidence for a possible association from a family study of children, *Neuropsychopharmacology* **1**(4), 257-264.

Wenzel-Seifert, K., Liu, H. Y., and Seifert, R., 2002, Similarities and differences in the coupling of human beta1- and beta2-adrenoceptors to Gs(alpha) splice variants, *Biochemical Pharmacology* **64**(1), 9-20.

Whitehead, I. P., Khosravi-Far, R., Kirk, H., Trigo-Gonzalez, G., Der, C. J., and Kay, R., 1996, Expression cloning of lsc, a novel oncogene with structural similarities to the Dbl family of guanine nucleotide exchange factors, *J. Biol. Chem.* **271**(31), 18643-18650.

Wilkie, T. M., Gilbert, D. J., Olsen, A. S., Chen, X. N., Amatruda, T. T., Korenberg, J. R., Trask, B. J., De Jong, P., Reed, R. R., Simon, M. I., 1992, Evolution of the mammalian G protein alpha subunit multigene family, *Nat. Genet.* **1**(2), 85-91.

Wilson, P. T., and Bourne, H. R., 1995, Fatty acylation of alpha z. Effects of palmitoylation and myristoylation on alpha z signaling, *J. Biol. Chem.* **270**, 9667-9675.

Wittau, N., Grosse, R., Kalkbrenner, F., 2000, A. Gohla, G. Schultz, and T. Gudermann, The galanin receptor type 2 initiates multiple signaling pathways in small cell lung cancer cells by coupling to G(q), G(i) and G(12) proteins, *Oncogene* **19**(37), 4199-4209.

Wolfson, M., Bersudsky, Y., Hertz, E., Berkin, V., Zinger, E., and Hertz, L., 2000, A model of inositol compartmentation in astrocytes based upon efflux kinetics and slow inositol depletion after uptake inhibition, *Neurochemical Research* **25**(7), 977-982.

Xu, N., Bradley, L., Ambdukar, I., and Gutkind, J. S., 1993, A mutant alpha subunit of G12 potentiates the eicosanoid pathway and is highly oncogenic in NIH 3T3 cells, *Proceedings of the National Academy of Sciences of the United States of America* **90**(14), 6741-6745.

Yajima, Y., Akita, Y., and Saito, T., 1986, Pertussis toxin blocks the inhibitory effects of somatostatin on cAMP-dependent vasoactive intestinal peptide and cAMP-independent thyrotropin releasing hormone-stimulated prolactin secretion of GH3 cells, *J. Biol. Chem.* **261**(6), 2684-2689.

Yamane, H. K., Farnsworth, C. C., Xie, H. Y., Howald, W., Fung, B. K., Clarke, S., Gelb, M. H., and Glomset, J. A, 1990, Brain G protein gamma subunits contain an all-transgeranylgeranyl-cysteine methyl ester at their carboxyl termini, *Proceedings of the National Academy of Sciences of the United States of America* **87**(15), 5868-5872.

Yan, K., Kalyanaraman, V., and Gautam, N., 1996, Differential ability to form the G protein betagamma complex among members of the beta and gamma subunit families, *J. Biol. Chem.* **271**(12), 7141-7146.

Yang, C. Q., Kitamura, N., Nishino, N., Shirakawa, O., and Nakai, H., 1998, Isotype-specific G protein abnormalities in the left superior temporal cortex and limbic structures of patients with chronic schizophrenia, *Biol. Psychiatry* **43**, 12-19.

Yang, S., Zhang, L., and Huang, Y., 2001, Membrane association and conformational change of palmitoylated G(o)alpha, *FEBS Letters* **498**(1), 76-81.

Yoshida, T., Willardson, B. M., Wilkins, J. F., Jensen, G. J., Thornton, B. D., and Bitensky, M. W., 1994, The phosphorylation state of phosducin determines its ability to block transducin subunit interactions and inhibit transducin binding to activated rhodopsin, *J. Biol. Chem.* **269**(39), 24050-24057.

Yowe, D., Weich, N., Prabhudas, M., Poisson, L., Errada, P., Kapeller, R., Yu, K., Faron, L., Shen, M., Cleary, J., Wilkie, T. M., Gutierrez-Ramos, C., and. Hodge, M., 2001, RGS18 is a myeloerythroid lineage-specific regulator of G-protein-signalling molecule highly expressed in megakaryocytes, *Biochemical Journal* **359**(Pt 1), 109-118.

Yung, L. Y., Tsim, S. T., and Wong, Y. H., 1995, Stimulation of cAMP accumulation by the cloned Xenopus melatonin receptor through Gi and Gz proteins, *FEBS Letters* **372**, 99-102.

Zheng, B., De Vries, L., and Farquhar, M. G., 1999, Divergence of RGS proteins: evidence for the existence of six mammalian RGS subfamilies, *Trends in Biochemical Sciences* **24**(11), 411-414.

Zheng, B., Chen, D., and Farquhar, M. G., 2000, MIR16, a putative membrane glycerophosphodiester phosphodiesterase, interacts with RGS16, *Proceedings of the National Academy of Sciences of the United States of America* **97**(8), 3999-4004.

Zheng, B., Ma, Y. C., Ostrom, R. S., Lavoie, C., Gill, G. N., Insel, P. A., Huang, X. Y., and M. G. Farquhar, 2001, RGS-PX1, a GAP for $G\alpha_s$ and sorting nexin in vesicular trafficking, *Science* **294**(5548), 1939-1942.

Zhou, J. Y., Siderovski, D. P., and Miller, R. J., 2000, Selective regulation of N-type Ca channels by different combinations of G-protein β/gamma subunits and RGS proteins, *Journal of Neuroscience* **20**(19), 7143-7148.

13. MONOAMINE TRANSPORTERS

Mohamed Jaber*

1. INTRODUCTION

The monoamine neurotransmitters, norepinephrine (NE), dopamine (DA), and serotonin (5-HT), have broad modulatory functions in the CNS that include mood, cognition, learning, motor activity, reward, aggression, sexual behavior, sleep, and appetite. Thus, it is not surprising that the pharmacological regulation of monoamine transmission has been found to influence a wide range of psychiatric disorders such as depression, attention deficit/hyperactivity disorder (ADHD), schizophrenia, drug abuse, Parkinson's disease, Tourette's syndrome, a spectrum of anxiety disorders, and anorexia nervosa (Carlsson, 1987; Duman, 1999; Ressler and Nemeroff, 1999).

Monoamine transporters are Na^+/Cl^- dependent plasma membrane proteins at nerve endings that rapidly reuptake the corresponding released neurotransmitter. Thus, they control the lifetime of monoamines and play a key role in determining the intensity and duration of monoamine transmission. This step limits the temporal persistence and the spatial spread of monoamines at their presynaptic and postsynaptic receptors and thus allow fine-tuning of neurotransmitter actions. Biochemical studies of the stoichiometry of monoamine transporters have shown that uptake of each monoamine molecule (positively charged at physiological pH) is coupled to cotransport of 2 Na^+ and 1 Cl^- (Krueger, 1990; McElvain and Schenk, 1992; Gu et al., 1994). Following uptake back into the cytoplasm of the released neurotransmitter, monoamines undergo a second step of transport required for their efficient recycling. This is performed by a vesicular transporter driven by a H^+ electrochemical gradient that packages monoamines from cytoplasm into secretory vesicles for their release by exocytosis.

* Mohamed Jaber, CNRS UMR 6187, Université de Poitiers, Pôle Biologie Santé, 40 av. du recteur Pineau, 86022 Poitiers Cedex, France.

Monoamine transporters have received a great deal of attention in the last 15 years mainly because they constitute crucial targets for antidepressants, psychostimulants and neurotoxins. In addition, they are implicated in various physiological and pathological conditions. This line of research have provided many key insights and opened a new and fruitful field for drug discoveries and genetic related disorders. For instance, the DA transporter (DAT) is suspected to play a pivotal role in the development of (i) addictive, (ii) neurotoxic, (iii) neurological, and (iv) physiological disorders related to DA. Indeed, (i) Psychostimulant drugs such as cocaine and amphetamine are powerful psychotropes that draw their reinforcing properties from their interaction with the DAT and the subsequent increase in DA overflow from terminals of DA neurons (Di Chiara and Imperato, 1988; Kuhar et al., 1991). (ii) All known DA neurotoxins, such as 1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine (MPTP) (Pifl et al., 1993) and 6-hydroxydopamine (6-OHDA), enter DA neurons via the DAT leading to selective degeneration of DA neurons and to Parkinson-like syndromes (Edwards, 1993; Miller et al., 1999). (iii) In Parkinson's disease, the number of DAT binding sites is reduced as one would suspect following loss of DA neurons; but possibly more significantly, the number of DAT per neuron seems to be also reduced compared to the normal situation (Uhl et al., 1994). This would suggest that DA neurons may undergo adaptive changes by reducing the number of DAT on their terminals, thus reducing uptake of DA in an attempt to increase the amount of extracellular DA. (iv) The DAT seems to be implicated in the etiology of ADHD as we will see below. In addition, some form of dopaminergic dysfunction may be related to schizophrenia as several neuroleptics, which are used in the successful management of some symptoms of this disorder, selectively block DA receptors. The DA hypothesis has further been strengthened by the fact that psychostimulants, such as amphetamine, which increases extracellular DA through interactions with the DAT, induce psychotic states resembling those observed in the positive symptoms of schizophrenia (euphoria, auditory hallucinations, and akathisia or the inability to remain inactive) (Kokkinidis and Anisman, 1980; Prasad et al., 2002).

2. HISTORICAL BACKGROUND

In 1932 Burn was among the firsts to propose that NE might be reuptaked (Burn, 1932) ; but it was the pioneered research work of Julius Axelrod and colleagues (1959) that triggered studies in the field of neurotransmitter reuptake, only 4 years following the serendipitous discovery of the first drug with antidepressant effects (Bloch et al., 1954). They were the first to propose that

NE can be reuptaked into the sympathetic nerve terminals using ^{3}H-adrenaline and than ^{3}H-NE. Soon afterwards, Iversen (1971) found that a similar mechanism occurs for the 5-HT transmission and proposed that this mechanism is central for inactivation of neurotransmission. Coyle and Snyder (1969) developed synaptosomal preparations to study reuptake in brain homogenates, a crucial step to identify the reuptake inhibitors efficiency. Kuhar (1973) showed that lesion of a noradrenergic pathway decreased the uptake of noradrenaline in synaptosomal preparations from target areas. Later on, radioligands were developed for the 5-HT transporter (SERT) (Langer et al., 1980) and than DAT (Kennedy and Hanbauer, 1983). These findings opened a new era in the discovery of antidepressant drugs and understanding the mechanism of action of psychostimulants. This line of investigation has become recently so popular that a book that deals with the antidepressant effects of Prozac (fluoxetine) was a best-seller for years and has been translated into several languages (Kramer, 1993). Currently, 5-HT selective uptake inhibitors (SSRI), such as Prozac, are amongst the most commonly used medications in the US (Nemeroff and Owens, 2002).

The history of the vesicular transporter field began with the discovery of neurotransmitter secretory vesicles themselves (Hillard, 1958). Chromaffin granules of adrenal medulla have been extensively used to study the function of these transporters where most of the biophysical, biochemical, pharmacological and cloning studies were initiated (for a review see Henry et al., 1998). The first description of ATP-dependant catecholamine uptake by isolated bovine chromaffin granules was given by Kirshener (1962) and by Carlsson et al. (1963). These transporters were shown to be inhibited by reserpine and tetrabenazine that deplete monoamine stores albeit through distinct mechanisms (Pletscher, 1977). Reserpine, which was initially used to reduce blood pressure, was also found to induce lethargy which suggested that amines are implicated in depression and provided the archetypal amine hypothesis of affective disorders (Frize, 1954). Tetrabenazine is still being used to treat movement disorders such as tics and dystonias and is known to deplete central amine stores.

3. GENE STRUCTURE AND FUNCTION

Cloning of the transporter genes has spurred rapid progress by several research groups that identified the transporters expression site, their specific pharmacology and gathered an extensive amount of data obtained in knock-out mice of these transporters. The purification of a rat GABA transporter protein (Radian et al., 1986) has led to the cloning of the first neurotransmitter transporter cDNA (Guastella et al., 1990). Using an expression cloning strategy Amara and colleagues isolated the first monoamine transporter cDNA, namely the NE transporter (NET) (Pacholczyk et al., 1991) that shared a high degree of sequence homology with the GABA transporter previously cloned, suggesting that they belong to a new multigene family. This finding led several groups to take advantage of the conserved sequences between the NE and GABA transporters to design degenerate primers for PCR reactions and identify additional members of this family. Members of this large family are distinguished by their strict functional dependence on inward co-transport on Na^+ and Cl^- ions. They share a significant degree of sequence homology and a similar transmembrane topology, with a 12 hydrophobic α-helical transmembrane domain with an amino and carboxy termini intracellularly oriented (Kyte and Doolittle, 1982). They are composed of 600 amino acids approximately and have a molecular masses in the order of 70 kDA. Glycosylation sites have been identified on the second large extracellular loop; they promote transporter stability, surface trafficking and transport activity (Melikian et al., 1996). Each of these transporters has several intracellular consensus sites for phosphorylation suggesting that their function or subcellular localisation may be regulated (reviewed by Zahniser and Doolen, 2001).

The monoamine subfamily consists of single genes coding for either the NET, the DAT, or the SERT (SLC6A3, SLC6A2 SLC6A4 respectively) located on different chromosomes.The close relationship of the transporter proteins and their transmembrane topology is reflected by the organization of their genes, where each TM is encoded by a single, homologous exons. This theoretical topology was challenged as several teams proposed that the first transmembrane domain (TM) forms a pore loop structure associated with the plasma membrane as described for ion channels where such structures form an ion filter (MacKinnon, 1995).

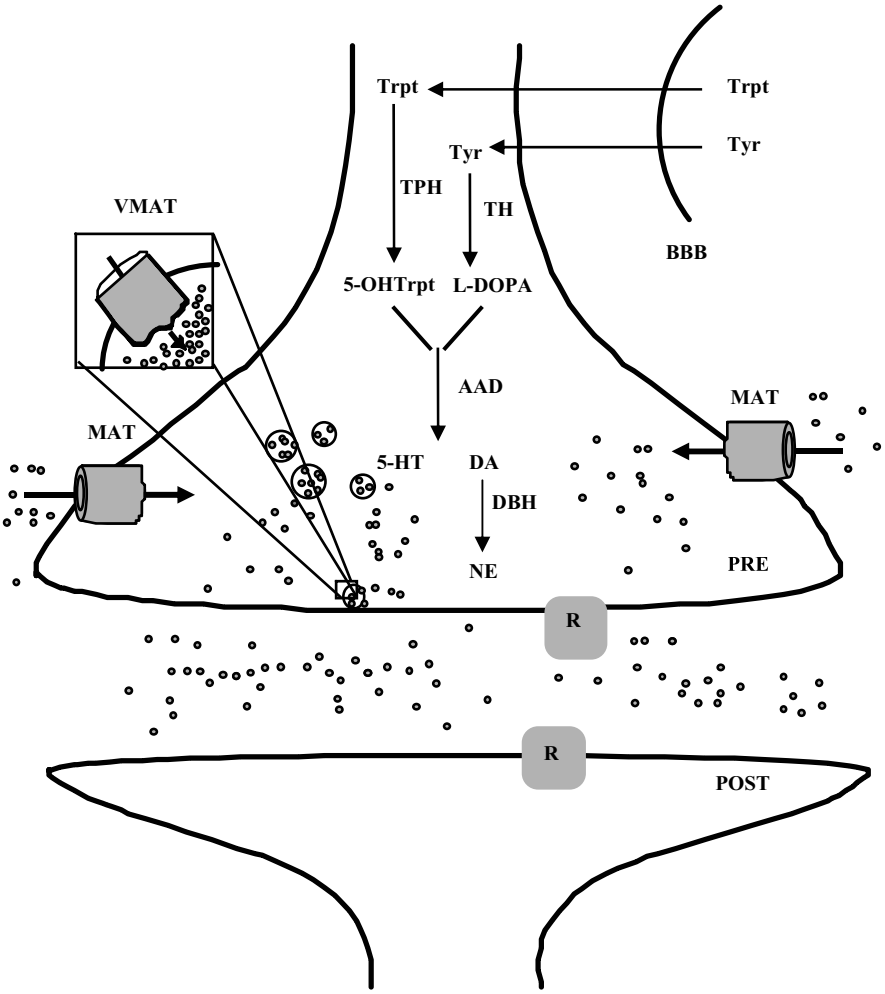


Figure 1. Dopamine (DA) is produced from the precursor tyrosine, which crosses the blood-brain barrier (BBB), by the rate limiting enzyme tyrosine hydroxylase (TH) than by the aromatic amino acid decarboxylase (AAD). Norepinephrine (NE) is obtained from dopamine by dopamine beta hydroxylase (DBH). Serotonin (5-HT) is synthetized from the precursor tryptophan (Trpt) by tryptophan hydroxylase (TPH) and AAD. These monoamines are sequestered into secretory vesicles by the vesicular monoamine transporters (VMAT). Following release, monoamines will activate pre and postsynaptic receptors before reuptake by monoamine transporters (MAT) that are specific to each neurotransmitter and that are localized presynaptically on the corresponding neurons, outside the synaptic cleft.

The gene for the human SERT has been mapped to chromosome 17q11.1-112 and was described to span 24 kb, and to contain 13 exons (Ramamoorthy et al., 1993). The human NET gene is localized to chromosome 16q12.2, spans 45 kb and was described as being composed of 16 exons giving rise to four splice variants, three of them only being functional (Gelernter et al., 1993; Pörzgen et al., 1998). Alternative splicing is of interest since it can provide a substrate for potential genetic variability through differential selection of splice variants by polymorphic sequences. In rat two isoforms were described, rNETa and rNETb which differ at their COOH termini (Kitayama et al., 1999). rNETb revealed no detectable transport function but reduced functional expression of rNETa when both isoforms were expressed in the same cell suggesting that rNETb may function as a dominant negative inhibitor of rNETa activity. Sequencing downstream of the C-terminal exon of the NET gene revealed 5 consensus polyadenylation signals and several adenylate/uridylate-rich elements, some of them containing the pentamer ATTTA, which is considered to be a mRNA instability determinant and may control cell-specific mRNA degradation and turnover rates of the transcripts (Porzgen et al., 1995, 1998). In addition, a possible role for C-terminal NET variants may be their involvement in targeting transporter proteins to plasma membrane.

The human DAT gene is located on chromosome 5p15.3 where it spans 65 kb consisting of 15 exons with no described alternative splicing (Giros et al., 1992; Vandenbergh et al., 1992). The deduced primary amino acid sequence contains multiple putative consensus sequences for phosphorylation by protein kinases, suggesting that DAT may be regulated by post-translational modifications which may alter the function of the protein and which may explain the pharmacological differences previously documented in various brain regions (Lew et al., 1992).

The identification of a specific DA toxin, MPTP, was due to its inadvertent administration by heroin addicts in the early 1980's (Langston et al., 1983) and was shown to induce parkinsonism in human and non-human primates which has led to the development of a valuable animal model for this disease (Burns et al., 1983). Neurotoxic effects of MPTP have been shown to be due to its oxidation product, 1-methyl-4-phenylpyridinium (MPP^+), which is actively transported into the presynaptic dopaminergic nerve terminals through the DAT (Irwin and Langston, 1985). Once inside the cell, MPP^+ is taken up into the mitochondria where it impairs respiration (DiMonte et al., 1983). This may lead to the formation of superoxide radicals (Rossetti et al., 1988) which in turn raise Ca^{2+} concentrations in the cytosol to cytotoxic levels, finally leading to cell death.

The ability of the vesicular transporters to sequester toxic substances, among which MPP^+, from the cytoplasm into vesicles has been used through a very elegant cloning strategies of selecting transfected CHO cells that express a protein providing cellular resistance to MPP^+ (Liu et al., 1992). Independently Erickson and co-workers (1992) have put into profit the ability of reserpine to inhibit vesicular transport and identified a reserpine-sensitive transporter by expression cloning. The two corresponding clones were named VMAT-1 and

VMAT-2, and code for distinct but related proteins with 12 putative transmembrane domains and a large intravesicular loop. These transporters constitute a distinct gene family of carriers than plasmamembrane transporters and accumulate cytoplasmic biogenic amines or neurotoxins by a H^+-driven uptake into storage vesicles. "Worm genetics" has played prominent role in the study of vesicular neurotransmitter transporters (reviewed by Rand et al., 2000). In C. Elegans, only a single VMAT gene was found indicating that VMAT-1 and VMAT-2 probably emerged as variants after duplication of a single gene. Interestingly, the vesicular transporters show some sequence similarity with several bacterial drug resistance genes. Accordingly, VMAT2 is sometimes referred to as a member of the toxin-extruding proton-translocating anti-porter gene family (TEXAN) (Schuldiner et al., 1995).

4. DISTRIBUTION WITHIN THE CNS

Two major points emerge from studies of monoamine transporters distribution: (i), they seem to be exclusively expressed in corresponding neurons in the CNS to the exception of SERT that was also found in glia in the nucleus accumbens (Pickel and Chan, 1999) and (ii) their localization is perisynaptical rather than within the synaptic cleft which enable monoamines to relay information through classical synaptic transmission as well as volume and paracrine transmission (Garris et al., 1994).

In the adult rat brain, NET labeling was confined to noradrenergic neuronal somata, axons, and dendrites, including extensive arborizations within the hippocampus and cortex. While glial cells lacked NET immunoreactivity, CNS ependymal cells were positively labeled with a NET antibody (Schroeter et al., 2000). Ultrastructural studies revealed substantial cytoplasmic NET immuno-reactivity in axon terminals consistent with postulates of regulated trafficking controlling neurotransmitter clearance.

In situ hybridization and immunohistochemistry studies have shown a much more restricted distribution of the DAT than what had been previously proposed based on radioligand binding studies. DAT mRNA was found only in DA synthesizing neurons and the corresponding protein is localized within dopaminergic innervation of several regions including ventral mesencephalon, medial forebrain bundle and dorsal and ventral striatum (Ciliax et al., 1995). Neuronal processes expressing the DAT were also found in several laminae in the cingulate cortex, with notable fiber densities in the superficial aspects of lamina I and laminae II/III. At the subcellular level, the DAT was detected both at the plasma membrane as well as at the rough and smooth endoplasmic reticulum, Golgi complex, and multivesicular bodies. However, the DAT was not identified within any synaptic active zones suggesting that striatal DA reuptake may occur outside of synaptic specializations once DA diffuses from the synaptic cleft (Hersch et al., 1997). In addition, the DAT was found in the somatodendritic region of midbrain DA neurons but was detected at the cell surface only in smaller, distal dendrites nerve terminal. This seems to be the case also for the SERT and NET (for a review see Liu et al., 1999).

The SERT is expressed preferentially into axons, where it appears concentrated at varicosities and terminal boutons, and is widely and heterogeneously distributed in the rat brain with high densities within the caudate-putamen, amygdaloid complex, cortical areas, substantia nigra, ventral pallidum, Islands of Calleja, septal nuclei, interpeduncular nucleus, trigeminal motor nucleus, the hippocampus, and olfactory nuclei (Sur et al., 1996).

While VMAT-1 was found to be restricted to endocrine cells, VMAT-2 is expressed in neurons and in some endocrine cells (Peter et al., 1995; Weihe et al., 1995). The lack of catecholamine storage in the CNS of VMAT-2 knock-out mice is consistent with the exclusive VMAT-2 expression to monoaminergic neurons in the brain. Monoamines are released both from relatively homogeneous synaptic vesicles 30–40 nm in diameter found at the nerve terminal and mediating rapid release and by large dense-core vesicles that occur in dendrites as well as axons and release more slowly and in response to distinct stimuli (Bruns and Jahn, 1995). Accordingly, VMAT-2 was found in both vesicles with a preferential localization in the large dense-core vesicles. The presence of VMAT-2 in distinct populations of secretory vesicles that reside at different sites in the cell, may allow the cells to respond to different stimuli and release their contents at different rates. Vesicular monoamine transport is regulated by protein kinase A that changes its subcellular location and membrane trafficking rather than its intrinsic activity (Nakanishi et al., 1995).

5. REGULATION OF MONOAMINE TRANSPORT

With the exception of the 5-HT_3 receptor which is a ligand-gated cation channel that mediate fast neurotransmission, monoamines interact with G protein-coupled receptors (GPCR) that mediate slow synaptic transmission to produce their effects. Thus, the extend of receptor stimulation and monoamine neuro-transmission in whole depends greatly on uptake efficiency. The importance of monoamine uptake has been underscored by findings in knock out mice, as we will describe below, and by compelling evidence that psychostimulants and antidepressants, that inhibit monoamine reuptake, greatly increase the corres-ponding neurotransmission. Being the key element regulating neurotransmission, regulation of transporter function should greatly affect synaptic transmission (reviewed by Zaniser and Doolen, 2001). Studies using irreversible inhibitors as well as antisense oligonucleotides directed against monoamine transporters mRNAs suggest that the respective protein's half life is of approximately 6 days (Silvia et al., 1997; Do Régo et al., 1999). However, the regulation of activity of these transporters can occur within seconds to minutes by membrane potential, autoreceptors, protein kinases, protein phosphatases, direct interaction with the actin cytoskeleton, oligomerization, membrane trafficking and, of course, transcription.

Membrane potential was one of the first stimuli identified that transiently regulated the activity of monoamine transporters. Indeed, early studies have suggested that monoamine transporters are dynamically regulated in a voltage-dependent manner (Dubocovich and Langer, 1976). These studies were recently

confirmed in experiments using the oocytes expression system (Sonders et al., 1997; Galli et al., 1998). It was found that while hyperpolarization results in increased reuptake, depolarization seems to be associated with decreased reuptake, thus allowing the neurotransmitter to diffuse far enough to reach the pre- and post-synaptic receptors. In the same line, the number of active monoamine transporters seems to be regulated by the extracellular levels of the respective neurotransmitters. For instance, when reserpine is applied, the NET were reduced by more than 50% and when MAO inhibitors are applied they increase by 30% (Lee et al., 1983). Similar conclusions can be drawn from the DAT studies where repeated administration of the DA precursor L-dihydroxy-phenylalanine (L-Dopa) induced a decrease in the DAT levels while administration of α-methyl-paratyrosine, an inhibitor of tyrosine hydroxylase, the rate limiting enzyme of DA synthesis, induced an increase (Ikawa et al., 1993).

At the plasma membrane, members of the Na^+/Cl^- -dependent transporters form dimers or tetramers that may be critical to their functional capacity (Milner et al., 1994; Kilic and Rudnick, 2000) as so do many GPCRs. Activation of protein kinase C inhibits uptake, currents and binding probably by altering cell surface trafficking of monoamine transporters (Zhu et al., 1997; Loder and Melikian, 2003). Phorbol ester activation of PKC results in decreased transporter capacity and a parallel decrease in the amount of DAT on the cell surface that is attributable to intracellular transporter sequestration in recycling, but not degradative endocytic pathway (Melikian and Buckley, 1999). Amphetamine-mediated DA release through the DAT was found to be highly dependent on protein kinase C activity (Kantor and Gnegy, 1998) which has led several investigators to propose that PKC regulates not only cell surface distribution of monoamine transporters, thus decreasing substrate uptake, but also enhances reverse transport (Zahniser and Doolen, 2001). Neurotransmitter uptake is activity-dependent since phosphorylation and sequestration of the SERT were substantially impacted by 5-HT, amphetamine, cocaine or antidepressants ligand binding probably by preventing PKC phosphorylation (Ramamoorthy and Blakely, 1999). In concordance with PKC studies, the monoamine transporters, which may be constitutively phosphorylated, seem to form a complex with protein phosphatase 2A, suggesting that phosphatases participate in the regulation of monoamine transporters (Bauman et al., 2000). Substrate occupancy and transport activity seem to promote retention of the transporters at the cell surface probably by inducing a conformation that enhances access of phosphatases thereby reducing phosphorylation by PKC and subsequent internalization.

Psychostimulants can produce long-term changes in the DAT expression. A single amphetamine injection induces a down regulation of the DAT that reflects transporter internalization (Saunders et al., 2000). Chronic blockade of DA uptake by prolonged use of cocaine, increase the levels of the DAT as seen in the striatum and accumbens in human autopsy samples (Little et al., 1993; Staley et al., 1994) and DAT levels were found increased during abstinence as evidenced with PET Scans (Malison et al., 1998). Regarding the SERT, its regulation by chronic antidepressant administration was the focus of a

substantial amount of studies that yielded conflicting results. Perhaps the only general agreement is that these treatments reduce the number of SERT and increase their desensitization (Pineyro et al., 1994; Benmansour et al., 1999). Similar conclusions can de drawn for the NET where results generally agree that its long term blockade produce a transient decrease in its level.

6. CELLULAR MOLECULAR AND BEHAVIOURAL CONSEQUENCES OF KNOCK-OUT OF THE MONOAMINE TRANSPORTER GENES

Considerable progress has been made in the molecular characterization of the monoamine transporters that provided the means for targeted approaches of studying many key features such as uptake processes, psychostimulants and antidepressants action, and monoamines related neurological and psychiatric disorders. Although much has been described regarding the role of monoamine transporters in re-uptaking respective neurotransmitters and in the action of various psychostimulants and antidepressants, the relative contribution of each of these transporters in the physiological and pharmacological regulation of neurotransmission was not quit established mainly because drugs that target these transporters often lack selectivity. The gene inactivation procedure *in vivo*, or knock out, permits the creation of strains of mice specifically lacking a designated gene. This technique has been applied to inactivate the expression of DAT, NET, SERT and VMAT-2. A striking example of the relevance of such studies in understanding monoamine transmission has been obtained from DAT knock-out mice that we have helped characterize. Our results established not only the central importance of the DAT as the key element controlling extracellular DA levels but its role as an obligatory target for the behavioral and biochemical action of amphetamine and cocaine.

Using $DAT^{-/-}$, we have demonstrated that the DAT is involved in the lifetime control of extracellular DA (Giros et al., 1996; Jones et al., 1998; Benoit-Marand et al., 2000), maintenance of pre-synaptic DA functions such as autoreceptor regulation (Jones et al., 1999, Benoit-Marand et al., 2000), DA synthesis (Jaber et al., 1999) and storage mechanisms (Jones et al., 1998) as well as post synaptic regulation such as receptor endocytosis (Dumartin et al., 2000) and gene expression regulation (Fauchey et al., 2000a, 2000b; Le Moine et al., 2002). The synthesis, storage, and degradation of DA has been markedly changed suggesting that the transporter is an important factor regulating homeostasis of DA in the basal ganglia (Jones et al., 1998; Jaber et al., 1999). $DAT^{-/-}$ mice present a hyperactive DA phenotype that is reflected behaviorally by several abnormalities due to a dysfunction within (i) the dopaminergic mesolimbic system (locomotor hyperactivity, lack of habituation to novelty and increased response to environmental stimuli) (Spielewoy et al., 2000), (ii) the dopaminergic nigrostriatal system (motor and sensorimotor integration deficits) (Fernagut et al., 2002 and 2003) and (iii) the hypothalamo-infundibular system (decreased growth hormone and prolactin secretion) (Bosse et al., 1997).

An obvious phenotype observed in mice lacking the DAT is the remarkable increase in their spontaneous locomotor activity during both day and night

(Giros et al., 1996). This increase is of the same magnitude as in normal mice treated with very high doses of amphetamine and cocaine which are known to produce this locomotor effect by increasing the amount of DA available in the basal ganglia (Giros et al., 1996). The characterization of the biochemical and molecular adaptation mechanisms in these animals made the marked increase in locomotor activity surprising for many reasons. Most of the adaptive changes so far documented in these animals would all be expected to dampen dopaminergic responsiveness. For instance, *in situ* hybridization and ligand binding experiments revealed that $DAT^{-/-}$ mice have as little as 50% of the normal levels of both D1 and D2 receptors, the main dopaminergic receptors in the basal ganglia. The biochemical basis for the paradoxical hyperactivity of $DAT^{-/-}$ mice was revealed by examining the dynamics of the neurotransmitter DA. Using cyclic voltammetry, a technique capable of measuring real time stimulated release and reuptake of DA, it was found that, in $DAT^{-/-}$ mice, DA remains in the extracellular space 300 times longer than in normal animals and diffusion is the only mechanism left for the clearance of DA. Inhibitors of DA degradative enzymes or blockers selective for other biogenic amine transporters do not influence the kinetics of clearance (Benoit-Marand et al., 2000). These findings establish the crucial role of the DAT in maintaining a normal dopaminergic tone such that its loss cannot be compensated by any adaptive changes even of a magnitude never previously observed.

An interesting contrast can be drawn between $DAT^{-/-}$ mice in which as little as 5% of intracellular DA leads to 500% of extracellular DA and subsequent hyperactivity and patients with Parkinson's disease in whom similarly low levels of DA cause debilitating impairment in locomotion. Therapeutic strategies aimed at mimicking some of the properties or adaptive changes of $DAT^{-/-}$ mice could prove beneficial to Parkinson's patients. These strategies might include high affinity DAT blockers, alone or in conjunction with agents that increase synthesis and/or retard degradation of DA.

Besides providing strong direct evidence for a role of the DAT in the tight control of DA homeostasis and transmission, the $DAT^{-/-}$ mice have provided key elements in understanding the way psychostimulants act on the DA system. Cocaine and amphetamine are known to increase DA levels through their action on the DAT. Cocaine has been known to act by blocking the DAT and thus inhibiting DA reuptake which ultimately leads to an increase in extracellular DA levels. However, the action of amphetamine seems to relate mainly to its ability to promote release of DA. Whether this action of amphetamine is solely dependent on the DAT was not firmly established (Eschleman et al., 1994). Behaviorally $DAT^{-/-}$ mice do not respond to doses of cocaine (40 mg/kg) and amphetamine (10 mg/kg) by further increasing their locomotion, when these drugs are given in their home cages. These drug doses are high enough to markedly increase the locomotor activity of normal animals followed in time by an increase in stereotyped behaviors. These findings are consistent with cyclic voltammetry data which show that, in the absence of the DAT, amphetamine is completely ineffective in promoting release of DA despite being able to abolish intracellular stores of the neurotransmitter (Giros et al., 1996). Thus, the DAT is required for the DA releasing action of amphetamine. These conclusions have

also been corroborated by results obtained from studies using short and long term unilateral DAT antisense treatments in the substantia nigra of rats (Silvia et al., 1997). Indeed, rotational behavior results using this model demonstrated that cocaine acts as a DAT blocker, whereas amphetamine acts as a DA releaser and this release is DAT-dependent.

Regarding the vesicular transporters, disruption of the gene encoding VMAT-2 results in perinatal death due to a failure to release DA, NE and 5-HT (Masson et al., 1999 for a review). VMAT-2 heterozygotes appear normal but demonstrate substantial deficits in the storage and release of monoamines as well as alterations in behaviour.

7. MONOAMINE TRANSPORTERS AND NEUROTOXICITY

As discussed above, studies of the mechanisms of the toxic effects of DA related neurotoxins such as 6-hydroxydopamine, MPTP and its metabolite MPP^+ have focused on the DAT and VMAT-2. The DAT has long been known to be a neuronal gate to neurotoxins. It uptakes DA-specific neurotoxins from the extracellular space into the cytosol of DA neurons. Neurotoxins are then concentrated into synaptic vesicles by VMAT-2 protecting the neurons by lowering free cytoplasmic concentrations of these substrates (reviewed by Uhl, 1998). Consequently, the ratio of DAT to VMAT-2 could impact the function and ultimately survival of the DA neurons following administration of potentially toxic compounds inducing experimental parkinsonian syndromes (Pifl, 1993; Sanghera et al., 1997). Interestingly, the levels of DAT and VMAT-2 expression in dopaminergic neurons parallel the extension of neuronal loss in Parkinson disease. For instance, in monkeys that have been administered MPTP, the caudate and the putamen, which have the highest levels of DAT, are the most severely affected whereas the thalamus and amygdala, that have high levels of VMAT-2 and low levels of DAT, are virtually unaffected (Miller et al., 1998). In addition, a correlation can be drawn between the dopaminergic brain areas affected by the extend neuronal loss in Parkinson disease patients and the respective density of DAT. Thus, a dysfunctional transport process, as observed with ageing for instance, may contribute ton an increased susceptibility to exogenous stressors such as MPP^+-like neurotoxins.

If the DAT appears implicated in the MPTP toxicity, its role has been overall determined by *in vitro* studies. Using $DAT^{-/-}$ mice, we demonstrated at the cell body level, the direct evidence of the DAT requirement for *in vivo* MPTP-induced nigral degeneration (Bezard et al., 1999). Indeed, the number of DA neurons of the substantia nigra compacta of the $DAT^{-/-}$ mice was not affected by the treatment with MPTP. In addition, if the absence of DAT confers to $DAT^{-/-}$ mice a total neuroprotection, the decrease in number of DAT sites in $DAT^{+/-}$ mice to half of normal levels provides decreased accessibility of MPP^+ into the neuron. Accordingly, VMAT-$2^{+/-}$ mice showed an increased vulnerability to MPTP (Gainetdinov et al., 1998).

These findings suggests that, if a unknown endogenous neurotoxin is responsible of this human disease, individual vulnerability may be related to

levels of DAT expressed. If true, a simple assessment of these levels may prove beneficial in preventive medicine or early diagnosis of the disease. In addition, decreasing DA uptake by DAT blockers in patients with Parkinson's disease might be beneficial not only to increase DA levels at the synapse but also to prevent an eventual endogenous or exogenous toxin to enter DA neurons. Similar results have been obtained with methamphetamine that enters also the DA neurons through the DAT and by diffusion through the membrane due to its lipophilic nature (Fumagalli et al., 1998). The neurotoxic effects of methamphetamine on the DA system were abolished in $DAT^{-/-}$ mice and were increased in VMAT-$2^{+/-}$ mice (Fumagalli et al., 1999).

In parallel to these studies, we focused more recently on the consequences of prolonged hyperdopaminergia on neuronal toxicity. The interest of these studies arise from the increasing evidence that DA exerts a permissive action on striatal excitoxic processes (Reynolds et al., 1998; Jakel and Maragos, 2000). On one hand, the protective effect of nigrostriatal dopaminergic transmission disruption upon striatal excitotoxic damage has been observed; on the other hand, increased dopaminergic transmission by methamphetamine enhances 3-NP striatal toxicity (Reynolds et al., 1998). In addition, alteration in the number of striatal neurons in human long-term methamphetamine and cocaine abusers has been recently proposed (Ernst et al., 2000; Volkow et al., 2001). We have shown a spontaneous impaired functioning of the nigrostriatal system in $DAT^{-/-}$ hyperdopaminergic mice, as illustrated by motor and sensorimotor integration deficits, despite their apparent hyperactivity. We provided evidence suggesting that constitutive elevated striatal DA tone as found in $DAT^{-/-}$ mice enhances the histopathological and behavioural consequences of intoxication with 3-NP, a toxin that inhibits mitochondrial respiration, since these mice showed a significant impairment in a rotarod task and a significant reduction of the striatal volume, neuronal density and absolute number estimates of striatal neurons only in $DAT^{-/-}$ mice (Fernagut et al., 2002, 2003). These observations are of clinical relevance regarding the role of endogenous DA or dopaminergic treatments on the neurodegenerative process of diseases affecting the striatum, such as Huntington's disease (HD) and nigrostriatal degeneration (Tison et al., 1995) or striatal dysfunction in methamphetamine and cocaine abusers (Ernst et al., 2000; Volkow et al., 2001).

In relation with our previous work regarding the genetic influence on neurotoxin susceptibility, we have initiated a study to investigate the effects of life experience on an experimental model of Parkinson's disease. We compared the effect of MPTP in mice raised in either a standard or an enriched environment. Our findings showed that adult mice raised in an enriched environment for only two months are 200% more resistant to MPTP. Indeed, while mice raised in a standard environment showed a 75% loss of DA neuron, mice raised in an enriched environment showed only 40% of such loss. This is achieved by down regulating the expression of the DAT (Bezard et al., 2003). These data provide a direct demonstration that a positive short life experience may have beneficial consequences on the probability to develop later on neurological disorders such as Parkinson's disease.

The substituted amphetamine, (+)-3,4-methylenedioxymethamphetamine (MDMA), is an indirect sympathomimetic highly lipophilic with effects on the serotoninergic system (Rattray, 1991). It has been suggested that the primary mechanism of action of MDMA involves the displacement of the biogenic amines from their storage vesicles and ultimately the enhancement of the release of endogenous 5-HT from presynaptic nerve terminals, presumably via reversal of the plasma membrane 5-HT transport (Rudnick and Wall, 1992). The net effect of this agent is an increase in 5-HT, as well as DA, in the synapse and a subsequent increase in locomotor activity. These effects were completely absent in 5-HTT$^{-/-}$ mutants (Bengel et al., 1998). Long term administration of MDMA results in toxic degeneration of serotoninergic terminals that can be prevented by 5-HT uptake inhibitors (for a review see, Lesch et al., 1996).

8. POLYMORPHISMS, PHARMACOGENETICS AND BRAIN DISORDERS

Genetic factors contribute to the risk of psychopathology in many psychiatric conditions but the specific genes involved and their relative contribution are yet to be determined. Because monoamines have broad modulatory functions in the CNS and dysfunction in the monoamine system is implicated in several affective, psychiatric, and neurological disorders, etiological research has focused significantly on the investigation of genetic factors that may predispose to these disorders. Given the central role of the monoamine transporters, DAT, SERT, and NET genes have been extensively screened for genetic polymorphisms (reviewed by Hahn and Blakely, 2002; Glatt and Reus, 2003). Polymorphisms can affect monoamine transporters function through differential regulation of gene expression, altered trafficking, modified physiological or pharmacological substrate binding, and uptake dysfunction.

Most genomic variation is attributable to single nucleotide polymorphisms (SNPs, pronounced “snips”) where two alternate bases occur at one position. Those of interest are located in the promoter region of a gene as well as in exonic or intronic regions, with a range of 3-50 SNPs per 10 kilobases. Structural polymorphisms in monoamine transporters causing substantial changes in the structure of the transporters that would significantly alter uptake are rare. This suggests that these transporters play key role in regulating transmission and that the corresponding genes are under a selective pressure. Polymorphisms that do not alter amino acid sequence of transporters such as synonymous SNPs, non-coding SNPs, variable number of tandem repeats (VNTR) regions are more common. They can modulate levels of the transporters at the transcriptional or translational level or alter transporter trafficking and thus influence reuptake efficiency.

In spite of the large number of association studies between psychiatric disorders and monoamines transporter genes that have been performed so far, it is difficult, to a few exceptions, to draw a general conclusion as discrepancies are often found. These inconsistency may be related to variable diagnosis

criteria, which are often obtained only by clinical interview, methodological differences, population heterogeneity, ethnical diversity, and lack of statistical power to detect small gene effects. In addition, complex disorders, such as psychiatric illnesses, are believed to require multiple deleterious genetic variants that may act in combination with environmental factors. Nonetheless, the best examples of successful linkage found between the monoamine transporter genes and neurological and psychiatric disorders are mutations in the NET gene that were recently found to contribute to an autonomic nervous system disorder, polymorphisms in the SERT gene that are linked to impulsive behavior with suicidal attempts and alcoholism co-morbidity and polymorphisms in the DAT gene that have been linked to ADHD.

Several SNPs were described for the NET gene and that seem to participate in the homeostasis of blood pressure (Halushka et al., 1999). A structural polymorphism was found with the NET that seems to be implicated in orthostatic intolerance, a pathology characterized by a standing-involved increase in heart rate (Shannon et al., 2000). This significant correlation between a NET gene polymorphism and orthostatic intolerance results in a proline substitution for an alanine at position 457 that is believed to impair reuptake, decrease expression and alter transporter trafficking.

The lengthy scientific history linking 5-HT and behavior in patients with neuropsychiatric disorders has lead many researchers to investigate SERT polymorphisms. Two repetitive sequence elements within the SERT were found to display polymorphic repeats consisting of a variable number of tandem repeat (VNTR) that varies greatly within different ethnic populations. The first one is a VNTR polymorphism that was found in the intron 3 of the SERT gene and is composed of 17 bp repeats, the most prevalent being the 10 and 12 repeats (Lesch et al., 1994). The other SERT polymorphism is named 5-HTTLPR and is due to a 44 bp deletion (SS)/insertion (LL) in the 5' region that results in differential expression of SERT gene (Lesch et al., 1994; Heils et al., 1996). Homozygote (SS) and heterozygote (SL) forms are associated with fewer binding sites than the homozygote (LL). This has led to the hypothesis that patients homozygous for the long variant may require higher doses of antidepressants to achieve a pharmacotherapeutic effect. A growing number of studies indicate that patients with major depressive disorder possessing the 5-HTTLPR L allele may exhibit a better long-term outcome when treated with antidepressants. For instance, clinical improvement of depressive symptoms was more significant for carriers of the L allele [L/L and L/S genotypes] than for those possessing the S/S genotype during long-term treatment with antidepressants. In addition, a response to treatment was also significantly more frequent in carriers of the L allele than in those with the S/S genotype (Zanardi et al., 2000; Lee et al., 2004).

Although non consensus in the literature on the association of the 5-HTTLPR genotype with a particular mood disorder can be drawn, many studies converge towards an implication of the S allele with impulsive violent behavior co-morbid with alcohol dependence or suicide attempts (see Arango et al., 2003 and Anguelova et al., 2003 for a review). Indeed, consistent evidence suggest that suicide behavior has a genetic component. While multiple

neurotransmitters alterations are likely present in the brain of suicide victims, 5-HT alterations have most consistently been found. For instance, less 5-HT binding was found in the prefrontal cortex of suicide victims. Among the candidate 5-HT genes investigated, the most relevant may well be the SERT where a positive genetic association was often found between the S allele of the 5-HTTLPR and the suicidal behavior (Bondy et al., 2000; Courtet et al., 2001). Moreover, Gorwood et al. (2000) found that the S allele is associated with severe suicide attempts in alcoholic-dependent patients, this association is stronger for numerous suicide attempts with high lethality.

The DAT gene is also subject to VNTR in intron 8 and the 3' untranslated region that harbors a 40 bp repeats of 3 to 11 copies, with the 9 and 10 copies being the most frequent (Vandenbergh, 1992). Conflicting results were obtained in linkage studies focused on the 9-10 repeat alleles and cocaine abuse, affective disorders, or alcoholism (Hahn and Blakely, 2002). However, it seems to be established that alleles with 10 copies of the 40 bp repeat unit are associated with ADHD. ADHD is a childhood onset, clinically heterogeneous disorder characterized by excessive motor activity, impulsiveness, and inattention. 4% of school-aged children in the U.S. are currently treated with amphetamine-like psychostimulant drugs to manage this disorder that is diagnosed based only on the DSM IV criteria. Several lines of evidence implicate a DA system dysfunction in the pathogenesis of ADHD (Swanson et al., 2000). For instance, efficient control of ADHD symptoms is obtained with psychostimulant drugs such as methylphenidate that target the DAT and increase DA levels. Exposure to lead during childhood seems to be associated with ADHD (Brockel and Cory-Slechta, 1998) and lead is uptaken by the DAT (Boykin et al., 1991). Abnormal levels of DAT are found in the brains of subjects with ADHD (Dougherty et al., 1999). Finally, mice lacking the DAT show several symptoms relevant to ADHD such as cognitive impairments and hyperactivity which is decreased by psychostimulants injections (Gainetdinov et al., 1999). Few studies have addressed the relationship between genetic markers at the DAT locus and response to methylphenidate in ADHD patients. Although a significant effect of the 40 bp VNTR on response to methylphenidate has been detected in most of these reports, the findings are still inconsistent regarding both the allele involved and the drug response (See Madras et al., 2002 and DiMaio et al., 2003 for a review).

9. CONCLUSION

The prescription of psychoactive drugs in the beginning of this 21st century still relies on clinical investigation rather than on new findings in the psycho-pharmacogenomic field. Although results obtained from pharmacogenetics approaches remain preliminary, they do provide a novel dissection of the heterogeneity of psychotropic drug response and may help in the near future to individualize pharmacological therapy. Better phenotype definition and emerging genomic technologies that include bioinformatics, statistical methods, human genomic information, functional genomics, and pharmacogenomics

could provide new paradigm of drug discovery research and novel strategy of medical care. We should keep in mind, however, that the genetic sequence cannot effectively predict post-transciptional and post-translantional modifications and that it is likely that a variety of genetic as well as environmental pathways are involved in the susceptibility to a given psychiatric disorder.

10. ACKNOWLEDGMENTS

Work in the author's laboratory is supported by the Centre National de la Recherche Scientifique, Université of Poitiers, Région Poitou-Charentes, Association France Parkinson and Fondation de la Recherche Médicale.

11. REFERENCES

Axelrod, J., Weil-Malherbe, H., and Tomchick, R., 1959, The physiological disposition of ^{3}H-epinephrine and its metabolite metanephrine, *J. Pharmacol. Exp. Ther.* **127**:251.

Anguelova, M., Benkelfat, C., and Turecki, G., 2003, A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: II Suicidal behavior, *Mol. Psy.* **8**:646.

Arango, V., Huang, Y.-Y, Underwood, M. D., and Mann, J. J., 2003, Genetics of the serotoninergic system in suicidal behavior, *J. Psychi. Res.* **37**:375.

Bauman, A. L., Apparsundaram, S., Ramamoorthy, S., Wadzinski, B. E., Vaughan, R. A., and Blakely, R. D., 2000, Cocaine and antidepressant-sensitive biogenic amine transporters exist in regulated complexes with protein phosphatase 2A, *J. Neurosci*. **20**:7571.

Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., Heils, A., Mossner, R., Westphal., H., and Lesch, K. P., 1998, Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice, *Mol Pharmacol*. **53**:649.

Benmansour, S., Cecchi, M., Morilak, D. A., Gerhardt, G. A., Javors, M. A., Gould, G. G., and Frazer, A., 1999, Effects of chronic antidepressant treatments on serotonin transporter function, density and mRNA level, *J. Neurosci*. **19**:10494.

Benoit-Marand, M., Jaber, M., and Gonon, F., 2000, Release and elimination of dopamine in vivo in mice lacking the dopamine transporter: functional consequences, *Eur. J. Neurosci*. **12**:2985.

Bezard, E., Gross, C. E., Fournier, M. C., Dovero, S., Bloch, B., and Jaber M., 1999, Absence of MPTP-induced neuronal death in mice lacking the dopamine transporter, *Exp. Neurol.* **155**:268.

Bezard, E., Dovero, S., Belin, D., Duconger, S., Jackson-Lewis, V., Przedborski, S., Piazza, P. V., Gross, C. E., and Jaber, M., 2003, Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: involvement of dopamine transporter and trophic factors, *J. Neurosci.* **23**:10999.

Bloch, R. G., Dooneief, A. S., Buchberg, A. S., and Spellman, S., 1954, The clinical effect of isoniazid and iproniazid in the treatment of pulmonary tuberculosis, *Ann. Intern. Med.*, **40**:881.

Bondy, B., Kuznik, J., Baghai, T., Schule, C., Zwanzger, P., Minov, C., de Jonge, S., Rupprecht, R., Meyer, H., Engel, R. R., Eisenmenger, W., Ackenheil, M., 2000, Lack of association of serotonin-2A receptor gene polymorphism (T102C) with suicidal ideation and suicide, *Am. J. Med. Genet.* **96**:831.

Bosse, R., Fumagalli, F., Jaber, M., Giros, B., Gainetdinov, R. R., Wetsel, W. C., Missale, C., and Caron, M. G., 1997, Anterior pituitary hypoplasia and dwarfism in mice lacking the dopamine transporter, *Neuron* **19**:127.

Boykin, M. J., Chetty, C. S., and Rajanna, B., 1991, Effects of lead on kinetics of ^{3}H-dopamine uptake by rat brain synaptosomes, *Ecotoxicol. Environ. Saf*. **22**:88.

Brockel, B. J., and Cory-Slechta, D. A., 1998, Lead, attention, and impulsive behavior: changes in a fixed-ration-waiting-for-reward paradigm, *Pharmacol. Biochem. Behav*. **60**:545.

Bruns, D., and Jahn, R., 1995, Real-time measurement of transmitter release from single synaptic vesicles, *Nature* **377**:62.

Burn, J. H., 1932, The action of tyramine and ephedrine, *J. Phamacol. Exp. Ther*. **46**:75.

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. J., and Kopin, I. J., 1983, A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *Proc. Natl. Acad. Sci. USA* **80**:4546.

Carlsson, A., Hillarp, N. A., and Waldek, B., 1963, Analysis of the Mg^{++}-ATP-dependent storage mechanism in the amine granules of the adrenal medulla, *Acat Physiol. Scand*. (suppl.) **215**:1.

Carlsson, A., 1987, Perspectives on the discovery of central monoaminergic neurotransmission, *Annu. Rev. Neurosci*. **10**:19.

Ciliax, B. J., Heilman, C., Demchyshyn, L. L., Pristupa, Z. B., Ince, E., Hersch, S. M., Niznik, H. B., and Levey, A. I., 1995, The dopamine transporter: immunochemical characterization and localization in brain, *J. Neurosci*. **15**:1714.

Courtet, P., Baud, P., Abbar, M., Boulenger, J. P., Castelnau, D., Mouthon, D., Malafosse, A., Buresi, C., 2001, Association between violent suicidal behavior and the low activity allele of the serotonin transporter gene, *Mol. Psychiatry* **6**:338.

Coyel, J. T., and Snyder S. H., 1969, Catecholamine uptake by synaptosomes in homogenates of rat brain: stereospecificity in different areas, *J. Phamacol. Exp. Ther*. **170**:221.

Di Chiara, G., and Imperato, A., 1988, Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats, *Proc. Natl. Acad. Sci. USA*. **85**:5274.

Di Maio, S., Grizenko, N., and Joober, R., 2003, Dopamine genes and attention-deficit hyperactivity disorder: a review, *J. Psychiatry Neurosci*. **28**:27.

Di Monte, D., Sandy, M. S., Ekstrom, G., and Smith, M. T., 1986, Comparative studies on the mechanisms of paraquat and 1-methyl-4-phenylpyridine (MPP+) cytotoxicity, *Biochem. Biophys. Res. Commun.* **137**:303.

Do Régo, J.-C., Syringas, M., Leblond, B., Costentin, J., and Bonnet, J.-J., 1999, Recovery of dopamine neuronal transporter but lack of change of its mRNA in substantia nigra after inactivation by a new irreversible inhibitor characterized *in vitro* and *ex vivo* in the rat, *Br. J. Pharmacol.* **128**:51.

Dougherty, D. D., Bonab, A. A., Spencer, T. J., Rauch, S. L., Madras, B. K., and Fischman, A. J., 1999, Dopamine transporter density in patients with attention deficit hyperactivity disorder, *Lancet* **354**:2132.

Dubocovich, M. L., and Langer, S. Z., 1976, Influence of the frequency of nerve stimulation on the metabolism of ^{3}H-norepinephrine released from the perfused cat spleen: differences observed during and after the period of stimulation, *J. Phamarmacol. Exp. Ther*. **198**:83.

Duman, R. S., 1999, The neurochemistry of mood disorders: preclinical studies, in: *Neurobiology of Mental Illness,* D. S. Charney, E. J. Nestler, B. S. Bunney, eds., Oxford University Press, New York, pp.333–347.

Dumartin, B., Jaber, M., Gonon, F., Caron, M. G., Giros, B., Bloch, B., 2000, Dopamine tone regulates D1 receptor trafficking and delivery in striatal neurons in dopamine transporter-deficient mice, *Proc. Natl. Acad. Sci. USA* **97**:1879.

Edwards, R. H., 1993, Neural degeneration and the transport of neurotransmitters. [Review]. *Ann. Neurol.* **34**:638.

Erickson, J. D., Eiden, L. E., and Hoffman, B. J., 1992, Expression cloning of a reserpine-sensitive vesicular monoamine transporter, *Proc. Natl. Acad. Sci. USA* **85**:5274.

Ernst, T., Chang, L., Leonido-Yee, M., and Speck, O., 2000, Evidence for long-term neurotoxicity associated with methamphetamine abuse: A 1H MRS study. *Neurology*, **54**:1344.

Eshleman, A. J., Henningsen, R. A., Neve, K. A., and Janowsky, A., 1994, Release of dopamine via the human transporter, *Mol. Pharmacol.* **45**:312.

Fauchey, V., Jaber, M., Caron, M. G., Bloch B., and Le Moine C., 2000, Differential regulation of the dopamine D1, D2 and D3 receptor gene expression and changes in the phenotype of the striatal neurons in mice lacking the dopamine transporter, *Eur. J. Neurosci.* **12**:19.

Fauchey, V., Jaber, M., Bloch, B., and Le Moine, C., 2000, Dopamine control of striatal gene expression during development: relevance to knockout mice for the dopamine transporter, *Eur. J. Neurosci*. **12**:3415.

Fernagut, P. O., Diguet, E., Jaber, M., Bioulac, B., and Tison, F., 2002, Dopamine transporter knock-out mice are hypersensitive to 3-nitropropionic acid-induced striatal damage, *Eur. J. Neurosci.* **15**:2053.

Fernagut, P. O., Chalon, S., Diguet, E., Guilloteau, D., Tison, F., and Jaber, M., 2003, Motor behaviour deficits and their histopathological and functional correlates in the nigrostriatal system of dopamine transporter knockout mice, *Neuroscience* **116**:1123.

Frize, E., 1954, Mental depression in hypertensive patients treated for long periods with high doses of Reserpine, *N. Engl. J. Med*. **251**:1006.

Fumagalli, F., Gainetdinov, R. R., Valenzano, K. J., and Caron, M. G., 1998 Role of dopamine transporter in methamphetamine-induced neurotoxicity: evidence from mice lacking the transporter, *J. Neurosci*. **18**:4861.

Fumagalli, F., Gainetdinov, R. R., Wand, Y.-M., Valenzano, K. J., and Caron, M. G., 1999, Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice, *J. Neurosci*. **19**:2424.

Gainetdinov, R. R., Fumagalli, F., Wang, Y.-M., Jones, S. R., Levey, A. I., Miller, G. W., and Caron, M. G., 1998, Increased MPTP neurotoxicity in vesicular monoamine transporter 2 heterozygote knock-out mice, *J. Neurochem*. **70**:1973.

Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Levin, E. D., Jaber, M., Caron, M. G., 1999, Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity, *Science* **283**:397.

Galli, A., Blakely, R. D., and DeFelice, L. J., 1998, Patch-clamp and amperometric recordings from norepinephrine transporters: channel activity and voltage-dependent uptake, *Proc. Natl. Acad. Sci USA* **95**:13260.

Garris, P. A., Ciolkowski, E. L., Pastor, P., and Wightman, R. M., 1994, Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain, *J. Neurosci*. **14**:6084.

Gelernter, J., Kruger., S., Pakstis, A. J., Pacholczyk, T., Sparkes, R. S., Kidd, K. K., and Amara, S., 1993, Assignment of the norepinephrine transporter protein (NET1) locus to chromosome 16, *Genomics* **18**:690.

Giros, B., el Mestikawy, S., Godinot, N., Zheng, K., Han, H., Yang-Feng, T., and Caron, M. G., 1992, Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter, *Mol. Pharmacol*. **42**:383.

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., and Caron, M. G., 1996, Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter, *Nature* **379**:606.

Glatt, C. E., and Reus, V. I., 2003, Pharmacogenetics of monoamine transporters, *Pharmacogenomics* **4**:1.

Gorwood, P., Batel, P., Adès, J., Hamon, M., and Boni, C., 2000, Serotonin transporter gene polymorphisms, alcoholism, and suicidal behavior, *Biol. Psy*. **48**:259.

Gu, H., Wall, S. C., and Rudnick, G., 1994, Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence, *J. Biol. Chem*. **269:**7124.

Guastella, J., Nelson, N., Nelson, H., Czyzk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. A., and Kanner, B. I., 1990, Cloning and expression of a rat GABA transporter, *Science* **249**:1303.

Hahn, M. K., and Blakely, R. D., 2002, Monoamines transporter gene structure and polymorphisms in relation to psychiatric and other complex disorders, *Pharmacogen. J*. **2**:217.

Halushka, M. K., Fan, J. B., Bentley, K., Hsie, L., Shen, N., Weder, A., Cooper, R., Lipshutz, R., and Chakravarti, A., 1999, Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis, *Nat. Genet.* **22**:239.

Heils, A., Teufel, A., Petri, S. Stober, G., Riederer, P., Bengel, D., and Lesch, K. P., 1996, Allelic variation of human serotonin transporter gene expression, *J. Neurochem*. **66**:2621.

Henry, J. P., Sagné, C., Bedet, C., and Gasnier, B., 1998, The vesicular monoamine transporter: from chromaffin granule to brain, *Neurochem. Int*. **21**:227.

Hersch, S. M., Yi, H., Heilman, C. J., Edwards, R. H., and Levey, A. I., 1997, Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra, *J. Comp. Neurol*. **388**:211.

Hillard, N. A., 1958, Isolation and some biochemical properties of the catecholamine granules in the cow adrenal medulla, *Acta Physiol. Scand*. **43**:82.

Ikawa, K., Watanabe, A., Kaneno, S., and Toru, M., 1993, Modulation of [^{3}H]mazindol binding sites in rat striatum by dopaminergic agents, *Eur. J. Pharmacol*. **250**:261.

Iversen, L. L., 1971, Role of transporter uptake mechanisms is synaptic neurotransmission, *Br. J. Phamacol*. **41**:571.

Jaber, M., Dumartin, B., Sagné, C., Haycock, J. W., Roubert, C., Giros, B., Bloch, B., and Caron, M. G., 1999, Differential regulation of tyrosine hydroxylase in the basal ganglia of mice lacking the dopamine transporter, *Eur. J. Neurosci*. **11**:3499.

Jakel, R. J., and Maragos, W. F., 2000, Neuronal cell death in Huntington's disease: a potential role for dopamine. *Trends Neurosci.* **23**:239.

Jones, S. R., Gainetdinov, R., Jaber, M., Giros, B., Wightman, R. M., and Caron, M. G., 1998, Profound neuronal plasticity in response to inactivation of the dopamine transporter, *Proc. Natl. Acad. Sci. USA* **31**:4029.

Kantor, L., and Gnegy, M. E., 1998, Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices , *J. Pharmacol. Exp. Ther*. **284**:592.

Kennedy, L. T., and Hanbauer, I., 1983, Sodium-sensitive cocaine binding to rat striatal membrane: possible relationship to dopamine uptake sites, *J. Neurochem*. **41**:172.

Kilic, F., and Rudnick, G., 2000, Oligomerization of serotonin transporter and its functional consequences, *Proc. Natl. Acad. Sci. USA* **97**:3106.

Kirshner, N., 1962, Uptake of catecholamines by a particulate fraction of the adrenal medulla, J. *Biol. Chem*. **237**:2311.

Kitayama, S., Ikeda, T., Mitsuhata, C., Sato, T., Morita, K., and Dohi, T., 1999, Dominant negative isoform of rat norepinephrine transporter produced by alternative RNA splicing, *J. Biol. Chem*. **16**:10731.

Kokkinidis, L., and Anisman, H., 1980, Amphetamine models of paranoid schizophrenia: an overview and elaboration of animal experimentation, *Psychol. Bul*. **88**:551.

Kramer, P., 1993, Listening to Prozac, Viking, New York.

Krueger, B. K., 1990, Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus, *J. Neurochem*. **55**:260.

Kuhar, M. J., 1973, Neurotransmitter uptake: a tool for identifying neurotransmitter-specific pathway, *Life Sci*. **33**:1623.

Kuhar, M. J., Ritz, M. C., and Boja, J.W., 1991, The dopamine hypothesis of the reinforcing properties of cocaine, *Trends Neurol. Sci*. **14**:299.

Kyte, J., and Doolittle, R. F., 1982, A simple method for displaying the hydropathic character of a protein, *J. Membr. Biol*. **157**:105.

Langer, S. Z., Moret, C., Raisman, R., Dubocovich, M. L., and Briley, M., 1980, High-affinity [^{3}H] imipramine binding in rat hypothalamus: association with uptake of serotonin but not of Norepinephrine, *Science* **210**:1133.

Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I., 1983, Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, *Science* **219**:979.

Lee, C.-M., Javitch, J. A., and Snyder, S. H., 1983, Recognition sites for norepinephrine uptake: regulation by neurotransmitter, *Science* **220**:626.

Lee, M. S., Lee, H. Y., Lee, H. J., and Ryu, S. H., 2004, Serotonin transporter promoter gene polymorphism and long-term outcome of antidepressant treatment, *Psychiatr. Genet.* **14**:111.

Le Moine, C., Fauchey, V., and Jaber, M., 2002, Opioid receptor gene expression in dopamine transporter knock-out mice in adult and during development, *Neuroscience* **112**:131.

Lesch, K. P., Balling, U., Gross, J. Strauss, K., Wolozin, B. L., Murphy, D. L., and Riederer, P., 1994, Organisation of the human serotonin transporter gene, *J. Neural Transm. Gen. Sect.* **95**:157.

Lesch, K. P., Heils, A., and Riederer, P., 1996, The role of neurotransporters in exitotoxicity, neuronal cell death and other neurodegenerative processes, *J. Mol. Med*. **74**:365.

Lew, R., Patel, A., Vaughan, R. A., Wilson, A., and Kuhar, M. J., 1992, Microheterogeneity of dopamine transporters in rat striatum and nucleus accumbens, *Brain Res*. **584**:266.

Little, K. Y., Kirkman, J. A., Caroll, F. I., Clark, T. B., and Duncan, G. E., 1993, Cocaine use increases [^{3}H]WIN 35428 binding sites in human striatum, *Brain Res*. **628**:17.

Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Prive, G. G., Eisenberg, D., Brecha, N., and Edwards, R. H., 1992, A cDNA that suppresses MPP+ toxicity encodes a vesicular amine transporter, *Cell* **70**:539.

Liu, Y., Krantz, D. E., Waites, C., and Edwards, R. H., 1999, Membrane trafficking of neurotransmitter transporters in the regulation of synaptic transmission, *Trends Cell. Biol*. **9**:356.

Loder, M. K., and Melikian, H. E., 2003, The dopamine transporter constitutively internalizes and recycles in a protein kinase C-regulated manner in stably transfected PC12 cell lines, *J. Biol. Chem*. **278**:22168.

MacKinnon, R., 1995, Pore loops: an emerging theme in ion channel structure, *Neuron* **14**:889.

Madras, B., Miller, G. M. and Fischman, A. J., 2002, The dopamine transporter: relevance to attention deficit hyperactivity disorder (ADHD), *Behav. Brain Res*. **130**:57.

Malison, R. T., Best, S. E., van Dyck, C. H., McCance, E. F., Wallace, E. A., Laruelle, M., Baldwin, R. M., Seibyl, J. P., Price, L. H., Kosten, T. R., and Innis, R. B., 1998, Elevated striatal dopamine transporters during acute cocaine abstinence as measured by [125I] b-CIT SPECT, *Am. J. Psychiatry* **155**:832.

Masson, J., Sagné, C., Hamon, M. and El Mestikawi S., 1999, Neurotransmitter transporters in the central nervous system, *Pharmacol. Rev*. **51**:439.

McElvain, J. S., and Schenk, J. O., 1992, A multisubstrate mechanism of striatal dopamine uptake and its inhibition by cocaine, *Biochem. Pharmacol*. **43**:2189.

Melikian, H. E., Ramamoorthy, S., Tate, C. G., and Blakely, R. D., 1996, Inability to N-glycosylate the human norepinephrine transporter reduces protein stability, surface trafficking, and transport activity but not ligand recognition, *Mol. Pharmacol*. **50**:266.

Melikian, H. E., and Buckley, K. M., 1999, Membrane trafficking regulates the activity of the human dopamine transporter, *J. Neurosci*. **19**:7699.

Miller, G. M., Gainetdinov, R. R., Levey, A. I., and Caron, M. G., 1999, Dopamine transporters and neuronal injury, *Trends Pharmacol. Sci*. **20**:424.

Miller, G. W., Erickson, J. D., Perez, J. T., Penland, S. N., Mash, D. C., Rye, D. B., and Levey, A. I., 1999, Immunochemical analysis of vesicular monoamine transporter (VMAT2) protein in Parkinson's disease, *Exp. Neurol*. **156**:138.

Milner, H. E., Béliveau, R., and Jarvis, S. M., 1994, The in situ size of the dopamine transporter is a tetramer as estimated by radiation inactivation, *Bioch. Biophys*. **1190**:185.

Nakanishi, N., Onozawa, S., Matsumoto, R., Hasegawa, H., and Minami, N., 1995, Vesicular monoamine uptake by digitonin-permeabilized PC12 cells: inhibitory effect of neuromodulators and drugs on the amine transport, *J. Biochem*. **118**:291.

Nemeroff, C. B., and Owens, M. J., 2002, Treatment of mood disorders, *Nat. Neurosci*. **5**(suppl.):1068.

Pacholczyk, T., Blakely, R. D., and Amara, S. G., 1991, Expression cloning of a cocaine-and antidepressant-sensitive human noradrenaline transporter, *Nature* **350**:350.

Peter, D., Liu, Y., Sternini, C., de Giorgio, R., Brecha, N., and Edwards, R. H., 1995, Differential expression of two vesicular monoamine transporters, *J. Neurosci*. **15**:6179.

Pickel, V. M., and Chan, J., 1999, Ultrastructural localization of the serotonin transporter in limbic and motor compartments of the nucleus accumbens, *J. Neurosci*. **19**:7356.

Pifl, C., Giros, B., and Caron, M. G., 1993, Dopamine transporter expression confers cytotoxicity to low doses of the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium, *J. Neurosci*. **13**:4246.

Pineyro, G., Blier, P., Dennis, T., and de Montigny, C., 1994, Desensitization of the neuronal 5-HT carrier following its long-term blockade, *J. Neurosci*. **14**:3036.

Pletscher, A., 1977, Effect of neuroleptics and other drugs on monoamine uptake by membrane of adrenal chromafin granules, *Br. J. Pharmacol*. **59**:419.

Pörzgen, P., Bonisch, H., and Bruss, M., 1995, Molecular cloning and organization of the coding region of the human norepinephrine transporter gene, *Biochem. Biophys. Res. Commun*., **215**:145. Erratum in: *Biochem. Biophys. Res. Commun*. 1996, **227**:642.

Pörzgen, P., Bonisch, H., Hammermann, R., and Bruss, M., 1998, The human noradrenaline transporter gene contains multiple polyadenylation sites and two alternatively spliced C-terminal exons, *Biochim. Biophys. Acta*. **398**:365.

Prasad, S., Semwal, P., Deshpande, S., Bhatia, T., Nimgaonkar, V. L., and Thelma, B. K., 2002, Molecular genetics of schizophrenia: past, present and future*, J. Biosci*. **27**:35.

Radian, R., Bendahan, A., and Kanner, B. I., 1986, Purification and identification of the functional sodium- and chloride-coupled γ-aminobutyric acid transport glycoprotein from rat brain, *J. Biol. Chem.* **261**:15437.

Ramamoorthy, S., Bauman, A. L., Moore, K. R., Han, H., Yang-Feng, T., Chang, A. S., Ganapathy, V., and Blakely, R. D., 1993, Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization, *Proc. Natl. Acad. Sci. USA* **90**:2542.

Ramamoorthy, S., and Blakely, R. D., 1999, Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants, *Science* **28**:763.

Rand, J. B., Duerr, J. S., and Frisby, D. L., 2000, Neurogenetics of vesicular transporters in C. elegans, *Faseb J.* **14**:2414.

Rattray, M., 1991, Ecstasy: towards an understanding of the biochemical basis of the action of MDMA, *Essays Biochem*. **26**:77.

Ressler, K. J., and Nemeroff, C. B., 1999, Role of norepinephrine in the pathophysiology and treatment of mood disorders, *Biol. Psychiatry* **46**:1219.

Reynolds, D. S., Carter, R. J. and Morton, A. J., 1998, Dopamine modulates the susceptibility of striatal neurons to 3-nitropropionic acid in the rat model of Huntington's disease, *J. Neurosci.* **18**:10116.

Rossetti, Z. L., Sotgiu, A., Sharp, D. E., Hadjiconstantinou, M., Neff, N. H., 1988, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and free radicals in vitro, *Biochem. Pharmacol*. **37**:4573.

Rudnick, G., and Wall, S. C., 1992, The molecular mechanism of "ecstasy" (3,4-methylenedioxymethamphetamine), MDMA: serotonin transporters are targets for MDMA induced serotonin release, *Proc. Natl. Acad. Sci. USA* **89**:1817.

Sanghera, M. K., 1997, Dopamine transporter mRNA levels are high in midbrain neurons vulnerable to MPTP, *Neuroreport* **8**:3327.

Saunders, C., Ferrer, J. V., Shi, L., Chen, J., Merril, G., Lamb, M. E., Leeb-Lundberg, L. M. F., Carvelli, L., Javitch, J. A., and Galli, A., 2000, Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism, *Proc. Natl. Acad. Sci. USA* **97**:6850.

Schroeter, S., Apparsundaram, S., Wiley, R. G., Miner, L. H., Sesack, S. R., and Blakely, R. D., 2000, Immunolocalization of the cocaine- and antidepressant-sensitive l-norepinephrine transporter, *J. Comp. Neurol*. **420**:211.

Schuldiner, S., Shirvan, A., and Lineal, M., 1995, Vesicular neurotransmitter transporters: from bacteria to humans, *Physiol. Rev*. **75**:369.

Shannon, J. R., Flattem, N. L., Jordan J., Jacob, G., Black, B. K., Biaggioni, I., and Blakely, R. D, 2000, Orthostatic intolerance and tachycardia associated with norepinephrine-transporter deficiency, *N. Engl. J. Med.* **342**:541.

Sonders, M. S., Zhu, S. J., Zahniser, N. R., Kavanaugh, M. P., and Amara, S. G., 1997, Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychomotorstimulants, *J. Neurosci*. **17**:960.

Spielewoy, C., Gonon, F., Roubert, C., Fauchey, V., Jaber, M., Caron, M. G., Roques, B. P., Hamon, M., Betancur, C., Maldonado, R., and Giros B., 2000, Increased rewarding properties of morphine in dopamine-transporter knockout mice, *Eur. J. Neurosci.* **12**:1827.

Staley, J. K., Hearn, W. L., Ruttenber, A. J., Wetli, C. V., and Mash, D. C., 1994, High affinity cocaine recognition sites on the dopamine transporter are elevated in fatal cocaine overdose victims, *J. Phamacol. Exp. Ther*. **271**:1678.

Swanson, J. M., Flodman, P., Kennedy, J., Spence, M. A., Moyzis, R., Schuck, S. Murias, M., Moriarity, J., Barr, C., Smith, M., and Posner, M., 2000, Dopamine genes and ADHD, *Neurosci. Biobehav. Rev*. **24**:21.

Sur, C., Betz, H., and Schloss, P., 1996, Immunocytochemical detection of the serotonin transporter in rat brain, *Neuroscience* **73**:217.

Silvia, C. P., Jaber, M., King, G. R., Ellinwood, E. H., and Caron, M. G., 1997, Cocaine and amphetamine elicit differential effects in rats with a unilateral injection of dopamine transporter antisense oligodeoxynucleotides, *Neuroscience* **76**:737.

Tison, F., Wenning, G. K., Daniel, S. E. and Quinn, N. P., 1995, The pathophysiology of parkinsonism in multiple system atrophy, *Eur. J. Neurol.* **2**:435.

Uhl, G. R., 1998, Hypothesis: the role of dopaminergic transporters in selective vulnerability of cells in Parkinson's disease, *Ann. Neurol*. **43**:555.

Uhl, G. R., Walther, D., Mash, D., Faucheux, B., and Javoy-Agid, F., 1994, Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons, *Ann. Neurol*. **35**:494.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Wong, C., and Logan, J., 2001, Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers, *Am. J. Psychiatry* **158**:383.

Vandenbergh, D. J., Persico, A. M., Hawkins, A. L., Griffin, C. A., Li, X., Jabs, E. W., and Uhl, G. R., 1992, Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR, *Genomics* **14**:1104.

Weihe, E., Shäfer, M. K. H., Erickson, J. D., and Eiden, L. E., 1995, Localization of vesicular monoamine transporter isoforms (VMAT-1 and VMAT-2) to endocrine cells and neurons in rat, *J. Mol. Neurosci*. **5**:149.

Zahniser, N. R., and Doolen, S., 2001, Chronic and acute regulation of Na^+/Cl^--dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors and signalling systems, *Pharmacol. Ther*. **92**:21.

Zanardi, R., Benedetti, F., Di Bella, D., Catalano, M., and Smeraldi, E., 2000, Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene, *J. Clin. Psychopharmacol.* **20**:105.

Zhu, S.-J., Kavanaugh, M. P., Sonders, M. S., Amara, S. G., and Zahniser, N. R., 1997, Activation of protein kinase C inhibits uptake, currents and binding associated with the Human dopamine transporter expressed in *Xenopus* Oocytes, *J. Pharmacol. Exp. Ther*. **282**:1358.

14. DOPAMINE RECEPTORS

Structure, function and implication in psychiatric disorders

Pierre Sokoloff, Ludovic Leriche and Bernard Le Foll[1]

1. INTRODUCTION

Dopamine is an important neurotransmitter in the brain and controls various functions, including motor activity, cognition, motivation, emotion, food intake and endocrine secretions. The dopaminergic systems responsible for these functions have received much attention, namely because their dysfunctions are involved in the etiology or treatment of several pathological conditions, including schizophrenia, Parkinson's disease, Tourette's syndrome, attention-deficit and hyperactivity disorder and hyperprolactinemia. It has long been thought that only two receptor subtypes, the D1 and D2 receptors, which were initially defined on the basis of their distinct transduction mechanisms and pharmacological profiles (Spano et al. 1978; Kebabian and Calne 1979), mediated the pleiotropic actions of dopamine. At the time, it was recognized that the target of antiparkinsonian drugs and of antipsychotic drugs was the D2 receptor. In spite of various proposals for additional subtypes, the dual classification of dopamine receptors has gained general acceptance. The cloning of a D_2 receptor cDNA (Bunzow et al. 1988) and the subsequent demonstration that two splicing variants of this receptor exist, the D_{2S} and D_{2L}, the latter with an additional 29-aminoacid sequence (Giros et al. 1989), was followed by the cloning of a D_1 receptor cDNA (Dearry et al. 1990); (Sunahara et al. 1990); (Zhou et al. 1990; Monsma et al. 1991). Thus, molecular cloning outcomes met

[1]P. Sokoloff and L. Leriche, INSERM-Univ. René Descartes, UMR-S 573, Unité de Neurobiologie et Pharmacologie Moléculaire, Centre Paul Broca, 75014 Paris, France. P. Sokoloff, present address: Centre de Recherche Pierre Fabre, 81106 Castres cedex, France. B. Le Foll, National Institutes of Health, National Institute on Drug Abuse, Intramural Research Program, Behavioral Neuroscience Branch, Preclinical Pharmacology Section, Baltimore, MD 21224.

the expectations regarding the diversity of dopamine receptors, as perceived at the time. However, only one week after publication of the reports on cloning of the D_1 receptor, a novel dopamine receptor subtype was identified, the D_3 receptor (Sokoloff et al. 1990). It was followed soon later by the cloning of the D_4 (Van Tol et al. 1991) and D_5 (Sunahara et al. 1991) receptors, which were also far from being expected.

Structure and functions of dopamine receptors, and their implications to pathological conditions have been reviewed earlier (Civelli et al. 1993; Sokoloff et al. 1995; Missale et al. 1998), at a time when no highly specific tools were available. Notably, a wealth of novel information has been gained from the identification of highly selective ligands for some dopamine receptor subtypes, the generation of dopamine receptor subtype-targeted mutant mice and subtype-specific antibodies, and the advances of molecular genetics. This review intends to discuss this new information and to examine its implication for our knowledge of dopamine-related brain disorders and their treatment.

2. STRUCTURE OF DOPAMINE RECEPTORS

2.1. Gene structure and variants

Genes encoding dopamine receptors have significant sequence homology, which permitted their cloning by screening genomic or cDNA libraries at low stringency. Thus, D_1 and D_5 receptors resemble each other (~60% of sequence homology), and more markedly differ from D_2, D_3 and D_4 receptors (~25% sequence homology). Similarly, D_2, D_3 and D_4 receptors display strong homology (~50-70%). Their genomic organization also supports the classification of dopamine receptors into two subfamilies (Table 1). Genes encoding D_1 and D_5 (*DRD1* and *DRD5*) are intronless, whereas the coding sequence of the genes encoding the D_2, D_3 and D_4 receptors (*DRD2*, *DRD3* and *DRD4*) are interrupted by introns. Interestingly, the localization of introns is similar in the three receptor genes. This characteristic strongly suggests that the dopamine receptors derive from the divergence of two ancestors, which is also supported by phylogenetic analysis (Callier et al. 2003). The gene organization, as well as similar pharmacological properties and signal transduction (see below), make it usual to refer to the D1-like subfamily, containing D_1 and D_5 receptors and to the D2-like subfamily, containing D_2, D_3 and D_4 receptors.

The presence of introns within the coding sequences of genes coding D2-like receptors allows the generation of splicing variants. Indeed, the D_2 receptor has two variants, generated by alternative splicing of a 87-bp exon. The D_{2L} variant thus contains a 29 amino acid sequence that does not exist in the D_{2S} variant (Dal Toso et al. 1989; Giros et al. 1989). Several D_3 receptor mRNA variants have been described, which all encode for truncated, most likely inactive receptors (Giros et al. 1991; Fishburn et al. 1993; Schmauss et al. 1993; Griffon et al. 1996). One of these inactive D_3 receptor variants termed D_{3nf}, results from a deletion of 98-bp and encodes a D_3-like receptor with a different C-terminus (Schmauss et al. 1993). D_{3nf} mRNA was found abundant in the

human brain (Schmauss et al. 1993) and translated to protein (Liu et al. 1994). Interestingly, full-length D_3 receptor mRNA was found significantly decreased in certain cortical regions of the post-mortem brain of schizophrenic patients, whereas D_{3nf} could readily be detected. This may suggest defective alternative splicing in schizophrenia, yet to be confirmed.

Two *DRD3* polymorphic variants exist differing by the amino acid in the ninth position, which is either a serine or a glycine; a *Bal* I restriction site is created in the 9Gly *DRD3* variant (Lannfelt et al. 1992). *DRD3* sequences in three non-human species (accession numbers P52703 and AY407500; Werge et al. 2003) all carry the 9Gly codon, suggesting that 9Gly is the ancestral *DRD3* variant, and that a Gly9Ser mutation has appeared during human lineage and widespread. Current *DRD3* 9Ser allele frequency show considerable ethnic heterogeneity, ranging from 16% in a Congolese population, 40% in African American to 70% in Caucasians (Crocq et al. 1996; Lerer et al. 2002).

The human *DRD4* contains an unusual polymorphism characterized by a varying number (from 2 to 10) of imperfect 48-bp repeats (Van Tol et al. 1992). Sequencing of *DRD4* in 178 unrelated chromosomes identified 19 different repeats in 25 different haplotypes coding for 18 different predicted amino acid sequences (Lichter et al. 1993). The most prevalent *DRD4* alleles are the 4-, 7- and 2-repeat alleles, with global mean allele frequencies of 64.3%, 20.6% and 8.2% respectively(Chang et al. 1996). The variable 48-bp sequence of *DRD4* binds nuclear factors and may affect gene expression (Schoots and Van Tol 2003). Several other polymorphisms of *DRD4* have been described, notably two that either lead to truncated inactive variant or a receptor insensitive to dopamine (see Oak et al. 2000 for a complete description). Of notice is the fact that two human homozygotes, each carrying one of the two mutations, thus carrying essentially inactive D_4 receptors, have been identified. One has some psychiatric and somatic abnormalities, whereas the other one does not show any overt psychiatric or somatic abnormalities (Oak et al. 2000).

Table 1. Summary of dopamine receptor subtypes

	Receptor subfamily				
	D1-like		D2-like		
	D_1	D_5	D_2	D_3	D_4
Gene[a]	*DRD1*	*DRD5*[b]	*DRD2*	*DRD3*	*DRD4*
Human chromosome	5q35.1	4p15.2	11q23	3q13.3	11p15.5
Structural information	Intronless	Intronless	7 exons[c]	7 exons[c]	4 exons[c]
Number of aminoacids[d]	446 (h) 446 (r)	477 (h) 475 (r)	D_{2L}: 443 (h), 444 (r) D_{2S}: 414 (h), 415 (r)	400 (h) 446 (r)	387-515[e] (h) 386 (r)

Signal transduction mechanisms[f]	cAMP (+) $[Ca^{2+}]_I$ (+ or 0)	cAMP (+)	cAMP (-) $[Ca^{2+}]_I$ (+/-) K^+ outward curr. (+) AA release (+)	cAMP (-), Ca^{2+} curr. (-) MAP kinase (+) K^+ outward curr. (+)	cAMP (-), Ca^{2+} curr. (-) K^+ outward curr. (+) AA release (+)

[a] Nomenclature recommended by the Human Genome Organization; [b] Two pseudogenes have been identified

[c] Number of exons in the coding sequence; [d] h, human; r, rat; [e] The D_4 receptor contains a highly variable sequence, see text.; [f] +, stimulation; -, inhibition; 0, no effect; curr., currents.

2.2. Receptor structure and receptor complexes

All dopamine receptor subtypes belong to the G protein-coupled receptor (GPCR) family, the members of which are characterized by the occurrence of 7 predicted transmembrane domains (TM), separated by intra- and extracellular loops having hydrophilic amino acids at their boundaries. More specifically, dopamine receptors are classified in Family 1, which contains receptors having their ligand-binding site confined within the structure formed by the seven TMs (Bockaert and Pin 1999). Dopamine D1-like receptors have a short third intracellular loop and a long intracellular C-terminus, whereas D2-like receptors have a long third intracellular loop with a short C-terminus. The extracellular N-terminus contains several putative N-glycosylation site and the dopamine receptors are, indeed, N-glycosylated: their apparent molecular mass in electrophoresis decreases after treatment that remove N-linked sugars. Dopamine receptors contains potential sites for palmitoylation at the C-terminus, but palmitoylation has been formally demonstrated for the D_2 receptor only (Ng et al. 1994). This posttranslational process may be involved in the localization of the receptor to the plasma membrane.

Increasing evidence now suggests that GPCRs do not function isolated at the plasma membrane, but interact with various membrane or cytosolic proteins (in addition to G proteins that transduce the signal). Notably, the use of two-hybrid screening in Yeast and subsequent confirmation by pull-down and immunoprecipitation assays led to the identification of various dopamine receptor-interacting proteins that can regulate biosynthesis, signal transduction or receptor trafficking and recycling (Bergson et al. 2003). The first kind of protein-protein interaction undergone by dopamine receptors is dimerization. For instance, D_2 and D_3 receptors form homo- and heterodimers in transfected cells (Nimchinsky et al. 1997; Scarselli et al. 2001). Interestingly, the pharmacological properties of D_2/D_3 heterodimers differ from those of individual receptors. This feature may have physiological consequences in neurons in which the two receptors co-exist, for instance in dopamine neurons (see below).

Dopamine receptors also interact with other receptors for neurotransmitters. Thus, the D_1 receptor functionally interacts with glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype to inhibit NMDA receptor-gated

currents (Lee et al. 2002); interaction with the NMDA receptor also affects D_1 receptor trafficking (Fiorentini et al. 2003). D_1 and D_2 receptors interact with adenosine A_1 and A_{2A}, respectively and these interactions affect receptor trafficking and co-aggregation (Gines et al. 2000; Hillion et al. 2002). The D_5 receptor physically associates with the $GABA_A$ receptor to produce mutual inhibition of receptor function (Liu et al. 2000).

Localization of dopamine receptors in different cell compartments (plasma membrane, endosomes, biosynthesis and recycling vesicles) is determined by physical association of the receptor with cytoskeletal proteins, some of which have been identified (Bergson et al. 2003). Adaptor or chaperones also intervene in receptor trafficking, maintenance and signaling. For instance, a four-amino-acid spacing of hydrophobic residues, the FxxxFxxxF motif, is highly conserved among GPCRs, functions as an endoplasmic reticulum export signal for the D_1 receptor, through the involvement of DriP78, a new endoplasmic reticulum membrane-bound protein (Bermak et al. 2001). The D_5 receptor also contains a FxxxFxxxF motif, but it is unknown if a similar pathway targets the D_5 receptor at the plasma membrane. Calcyon is a 24-kilodalton single transmembrane protein, which localizes to dendritic spines of D_1 receptor-expressing pyramidal cells in prefrontal cortex. Calcyon interacts with the D_1 receptor and shifts D_1 receptors effector coupling to stimulate intracellular calcium release (Lezcano et al. 2000). Another recent example comes from the discovery that GIPC (GAIP Interacting Protein C-terminus) associates with the C-terminus of D_2, D_3, but not D_4 receptors and protects the receptor from degradation (Jeanneteau et al. 2004). Additionally, GIPC is an adaptor linking the D_2 receptor to RGS 19, one of the proteins regulating G protein activity (Jeanneteau et al. 2004).

From the examples mentioned above, we now realize that dopamine receptors, as well as other GPCR, are just components of macromolecular complexes, which ensure receptor compartmentalization, maintenance and trafficking and are dynamically regulated by various extracellular and intracellular signals.

3. DOPAMINE RECEPTOR EXPRESSION IN THE BRAIN AND ITS REGULATION

Dopaminergic neurons are organized in three main pathways (Björklund and Lindvall 1984). The nigrostriatal pathway originates from the substantia nigra, projects mainly to the caudate-putamen and controls initiation and planning of voluntary movements. The mesolimbocortical pathway originates from the ventral tegmental area and projects to the ventral parts of the caudate-putamen, the nucleus accumbens, the amygdala and the prefrontal cortex; it controls cognition, motivation and reward. The tuberoinfundibular pathway originates from the hypothalamus and controls endocrine functions of the anterior pituitary via the portal system.

Initially, in the absence of selective radioligands, the distribution of dopamine receptors in the brain has been assessed by *in situ* hybridization. D_1 receptor mRNA was found the most abundant, expressed in all dopaminergic

neuron-projecting areas of the nigrostriatal and mesolimbocortical pathways (Fremeau et al. 1991). D_1 receptor mRNA is also detected in hypothalamus and thalamus. In other regions where D1-like binding sites are detected, such as the substantia nigra, no D_1 receptor mRNA is found, suggesting that this receptor is present on afferences. This was later confirmed by using a subtype-specific anti-D_1 receptor antibody allowing the localization of the protein (Huang et al. 1992; Levey et al. 1993). A similar antibody also permitted examination of the subcellular localization of the D_1 receptor in normal conditions and after stimulation by dopamine. Direct D1-like agonists or endogenous dopamine released by amphetamine induce the translocation of D_1 receptor-immunoreactivty from the plasma membrane to endocytic vesicles and endosomes (Dumartin et al. 1998). An increase in cytoplasmic localization of D_1 receptor-immunoreactivity has also been found in the brain of levodopa-treated patients with Parkinson's disease (Muriel et al. 1999). These data show that the localization of the D_1 receptor, and therefore its postsynaptic function critically depends on the dopamine tone.

D_2 receptor mRNA is also abundantly expressed in all dopaminergic terminals areas (Meador-Woodruff et al. 1989; Bouthenet et al. 1991). In contrast with D_1 receptor mRNA, D_2 receptor mRNA is less abundant in cortical areas, but highly expressed in the dopamine cell bodies of the substantia nigra and ventral tegmental area. The short variant D_{2S} receptor is prominent in dopamine cell bodies (Khan et al. 1998). The localization of D_2 receptors has been confirmed using a subtype-specific anti-D_2 receptor antibody (Levey et al. 1993). D_2 receptor expression in the brain is dependent upon dopamine inputs, being increased after denervation in 6-hydroxydopamine (6-OHDA)-lesioned rats (Gerfen et al. 1990) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated monkeys (Falardeau et al. 1988), or after chronic treatment with antipsychotic drugs (Martres et al. 1992). In 6-OHDA-lesioned rats, D_2 receptor overexpression is reversed by antiparkinsonian treatment such as bromocriptine (Gerfen et al. 1990).

D_1 and D_2 receptor mRNAs prominently segregate in distinct striatal neuronal populations, the substance P/dynorphin- and enkephalin-containing neurons, respectively (Le Moine and Bloch 1995). More sensitive techniques, such as single cell RT-PCR (reverse transcription-polymerase chain reaction), have, however, detected co-localization (Surmeier et al. 1996). It is worth noting that immunohistochemistry studies with electron microscopy with subtype-selective anti- receptor antibodies have found that D_1 and D_2 receptor are mainly present on dendritic shafts and spines, but at extrasynaptic sites (Hersch et al. 1995; Caille et al. 1996), suggesting "volume transmission" (Zoli et al. 1998) for dopamine at these receptors.

In marked contrast with D_1 and D_2 receptors, the localizations of D_3, D_4 and D_5 receptors are restricted to certain dopamine terminal areas and the expression of these dopamine receptor subtypes is much less abundant, which has complicated the assessment of their brain expression. Initially, D_3 receptor mRNA has been found restricted in the most ventral parts of the striatal complex and in other limbic areas (Sokoloff et al. 1990; Bouthenet et al. 1991). The dorsal striatum expresses D_3 receptor mRNA levels undetectable by *in situ*

hybridization studies, but readily detectable with a more sensitive technique (Surmeier et al. 1996). The highest D_3 receptor mRNA expression is in the granule cells of the islands of Calleja, which are small neuronal structures embedded in the nucleus accumbens and olfactory tubercle, where their function remains unknown. D_3 receptor mRNA is also highly expressed in Purkinje cells of lobules 9 and 10 of the cerebellum. It should be noticed that dopamine innervation in does not make direct contact with granule cells in the islands of Calleja, suggesting that dopamine can act at D_3 receptors at some distance of the releasing sites; this is another case of volume transmission, which could be expected from the fact that the affinity of dopamine is the highest at the D_3 receptor than at other dopamine receptor subtypes. The development of D_3 receptor-selective tritiated (Lévesque et al. 1992), then iodinated (Burris et al. 1994) radioligands allowed the confirmation of the restricted expression pattern of D_3 receptors in rodent brains. However, *in situ* hybridization and receptor autoradiography studies have shown that D_3 receptor is more abundant in the dorsal striatum and various cortical areas, including the frontal cortex, of non-human primates (Morissette et al. 1998) and humans (Hall et al. 1996; Suzuki et al. 1998). This suggests the involvement of D_3 receptor in higher brain functions in developed species.

Immunological studies with a D_3 receptor-selective antibody demonstrated the presence of D_3 receptors in all mesencephalic dopaminergic neurons (Diaz et al. 2000). These receptors may function as autoreceptors controlling dopaminergic neuron activity, which is supported by studies using D_3 receptor-knockout mice (see below).

D_3 receptor expression is highly dependent on dopamine innervation. In 6-OHDA-lesioned rat, D_3 receptor expression is decreased in the shell of the nucleus accumbens of the denervated side (Lévesque et al. 1995). D_3 receptor density is also decreased in a non human primate model of Parkinson's disease, i.e. in MPTP-treated monkeys (Morissette et al. 1998) or in patients suffering from this disease (Ryoo et al. 1998). This change is paradoxical, since the D2 receptor is upregulated under these circumstances (see above) and was shown to depend on the deprivation of an anterogradly-transported factor from dopaminergic neurons, distinct from dopamine itself and its known peptide co-transmitters (Lévesque et al. 1995). In 6-OHDA-lesioned rats, repeated administration of levodopa progressively induces the overexpression of the D_3 receptor in the denervated nucleus accumbens and dorsal striatum (Bordet et al. 1997). This induction takes places in substance P-containing neurons of the direct striatonigral pathway, projecting to the substantia nigra pars reticulata, in which expression of the D_3 receptor protein is also induced (Bordet et al. 2000). The process is clearly attributable to stimulation of a D_1/D_5 receptor in the denervated striatum since a D1/D5-receptor agonist reproduces it and D_1/D_5-receptor antagonist prevents it. It is responsible for the development of behavioral sensitization to levodopa, i.e. a process by which the motor response is progressively enhanced by repeated administration (Engber et al. 1989; Carey 1991). Brain-derived neurotrophic factor (BDNF) is a factor controlling normal D_3 receptor expression during development and in adults and is responsible for

levodopa-induced D_3 receptor expression and behavioral sensitization (Guillin et al. 2001).

D_3 receptors are elevated in the post-mortem brain of patients with schizophrenia, who have not been under treatment for two months before death, but not of medicated patients (Gurevich et al. 1997). This suggests that increased D_3 receptor expression is a hallmark of the disease, and not its treatment, and that antipsychotic drugs normalize this expression. D_3 receptor mRNA and protein are also elevated in the post-mortem brain of human cocaine overdose fatalities (Staley and Mash 1996; Segal et al. 1997). D_3 receptor expression increases in rats receiving chronic nicotine administration (Le Foll et al. 2003) or after conditioning to cocaine (Le Foll et al. 2002) or nicotine (Le Foll et al. 2003). Since a high prevalence (about 30-40%) of lifetime substance abuse exists among patients with schizophrenia (Regier et al. 1990), who also are in majority cigarette smokers, increased D_3 receptor expression in both schizophrenia and drug addiction may reflect either an effect of abused drugs or an endophenotype that predisposes to both disorders.

Repeated handling and saline injections, which are presumably stressful manipulations in rats, decrease D_3 receptor expression in the nucleus accumbens (Lammers et al. 2000), a target for dopamine neurons involved in motivation, positive reinforcement and reward (Koob 1992; Salamone 1994). On the contrary, various antidepressant treatments, including antidepressant drugs of various classes and electroconvulsive shocks, increase D_3 receptor expression after chronic, but not acute administration (Maj et al. 1998; Lammers et al. 2000). This suggests that the D_3 receptor participates in the therapeutic response of antidepressants, especially on anhedonia.

D_4 receptor mRNA is found in various brain regions at low density compared with D_1 or D_2 receptors. It is most abundant in retina (Cohen et al. 1992), cerebral cortex, amygdala, hypothalamus and pituitary, but sparse in the basal ganglia, as assessed by RT-PCR and Northern blot (Valerio et al. 1994). This was confirmed by using higher resolution techniques, such as *in situ* hybridization (O'Malley et al. 1992; Meador-Woodruff et al. 1994; Meador-Woodruff et al. 1997) and immunohistochemistry (Mrzljak et al. 1996). These studies notably confirmed the presence of D_4 receptors in both pyramidal and non-pyramidal cells of the cerebral cortex, particularly layer V, and in the hippocampus. In cerebral cortex and hippocampus, non-pyramidal cells are γ-aminobutyric acid (GABA)-producing neurons (Mrzljak et al. 1996). D_4 receptors are also present in granule cells of the cerebellum (Mei et al. 1995).

Characterization of native D_4 receptors in brain using radioligands has been a difficult task, due to the lack of adequate ligands. Initially, the procedure to demonstrate the presence of and quantify D_4 receptors used ligand binding subtractive techniques, using [^{3}H]enomapride, which binds to D_2, D_3 and D_4 receptors and [^{3}H]raclopride, which binds to D_2 and D_3, but not D_4 receptor (Seeman et al. 1993). This procedure detected large amounts of D_4 receptors in the basal ganglia, which expresses only very low amounts of D_4 receptor mRNA or immunoreactivity. That raclopride-insensitive [^{3}H]enomapride binding sites represent genuine D_4 receptor was further challenged by the observation that raclopride-insensitive D2-like binding sites disappear in D_2 receptor-knock-out

mice (Seeman et al. 1997). The issue of the validity of subtractive techniques to label D_4 receptor is important because elevated D_4 receptors, as labeled by using these techniques, have been described in the brain of schizophrenic patients (Seeman et al. 1993). This finding was, nevertheless, challenged by other observations (for instance (Lahti et al. 1996). More recently, a D_4 receptor-selective radioligand, [^{3}H]NGD 94-1, has been developed, which demonstrates the non-striatal localization of D_4 receptors in the rat and human brain (Primus et al. 1997). [^{3}H]NGD 94-1 binding is greatest in entorhinal cortex, lateral septal nucleus, hippocampus and the medial preoptic area of the hypothalamus. An elevation (+ 46%) of [^{3}H]NGD 94-1 binding was found in the entorhinal cortex of both schizophrenic patients off antipsychotic drugs for at least 3 months prior to death and those on antipsychotic drugs at the time of death (Lahti et al. 1998).

The distribution of D_5 receptors has also been difficult to assess by *in situ* hybridization studies, because of the sequence similarities between D_5 receptors and D_1 receptors and the two D_5 pseudogenes (Sunahara et al. 1991). Studies in rat showed that D_5 receptor mRNA is restricted to the hippocampus, mammillary bodies and a thalamic nucleus initially identified as the anterior pretectal nucleus (Tiberi et al. 1991) and subsequently identified as the parafascicular nucleus (Meador-Woodruff et al. 1992). Higher and more organized levels of D_5 receptor mRNA expression was found in the human hippocampus, subicular complex and temporal cortex (Meador-Woodruff et al. 1994). Immunohistochemical studies of the D_5 receptor protein largely confirm and extend *in situ* hybridization studies, but also showed significant differences between the localization of D_1 and D_5 receptors at cellular levels. Thus, in the cortical pyramidal cells, whereas D_1 receptors are concentrated in dendritic spines, D_5 receptors are more localized in dendritic shafts (Bergson et al. 1995). In the striatum, D_1 and D_5 receptors are both present in medium-size spiny GABA-containing neurons, but only D_5 receptors are present in large cholinergic interneurons (Bergson et al. 1995). At the ultrastructural level, D_1 receptors are present on spines making asymmetrical, presumably non dopaminergic synapses, whereas both D_1 and D_5 receptors are present at small postsynaptic densities of symmetrical synapses typical of dopaminergic synapses (Khan et al. 2000).

From these studies, it appears that expression pattern of each of the five dopamine receptors markedly differs, even though co-localization occurs to some extent. This suggests that specific receptor subtypes subserve specific functions of dopamine.

4. SIGNAL TRANSDUCTION OF DOPAMINE RECEPTORS

Initially, coupling of dopamine receptors has been studied on brain slices or homogenates. These approaches led to the distinction of D1-like and D2-like receptors positively and negatively coupled to adenylyl cyclase, respectively. However, with the occurrence of several receptor subtypes in the same tissue and the paucity of highly subtype-selective ligands, notably among agonists, information regarding second-messenger system coupling and function of a

particular dopamine receptor subtype has been primarily obtained in heterologous expression systems after transfection of the respective cDNA (Table 1). Various recipients cell lines have been used, but most often fibroblasts; this raises the possibility that the functional characteristics found with these approaches may be influenced by or specific of a particular cellular environment, distinct from that in neuronal cells. Hence, conflicting results have often risen from the use of different heterologous systems. Nevertheless, in some particular cases reviewed below, cell-or tissue-specific expression of a receptor subtype permitted to unravel natural coupling of dopamine receptors in neurons.

In a variety of cell culture lines, D_1 and D_5 receptors preferentially couple to G proteins activating adenylyl cyclase, increasing the second messenger cyclic AMP (cAMP), and protein kinase A-dependent pathways (Dearry et al. 1990; Monsma et al. 1991). Interestingly, D_5 receptor appears to possess higher constitutive, i.e. agonist-independent, activity than the D_1 receptor (Tiberi and Caron 1994), a finding of which the functional significance remains to be ascertained. It is assumed that D_1 and D_5 receptors positively coupled to adenylyl cyclase via $G_S\alpha$. However, $G_{olf}\alpha$ is the G protein the most abundant in the basal ganglia and is more abundant after dopamine denervation (Herve et al. 1993), which also induces D1-like receptor hypersensitivity. This suggests that native D_1 and possibly D_5 receptors couple to $G_{olf}\alpha$ in neurons rather than to $G\alpha$.

Activation of D_1 (or D_5) receptors activates cAMP-dependent kinase (PKA); then the activated form of PKA phosphorylates various cellular protein targets. Among the well-characterized PKA-target is DARPP-32 (dopamine and cAMP-regulated phosphoprotein, 32 kDa), an important regulator of dopamine signaling in neurons (Svenningsson et al. 2004). DARPP-32 shares a high degree of amino acid sequence homology with inhibitor-1, an inhibitor of protein-phosphatase-1 (PP-1). Following D1-like receptor activation, phosphorylation of DARPP-32 by PKA at Thr^{34} converts it into a potent inhibitor of PP-1. DARPP-32 also serves as substrate for three distinct protein kinases, namely cdk5, CK1 and CK2 and is readily phosphorylated by these protein kinases under basal conditions. The overall consequence of phosphor-rylation by CK1 and CK2 is to increase the state of phosphorylation of DARPP-32 at Thr^{34}. Phosphorylation of DARPP-32 at Thr^{75} by cdk5 (through activation by its natural non-cyclin cofactor p35), inhibits PKA activity (Bibb et al. 1999). Hence, DARPP-32 appears as an important bi-functional regulator of the kinase/phosphatase balance. This pathway is subject of regulation by a variety of extracellular signals. Notably, it is involved in the long-term adaptations follow-ing chronic exposure to drugs of abuse like cocaine (Bibb et al. 2001). Moreover, dopamine-dependent cAMP pathway is involved in the regulation of a family of phosphoproteins sensitive to drugs of abuse (Dulubova et al. 2001). Various ion channels are substrates of PKA, activation of D_1/D_5 receptors also regulate Ca^{2+} and K^+ currents (see Wersinger et al. 2003, for a review). Stimulation of phosphoinositol (PI) hydrolysis by phospholipase C (PLC) and production of 1,4,5-triphosphate is another pathway modulating intracellular Ca^{2+} levels. PI hydrolysis is stimulated by application of D1-like receptor agonists on striatal slices (Undie and Friedman 1990). D_1 or D_5 coupling to PI hydrolysis could not be detected in transfected but not in Chinese hamster ovary (CHO) or baby

hamster kidney cells (Pedersen et al. 1994). However, a dopamine-induced and PKA-independent activation of PLC can be detected in transfected Ltk^- cells (Liu et al. 1992), This suggests that at least in some systems, D_1/D_5 signaling could activate PI hydrolysis in a manner that depends on direct coupling with the G_α.

Stimulation of D_2, D_3 or D_4 receptors, when expressed in transfected cells, inhibits the formation or accumulation of cyclic AMP (reviewed in Missale et al. 1998). As an example of the influence of the recipient cell environment on the response, D_3 receptors are poorly responsive to dopamine when expressed in fibroblasts (Sokoloff et al. 1990; Freedman et al. 1994; Tang et al. 1994). However, the D_3 receptor is readily coupled to adenylyl cyclase in the neuroblastoma X glioma hybrid cell line NG 108-15 (Griffon et al. 1997) or after transfection in fibroblasts of the adenylyl cyclase of type V (Robinson and Caron 1997). It is likely that the inhibition of cyclic AMP efflux measured in the striatum is mediated via D_2 receptors, which are the most abundant D2-like receptors in this structure. Nevertheless, coupling of a D2-like receptor to inhibition of adenylyl cyclase in neurons seems to be formally demonstrated only in the case of D_2 receptors in the anterior pituitary (Enjalbert and Bockaert 1983; Onali and Schwartz 1983; McDonald et al. 1984) and D_4 receptors present on photoreceptors of the retina (Cohen et al. 1992).

D2-like receptor stimulation affects a number of intracellular second messengers, and effectors, in addition to cAMP and adenylyl cyclase, including intracellular Ca^{2+}, arachidonic acid, Na^+/H^+ exchange, Na^+-K^+-ATPase, albeit with different efficiency depending on the receptor subtype (reviewed in Missale et al. 1998). An example of an integrated response mediated by D2-like receptors transfected cells is mitogenesis and cell differentiation, which involved somewhat different primary mechanisms according to the receptor subtype transfected (Pilon et al. 1994; Lajiness et al. 1995). Mitogenesis is certainly a dopamine-mediated response not relevant for neurons, but has been extensively used as a very sensitive functional response permitting the measurement of the intrinsic activity of compounds, including partial (Griffon et al. 1995) or inverse (Griffon et al. 1996) agonists. Different preferred primary effectors for D2-like receptors may be underlined by coupling preference for $G_{\alpha i/o}$ proteins, an issue that has not been fully addressed yet. Nevertheless, in the case of the two splice variants of the D_2 receptor, it has been shown that the D_{2S} receptor couples selectively to $G_{\alpha i2}$ and that the D_{2L} receptor couples selectively to $G_{\alpha i2}$, with an elegant approach using $G_{\alpha i}$ mutants resistant to modification by Pertussis toxin (Senogles 1994). An important structural determinant of the G_α selectivity resides in a pair of amino acid Arg^{233}-Ala^{234} located in the third intracellular loop of the receptor (Senogles et al. 2004).

The examples described above show that within a given dopamine receptor subfamily, the receptor subtypes may activate multiple pathways in transfected cells, in a manner highly dependent on the recipient cell line. In addition, two receptor subtypes (or isoforms) can activate the same intracellular pathway, but may do so with different efficiency or via distinct regulatory mechanisms. It is difficult to extrapolate the observations made in rather artificial expression systems to the native receptors in neurons. To circumvent the problem of the lack of highly subtype-selective agonists, another powerful approach has been to

combine patch-clamp recording of electro-physiological responses to non-selective agonists and phenotypical identification of neurons by single-cell RT-PCR profiling (Surmeier et al. 1996). Thus, D_2 receptor stimulation in enkephalin-expressing medium-size spiny neurons of the dorsal striatum inhibits a L-type Ca^{2+} channel, resulting in diminished excitability (Hernandez-Lopez et al. 2000). In the same kind of striatal neurons, D_4 receptor stimulation also inhibits Ca^{2+} currents (Surmeier et al. 1996). Natural coupling of D_4 receptors has also been reported for inhibition of L-type Ca^{2+} current in granule cells of the cerebellum, which express only this dopamine receptor subtype (Mei et al. 1995). Evidence for functional coupling of D_3 receptors could not be obtained with these approaches, insofar as all striatal neurons recorded express another D2-like receptor (Surmeier et al. 1996). However, the granule cells in the islands of Calleja express D_3 receptors, but not D_2 or D_4 receptors (Diaz et al. 1995; Primus et al. 1997); in this structure, dopamine and D_3 receptor potent agonists enhance intracellular conductance through gap junctions, which would increase the coupling between granule cells and thereby exert a concerted inhibition on the large hilar cells that convey ouputs of the islands of Calleja complex (Fallon et al. 1983).

5. PHARMACOLOGY OF DOPAMINE RECEPTORS

The dual classification of D1-like and D2-like subfamilies, based on sequence information and receptor coupling, is conserved when examining the pharmacological properties of dopamine receptor subtypes (Table 2). Agents previously identified as selective D1-like agonists (e.g. SKF 38393) or antagonists (e.g. SCH 23390) display a marked preference for D_1 and D_5 receptors, with respect to D_2, D_3 and D_4 receptors. Likewise, selective D2-like agonists (e.g. quinpirole) or antagonists (e.g. sulpiride) prefer D_2, D_3 and D_4 receptors to D_1 and D_5 receptors. However, most of the purported D1-like or D2-like "selective" compounds fail to discriminate the receptor subtypes in each subfamily. Thus, a direct and extended comparison of cloned human D_1 and D_5 receptors did not reveal any relevant difference of dissociation constants (K_i values) among a series of compounds. The most consistent difference is a higher affinity and potency of dopamine for the D_5 receptor, as compared to the D_1 receptors (Pedersen et al. 1994; Tiberi and Caron 1994)

Initial pharmacological studies with recombinant D_3 receptor showed that dopamine, as well as some of its agonists, display higher affinity at D_3 receptor than at D_2 receptor (Sokoloff et al. 1990; Sokoloff et al. 1992). Among antagonists, antipsychotics display very similar affinities at D_2 and D_3 receptors, but (+)AJ-76 and (+)UH 232, two aminotetralin derivatives acting as preferential dopamine autoreceptor antagonists (Svensson et al. 1986), show a little preference for the D_3 receptor, as compared to D_2 receptor (Sokoloff et al. 1990). In fact, (+) UH 232 has been shown to exhibit partial agonistic properties at the D_3 receptor (Griffon et al. 1995).

An important step towards identifying selective D_3 receptor ligands was the discovery that the dopamine agonist [^{3}H](+)7-OH-DPAT selectively binds in

vitro to the natural D_3 receptor (Lévesque et al. 1992), allowing to visualize this receptor in the rat (Lévesque et al. 1992) and human (Herroelen et al. 1994) brain slices and to confirm its pharmacological properties previously unraveled when expressed in recombinant cells. Three putative D_3 receptor antagonists, nafadotride (Sautel et al. 1995), PNU-99194A (Waters et al. 1993) and S14297 (Millan et al. 1995) have appeared later on, displaying 7-20 times higher affinity for the D_3 than the D_2 receptor. Nafadotride and PNU-99194A are pure antagonists in functional tests but S14297 actually acts as a full agonist on D_3 receptor (Perachon et al. 2000), which questions the nature of the effects shown with this compound. It follows that highly selective ligands were needed for assigning functional role(s) for the D_3 receptor. BP 897 was the first selective and potent D_3 receptor agonist (at least 70 times higher affinity at D_3 receptor as at other dopamine receptor subtypes), but it is a partial (intrinsic activity ~ 0.6) agonist (Pilla et al. 1999). Subsequently, novel and highly selective D_3 receptor antagonists have been identified (Table 2), like S33084 (Millan et al. 2000) or SB-277011-A (Reavill et al. 2000). Only two antagonists, domperidone and L741,626 were found to display some D_2 selectivity (table 2). Domperidone does not cross the blood-brain barrier and cannot be used for the *in vivo* characterization of the D_2 receptor-mediated response.

Table 2. Dopamine receptor affinity for antagonists

Drug	Affinity (K_i, nM)[a] Receptor subtype				
	D_1	D_2	D_3	D_4	D_5
Non selective:					
Amisulpride	>1,000	2.8	3.2	>1,000	>1,000
(+) Butaclamol	5	1	4.5	45	6.1
Chlorpromazine	35	5	4	16	33
Clozapine	35	145	238	29	343
Eticlopride	>10,000	0.1	0.25	25	>10,000
Flupentixol, *cis*	4	1.5	2.5	-	12
Fluphenazine	6	0.6	0.8	30	8
Haloperidol	150	2	5	6.5	170
Olanzapine	48	30	41	36	74
Pimozide	>10,000	3	4	30	-
Quetiapine	390	380	260	1,050	-
Raclopride	>50,000	1	1.2	2,100	-
Remoxipride[b]	>10,000	588	1,600	3,200	-
Risperidone	560	6	11	16	560
Spiperone	380	0.08	0.4	0.1	2,400
Sulpiride	>10,000	38	60	280	>10,000
Thioridazine	34	7	8	10	300
YM-09151-2	2,600	0.05	0.09	0.13	-
D_1-/D_5-selective:[c]					
SCH 23390	0.4	1,400	1,450	2,910	0.5
D_2-selective:					
Domperidone	>10,000	0.9	13	90	-

L741,626	790	4	63	320	630
D_3- selective:					
BP 897	3,000	61	0.9	300	-
GR 103,691	-	24	0.4	81	-
GR 218,231	>1,000	63	1	10,000	-
Nafadotride	890	3	0.3	1,780	-
NGD 2904	>10,000	217	1.4	>5,000	>10,000
S 33084	500	32	0.3	2,000	1,300
SB-277011-A	>1,000	1,030	10.5	>1,000	>1,000
U 99194A	-	2,280	223	>10,000	-
D_4-selective:					
FAUC 213	5,500	>3,400	5,300	2.2	-
L745,870	-	1,210	2,300	3.4	-
L750,667	-	>1,700	>4,500	0.5	-
NGD 94-1	>10,000	2,230	>10,000	4	-
RBI-257	2,830	568	145	0.33	>10,000
U 101387	>8,000	1,820-5,000	>2,500	4-29	-

[a]Data reviewed in Neve et al. (2003) and data from Schoemaker et al. (1997) and Pilla et al. (1999).
[b]Metabolites of this compound with much higher affinities for D_2 and D_3 receptors have been identified.
[c]Drugs that are selective for D_1 and D_5 receptors with respect to D_2, D_3 and D_4 receptors, but that do not distinguish D_1 from D_5 receptors.

Antipsychotic drugs display high affinities for D_2 and D_3 receptors and do not discriminate the two receptor subtypes; this suggests that both D_2 and D_3 receptors are blocked during treatment with these drugs. A recent placebo-controlled clinical trial with BP 897 in schizophrenia showed some antipsychotic effects of this compound correlated with exposure to the compound, as measured by BP 897 plasma concentrations (Lecrubier 2003).

Clozapine is an antipsychotic efficacious in patients who do not respond to other drugs and is almost devoid of extrapyramidal motor side effects; it is the only antipsychotic having a higher affinity at the D_4 than at the D_2 or D_3 receptors. The peculiar profile of clozapine at the D_4 receptor, the reported elevation of D_4 receptors in schizophrenia (Seeman et al. 1993), as well as the localization of D_4 receptors in the prefrontal cortex where dysfunctions are found in schizophrenia, prompted pharmaceutical companies to develop D_4-selective agents as antipsychotic drugs. To date, three placebo-controlled clinical studies with L-745,870 (Kramer et al. 1997), fananserin (Truffinet et al. 1999) and sonepiprazole (U-101387) (Corrigan et al. 2004) failed to demonstrate antipsychotic effects. We think that this result could be expected from the fact that the D_4 receptor is not a common target for antipsychotic drugs, since some potent antipsychotics, like fluphenazine, raclopride or amisulpride have much lower affinity at the D_4 than at the D_2 or D_3 receptors. Nevertheless, it could not be excluded that D_4-selective agents may find other indications, namely with attention deficit and hyperactivity disorder, for which a genetic association has been found (see below).

More limited differences have been reported in the pharmacology of receptor subtypes variants. The 9Gly variant of the D_3 receptor has a 7-time

higher affinity for dopamine than the 9Gly variant when expressed in insect cells (Lundstrom and Turpin 1996). A direct comparison between D_4 receptor variants with 2, 4 or 7 repeats shows that dopamine has a 2- to 3-fold higher potency in a functional assay at the longest variant (Asghari et al. 1995); however the dopamine potency at the D_4 receptor variant containing ten 48-bp repeats is lower than at the variant with 2 repeats. This suggests that there is no direct relationship between the number of 48-bp repeats and functional receptor pharmacology, a feature generally not taken into account in various genetic studies that have compared "short" and "long" alleles of this polymorphism.

6. D_2 AND D_3 RECEPTORS AS AUTORECEPTORS

Functionally, three classes of dopamine autoreceptors can be defined (for review, see (Wolf and Roth 1990). The soma and dendrites of midbrain DA neurons express autoreceptors that modulate rates of impulse activity (Bunney et al. 1973; Aghajanian and Bunney 1977). Nerve terminals of these neurons express autoreceptors that modulate DA synthesis (Kehr et al. 1972) and release (Farnebo and Hamberger 1971). Each of these three autoreceptors exhibits pharmacological characteristics of the D2-like receptor subfamily (Wolf and Roth 1990).

D_2 and D_3 receptor mRNA is present in dopamine neurons of the substantia nigra and ventral tegmental area (Meador-Woodruff et al. 1989; Bouthenet et al. 1991); D_2 receptor protein is detected in neurons of these structures (Levey et al. 1993). Moreover, some binding of [^{125}I]iodosulpride, a ligand with high affinity at both D_2 and D_3 receptors, persists in D_2 receptor-knockout mice (Mercuri et al. 1997). The presence of D_3 receptor protein in identified dopamine neurons has been confirmed using an anti-D_3 receptor antibody, of which the specificity has been assessed by using transfected cell lines, immunoprecipitation of recombinant dopamine receptors and D_3 receptor-knockout mice, in which it does not generate any immunolabeling (Diaz et al. 2000). Hence both D_2 and D_3 receptors may play the role of autoreceptors, the receptors expressed by dopamine neurons to provide feedback regulatory control. However, the level of expression of D_3 receptor mRNA is low compared to D_2 receptor mRNA (Bouthenet et al. 1991). The short D_{2S} receptor variant is prominent in dopamine neurons (Khan et al. 1998). D_1, D_4 or D_5 receptor mRNAs are not detected in the substantia nigra or ventral tegmental area, but D_1 receptor protein, detected by immunohistochemistry (Levey et al. 1993), is present in these regions on afferent neurons originating from the striatum. Since D_2/D_3 agonists hardly discriminate D_2 from D_3 receptors, the question arises about the nature of the receptor involved in the various autoreceptor activities.

The role of D_2 receptor as an autoreceptor is well established. The inhibitory effects of dopamine and of its D_2/D_3 agonist quinpirole on dopamine cell firing are completely abolished in D_2 receptor-knockout mice, whereas the basic electrophysiological properties of dopaminergic cells do not differ in mutant and wild-type mice (Mercuri et al. 1997). In synaptosomes from D_2 receptor-deficient mice, the inhibitory effects of several D_2/D_3 agonists on newly

synthesized [^{3}H]dopamine are also suppressed (L'hirondel et al. 1998). In normal conditions, D_2 receptor-knockout mice have normal extracellular levels of dopamine and its metabolites, but dopamine uptake was reduced (Dickinson et al. 1999). These results suggest that the D_2 receptor is a major functional presynaptic autoreceptor controlling phasic dopamine neuron activity. Interestingly, autoreceptor functions are deficient in mice lacking both long D_{2L} and short D_{2S} variants, but preserved in mice lacking the short D_{2S} variant only (Usiello et al. 2000; Wang et al. 2000; Centonze et al. 2002). This suggests that the D_{2S} receptor variant serves autoreceptor functions, in agreement with the prominence of this variant expression in dopamine neurons (Khan et al. 1998).

Nevertheless, dopamine extracellular levels are twice as high in D_3 receptor-knockout as in wild-type mice, whereas the tissue levels of dopamine and its metabolites are unchanged (Koeltzow et al. 1998); (Joseph et al. 2002). This suggests a control of dopamine release by the D_3 receptor. In contrast, inhibition by PD 128,907, a D_2/D_3 agonist with a preference for the D_3 receptor (Sautel et al. 1995), of electrical activity of dopamine neurons, of dopamine synthesis and release is intact *in vivo* in D_3 receptor-knockout mice (Koeltzow et al. 1998). However, the inhibitory effects of low doses of PD 128,907 on extracellular dopamine are reduced in D_3 receptor-knockout mice (Zapata et al. 2001). Dopamine synthesis rate appears normal in D_3 receptor-knockout, but, paradoxically, dopamine synthesis measured as L-DOPA accumulation under inhibition of cell firing was lowered (Joseph et al. 2002) and the effect of PD 128,907 on this index of was greater (Koeltzow et al. 1998). The apparent discrepancies between these studies may be explained by different knockout strategies to generate D_3 receptor mutant mice, to the different genetic background of these mice, to the use of highly sensitive techniques permitting to reveal subtle changes that were missed in initial studies and to development-related adaptive changes following constitutive gene invalidation (see Zapata et al. 2001 and Joseph et al. 2002 for an extensive discussion). The latter explanation is supported by the observation that D_3 receptor-knockout mice have reduced tyrosine hydroxylase mRNA levels and increased dopamine uptake, presumably resulting from hyperdopaminergia (Le Foll et al. 2005a).

The availability of the two highly selective D_3 receptor antagonists SB-277011A and S33084 has contributed to clarify the role of D_3 autoreceptors. SB-277011A antagonizes the reduction of dopamine efflux induced by quinelorane, another D_3-preferring agonist, in the nucleus accumbens, but not the striatum (Reavill et al. 2000). S33084 antagonizes the inhibitory effects of PD 128,907 on dopamine neuron cell firing in the ventral tegmental area and of extracellular dopamine levels in the frontal cortex (Millan et al. 2000). Of notice is the fact that neither SB-277011A nor S33084 has an effect when given alone, suggesting that the D_3 receptors that mediate inhibition of dopamine efflux in the nucleus accumbens are not occupied by dopamine under basal conditions. These data indicate that the D_3 receptor may, together with the D_2 receptor, control tonic dopamine neuron activities under some physiological or experimental circumstances. Among these are situations in which dopamine release is elicited, such as the exploratory activity in a novel environment, which is accompanied by an increased dopamine release (see below).

7. DOPAMINE RECEPTORS AND ANIMAL BEHAVIORS

Table 3. Phenotype of dopamine receptor mutant mice[a]

	Receptor subtype knockout				
	D_1	D_2	D_3	D_4	D_5
Spontaneous locomotion (after habituation)	Increased or decreased	Decreased	Increased or unchanged		Unchanged
Response to novelty			Rapid habituation	Decreased	
Anxiety-like behaviors				Increased	
Motor coordination		Decreased		Increased	Increased
Locomotor responses to psychostimulants	Markedly decreased			Increased	Decreased
Locomotor response to drug cues	n.t.	n.t.	Increased	n.t.	n.t.
Drug-conditioned place-preference	Unchanged (cocaine)	Absent (morphine)	Increased or decreased (morphine)		
Discriminative-stimulus effects of cocaine					Unchanged
Disruption of amphetamine-induced prepulse inhibition	n.t.	Absent	Unchanged	Unchanged	n.t.

[a] Data reviewed by Glickstein and Schmauss (2001) and Waddington et al. (2001). Other data from Le Foll et al. (2002), Katz et al. (2003), Elliot et al. (2003), Holmes et al. (2001) and Dulawa et al. (1999). n.t., not tested.

7. 1. Locomotor spontaneous activity and locomotor responses

Although, it has been shown for decades that a decrease of the dopaminergic tone and/or a blockade of dopamine receptors impairs motor function in animals, the involvement of specific receptor subtypes in these effects has been only recently revealed (see Holmes et al. 2004 for a review on studies with transgenic animals). Concurrent ablation of the D_1 and D_2 receptors is lethal during the second or third week after birth. This dramatic phenotype is likely to be related to altered feeding behavior and dysfunction of the gastrointestinal system (Kobayashi et al. 2004). Although, both D_1 and D_2 dopamine receptor antagonists disrupt basal locomotor activity in animals, it appears that the D_2 receptors are mainly involved in this process. D_2R-deficient mice present significant decrease of locomotor activity (Baik et al. 1995; Kelly et al. 1998; Kobayashi et al. 2004) and deficit in gait and motor coordination, suggesting the critical involvement of this receptor in Parkinson's disease (Jung et al. 1999; Aoyama et al. 2000). The D_{2L} receptor isoform seems particularly involved (Usiello et al. 2000).

D_1 receptor antagonists strongly disrupt locomotor activity in animals, but the results obtain with the D_1 receptor-deficient mice have been inconsistent. A

decreased (Drago et al. 1996; El-Ghundi et al. 1998; Smith et al. 1998; Tomiyama et al. 2002) or increased spontaneous activity of these mice has been reported (Xu et al. 1994; Centonze et al. 2003; McNamara et al. 2003). Compared to the D_1 and D_2 receptors, the D_3, D_4 and D_5 receptors do not appear to regulate spontaneous activity. The alterations found with D_3 and D_4 receptors under particular situations (see below) may instead reflect a different reactivity to presentation to novelty or to anxiogenic situations. The basal locomotor activity appears normal in mice lacking the D_5 receptor (Holmes et al. 2001; Elliot et al. 2003) or the D_4 receptor (Rubinstein et al. 1997; Kruzich et al. 2004).

Other spontaneous behaviors are under the control of dopamine transmission. For example, grooming behavior is mainly under the influence of D_1 receptor stimulation. Grooming behavior is increased by administration of D_1 receptor agonists and strongly decreased by D_1 receptor invalidation (Xu et al. 1994). The grooming behavior may be increased in D_3 receptor-deficient mice (Le Foll et al. 2005).

Administration of D_1 and D_2 receptor antagonists not only reduces spontaneous activity in rodents, but also inhibits locomotor hyperactivity induced by administration of drugs of abuse. Studies using transgenic animals have revealed a critical role for the D_1 receptor (Xu et al. 1994) and the D_{2L} isoform (Usiello et al. 2000) in the locomotor response to psychostimulant administration. In contrast to D_1 and D_2 receptors, the D_3, D_4 and D_5 receptors seem to affect in a more limited manner the locomotor activity induced by psychostimulant administration. Although it has been first reported that the D_3 receptor gene invalidation increased locomotor response to psychostimulant administration (Xu et al. 1997), this effect has not been consistently reported (Betancur et al. 2001; Le Foll et al. 2002). The locomotor response to psychostimulant administration is increased in D_4 receptor-deficient mice (Rubinstein et al. 1997; Katz et al. 2003; Kruzich et al. 2004) and slightly decreased in D_5 receptor-deficient mice (Elliot et al. 2003).

The behavioral procedures employed and the genetic background of the mice may be of importance to analyze the behavioral results. As an example, it should be noticed that D_3 receptor-deficient mice have been described as hyperactive in various behavioral paradigms. For example, Accili et al. (1996) first reported an increased basal locomotor activity in D_3 receptor-deficient mice (see also Steiner et al. 1997)). Subsequently, Xu et al. (1997) reported an increased activity during the first several minutes of assessment, but not thereafter, in D_3 receptor-deficient mice. This locomotor hyperactivity has been found during the night (Betancur et al. 2001), but not consistently during the day (Boulay et al. 1999; Betancur et al. 2001; Joseph et al. 2002). These differences may be due to the different strategies used to create these knock-out mice (Accili et al. 1996; Xu et al. 1997), but may also reflect a hyper-reactivity to presentation of a novel environment.

In D_4 receptor-deficient mice, some investigators report an attenuated response to novelty (Rubinstein et al. 1997; Dulawa et al. 1999; Katz et al. 2003). In contrast, in their home environment, these mice display normal behaviors (Dulawa et al. 1999).

7.2. Effects of drug of abuse and related behaviors

Dopamine has been involved in 'reward processes' (Wise and Bozarth 1987; Wise 2004), but the nature of this involvement has considerably evolved during the recent years. The initial idea of a role of dopamine, notably in the nucleus accumbens, as a direct mediator of the primary reinforcement of natural reinforcers, such as food and sex, or of drugs of abuse has been challenged by numerous observations suggesting that, instead, dopamine is acting as a modulator of several functions related to motivated behavior (Wise 2004; Salamone et al. 2005).

Although the D_1 receptor is clearly involved in the locomotor effects of drugs of abuse (Kalivas and Stewart 1991), its role in rewarding and reinforcing effects has been assessed by using pharmacological ligands that do not discriminate between D_1 and D_5 receptor subtypes. Surprinsingly, the reinforcing effects of cocaine are not affected in D_1 receptor-deficient mice, as assessed by the conditioned place preference procedure (Miner et al. 1995). In contrast, a reduction of voluntary ethanol consumption (El-Ghundi et al. 1998) and of responding for sucrose (El-Ghundi et al. 2003) has been reported with these mice. Recent evidence also suggests that the D_1 receptor may be an important determinant in brain stimulation reward and participate in coding for reward prediction and in spatial learning (Tran et al. 2005). Interestingly, psychostimulant-induced phosphorylation of DARPP-32, an intracellular signaling molecule thought to mediate the neural effects of psychostimulants, is also compromised in D_1 receptor-deficient mice (Svenningsson et al. 2003).

More evidence has been provided supporting the involvement of the D_2 receptor in the rewarding and reinforcing effects of drugs of abuse. Evidence for a critical role of the D_2 receptor in mediating opiate reward, as assessed in the conditioned place preference paradigm, has been provided in one study (Maldonado et al. 1997), but not in another (Dockstader et al. 2001). It has been proposed that the role of the D_2 receptor in opiate reward may be influenced by the dependence state of the animal (Dockstader et al. 2001). These findings have been recently confirmed using the morphine intravenous self-administration paradigm in mice (Elmer et al. 2002). Although the D_{2L} receptor isoform seems involved in morphine-induced conditioned place preference, its deletion does not significantly affect cocaine-induced conditioned place preference (Smith et al. 2002). The D_2 receptor-deficient mice show reduced ethanol preference, reduced ethanol consumption and reduced ethanol-induced conditioned place preference (Phillips et al. 1998; Cunningham et al. 2000; Risinger et al. 2000).

The D_3 receptor has distinct features suggesting its involvement in the reinforcing effects of abused drugs. D_3 receptors are expressed in the mesolimbic brain reward circuit (Lévesque et al. 1992; Diaz et al. 1995; Le Foll et al. 2002; Le Foll et al. 2005b; Sokoloff et al. 2005) and their density is elevated in the brains of long-term cocaine abusers (Staley and Mash 1996; Segal et al. 1997) and of animals chronically treated with cocaine or nicotine (Le Foll et al. 2002; Le Foll et al. 2003a). Early studies, using relatively non-selective D_3 receptor ligands, indicated a direct role for D_3 receptors in the reinforcing effects of psychostimulants (see Le Foll et al., 2000 for a review).

However, more recent studies using highly selective D_3 receptor ligands and D_3 receptor-deficient mice indicate that the D_3 receptors are not involved in the direct reinforcing effects of psychostimulants, but, instead, strongly modulate the influence of drug-associated environmental stimuli on drug-seeking behavior (Le Foll et al. 2005b).

D_3 receptor blockade reduces the effects of drug-associated environmental stimuli over behavior measured using various experimental procedures, including second-order self-administration (Pilla et al. 1999; Di Ciano et al. 2003), conditioned place preference (Vorel et al. 2002; Le Foll et al. 2005c) and Pavlovian conditioning procedures (Le Foll et al. 2002; Le Foll et al. 2003b; see also Le Foll et al. 2005c) for a review on influence of drug conditioning on nicotine's effects). The involvement of D_3 receptors has been confirmed by using dopamine D_3 receptor-deficient mice (Frances et al. 2004a). In contrast, effects of environmental stimuli associated with natural reinforcers, such as food, appear to be unaffected by modulation of D_3 receptors (Le Foll et al. 2005b; Sokoloff et al. 2005). All of these findings suggest that D_3 receptor ligands might represent potentially useful pharmacological tools for decreasing relapse to drug use in abstinent drug-abusers. Surprisingly, the D_3 receptor-deficient are hyperactive in the presence of drug-associated cues (Xu et al. 1997; Le Foll et al. 2002; Narita et al. 2003; Frances et al. 2004a; but see also Frances et al. 2004b) how the dose of morphine used during conditioning sessions strongly affects the reactivity to morphine-paired environmental cues in wild-type and D_3 receptor-deficient mice). This phenotype, which is opposite to the effect of acute administration of a D_3 receptor antagonist (Le Foll et al. 2002; Vorel et al. 2002; Ashby et al. 2003; Di Ciano et al. 2003; Le Foll et al. 2003b), could be explained by an increased dopaminergic tone in these mice (Koeltzow et al. 1998; Joseph et al. 2002).

Recently, it has been shown that the cannabinoid CB_1 receptor blockade is also able to reduce the influence of drug-associated environmental stimuli on the reinstatement of drug-seeking behavior (Le Foll et al. 2005b). Some effect of the CB1 antagonist, rimonabant, are diminished in D_3 receptor-deficient mice (Duarte et al. 2003), suggesting that D_3 receptors control cannabinoid CB_1 receptor-mediated processes.

7.3. Anxiety-like behaviors

Limited assessment of this phenotype has been reported. Anxiety-like behaviors appear normal in D_5 receptor-deficient mice (Holmes et al. 2001). Some reports suggest D_4 receptor-deficient mice may present increased anxiety-like behavior (Falzone et al. 2002), whereas D_3 receptor-deficient mice may present decreased anxiety-like behavior (Steiner et al. 1997). Further characterization of these behavioral abnormalities is needed to confirm these initial findings.

7.4. Depression-like behaviors

Dopamine has been proposed to play a critical role in depression and notably in anhedonia (Willner 1997). Administration of dopamine agonists such as pramipexole, a D_2/D_3 receptor agonist, is an effective treatment for depression in humans (Lattanzi et al. 2002; Ostow 2002). Recent studies have reported antidepressant-like activities of the dopamine D_2/D_3 agonist pramipexole in animal models of depression, such as the chronic mild stress model and in the forced swim test, suggesting that D_2/D_3 receptor agonists may represent a new class of antidepressant drugs. However, recent findings indicate that D_3 and D_4 receptors are not involved in the effects of pramipexole in the forced swim test (Siuciak and Fujiwara 2004; Basso et al. 2005). Moreover, in contrast to the antidepressant imipramine (Porsolt et al. 1978), which effectively increased performance (escape behavior) in the forced swim test, BP 897 had no effect on immobility (Le Foll et al. 2005c). Moreover, in the same test, D_3 receptor-invalidation had no effect and did not prevent the effects of imipramine (Le Foll et al. 2005c). Indeed, further studies are warranted to clarify the role of dopamine receptor subtypes in the antidepressant effects of drugs.

7.5. Schizophrenia-like behaviors

Psychostimulants can elicit symtoms resembling positive symptoms of schizophrenia (hallucinations, delusions) in long-term abusers and exacerbate psychotic symptoms in schizophreniac patients (Angrist et al. 1974; Carlsson 1988). Hence, inhibition of hyperactivity in response to psychostimulants is often used as a test predictive for antipsychotic activity in animals. The nature of the dopamine receptor subtype(s) involved in this effect is still elusive, most probably the D_2 subtype is involved. Nevertheless, antipsychotics, particularly atypical antipsychotics often associate antagonist activity at non-dopaminergic receptors, e.g. 5-HT_{2A} and/or 5-HT_{2C}, which may also participate to inhibition of psychotimulant-induced hyperactivity.

Deficit in glutamate neurotransmission has also been involved in schizophrenia. Indeed, blockade of NMDA receptors by phencyclidine (PCP, “angel dust”) or ketamine, produces psychotic symptoms, such as visual hallucinations, euphoric state and increased frontal blood flow (Jentsch and Roth 1999). Moreover, direct evidence for altered NMDA receptor function in schizophrenia has been recently reported (Emamian et al. 2004) and genetic linkage studies in schizophrenia show that the most plausible susceptibility genes (neuregulin 1, dysbindin, proline deshydrogenase and others) functionally interact with glutamate pathways (Harrison and Owen 2003). Genetically engineered mice with reduced NMDA receptor expression display abnormal behaviors such as locomotor hyperactivity and deficits in social interactions, which are corrected by clozapine (Mohn et al. 1999). Hence, the behavioral abnormalities produced by glutamate/NMDA receptor blockade represent a relevant pharmacological model of schizophrenia. Interestingly, BP 897 or nafadotride antagonize locomotor hyperactivity produced by administration of low doses of MK-801 (dizocilpine), a selective non-competitive antagonist at

glutamate/NMDA receptors. Furthermore, the MK-801-induced hyperactivity is attenuated and the effects of BP 897 and nafadotride are abolished in D_3 receptor-deficient mice (Leriche et al. 2003), suggesting that the D_3 receptor plays an important role in behavioral abnormalities induced by glutamate/NMDA receptor blockade.

Disruption of prepulse inhibition of acoustic startle is another schizophrenia-related behavior, which mimics deficit of sensorimotor gating that is present in a large proportion of schizophrenic patients and their first-degree relatives (Braff et al. 1992). The D_2, but not D_3 or D_4 receptors, is essential for the disruption of prepulse inhibition produced by amphetamine in mice (Ralph et al. 1999). However, the D_3 receptor is involved in the disruption of prepulse inhibition produced by isolation in rats, which is antagonized by SB-277011A (Reavill et al. 2000). Species differences in the dopaminergic modulation of prepulse inhibition between rats and mice exist, the D1-like receptors playing a more prominent role in mice compared to rats (Ralph-Williams et al. 2003).

8. DOPAMINE RECEPTOR GENES AND PSYCHIATRIC DISORDERS

8.1. Schizophrenia

The dopamine hypothesis of schizophrenia that postulates a dopaminergic dysfunction in this disorder, has been accepted for about 3 decades, but has for a long time been supported only by the indirect pharmacological evidence that all antipsychotic drugs are D_2/D_3 receptor antagonists, their efficacy being correlated with affinity for D_2/D_3 receptors. Moreover, indirect dopaminergic agonists such as amphetamine can induce psychosis in healthy subjects and precipitate symptoms in schizophrenic patients (Carlsson 1988). Recently, this hypothesis has now received more direct support by two different lines of research, both using imaging techniques. First, it has been shown that the synthesis of labeled dopamine or fluorodopamine in the brain, measured by means of PET after administration of radiolabeled DOPA or fluoroDOPA, is increased in drug-naive schizophrenic patients, compared to age-matched controls (Hietala et al. 1994; Dao-Castellana et al. 1997; Lindstrom et al. 1999; Gefvert et al. 2003). Second, SPECT and PET studies, using a sophisticated technique to measure the release of dopamine in the basal ganglia *in vivo*, have shown that, after an amphetamine challenge, the dopamine release is elevated in drug-naïve schizophrenic patients compared to age-matched controls, and that this elevation correlates to the induction of positive psychotic symptoms (Laruelle et al. 1996; Breier et al. 1997; Abi-Dargham et al. 1998; Laruelle et al. 1999).

Rates of schizophrenia are higher among relatives of patients than in the general population. Adoption and twin studies have shown that this increased risk is genetic, with a tenfold increase in risk associated with the presence of an affected first-degree family member. This genetic risk increases with each affected relative, to nearly 50% when both parents are affected (McGuffin et al. 1995), and 60%–84% when a monozygotic twin is affected (Cardno and

Gottesman 2000). The genetic transmission does not appear to follow simple Mendelian single-gene inheritance patterns. More probably, there are multiple susceptibility genes, each with small effect and acting in concert with epigenetic and environmental factors.

Therefore, the genes encoding dopamine receptors have been intensively studied for their possible involvement in vulnerability to schizophrenia.

8.1.1. Gene coding for the D_2 receptor

Schizophrenia is often treated with antipsychotic drugs, whose clinical potency was primarily related to dopamine D2 receptor affinity. Thus, the *DRD2* gene has been extensively studied as candidate for population and linkage studies in schizophrenia.

A first linkage study with two schizophrenia pedigrees has largely excluded the *DRD2* gene as a major candidate (Moises et al. 1991). This previous finding was confirmed by a recent study where the authors examined 90 trios from Portugal (Ambrosio et al. 2004).

There are positive case-control studies showing association between schizophrenia and the Cys^{311} variant (Arinami et al. 1994; Laurent et al. 1994; Shaikh et al. 1994; Arinami et al. 1996; Kaneshima et al. 1997; Jonsson et al. 2003). One study (Serretti et al. 2000) did not show that coding variants of the *DRD2* $Ser^{311}Cys$ play a major role in conferring susceptibility to major psychosis, including patients with schizophrenia, bipolar disorder, major depressive disorder, delusional disorder, psychotic disorder not otherwise specified. However, many studies failed to found association with the Cys^{311} allele (Nöthen et al. 1993; Hattori et al. 1994; Nanko et al. 1994; Nothen et al. 1994; Crawford et al. 1996; Ohara et al. 1996; Harano 1997; Hori et al. 2001; Himei et al. 2002).

Taking into account the discrepancy between all studies and the huge literature dealing with the subject, meta-analyses might be more informative. Jonsson et al. performed a meta-analysis of all published case-control studies comprising a total of 9,152 subjects ($\chi^2 = 11.37$, $df = 1$, $p < 0.001$; pooled odds ratio 1.43, 95% confidence interval = 1.16 – 1.78), then supporting the involvement of the *DRD2* $Ser^{311}Cys$ variant in the pathogenesis of schizophrenia (Jonsson et al. 2003). Finaly, Glatt et al. (Glatt et al. 2003), from all 24 published case-control studies (3,733 cases and 5,373 controls), calculated a pooled estimate of this association. The pooled odds ratio was 1.3 for the ^{311}Cys allele, which was highly significant ($p = 0.007$). Nevertheless, family-based studies are needed to confirm that the *DRD2* $Ser^{311}Cys$ polymorphism influences susceptibility to schizophrenia.

The other intensively studied polymorphism was the promoter –141C ins/del. positive case-control studies showing association between promoter –141C insertion allele in Japanese (Arinami et al. 1997; Ohara et al. 1998; Himei et al. 2002), Swedish (Jonsson et al. 1999), and Finish (Kampman et al. 2003) populations. In a British population, the –141C insertion allele was also associated with schizophrenia (Breen et al. 1999). Moreover, Schindler et al. reported an association and linkage disequilibrium between the –141C insertion

allele and schizophrenia in a genetically homogeneous Portuguese population (Schindler et al. 2002). However, in numerous other studies no association was found between the –141C ins/del polymorphism and schizophrenia (Li et al. 1998; Stober et al. 1998; Tallerico et al. 1999; Hori et al. 2001; Kapelski et al. 2002; Rohrmeier et al. 2003). Finally, in the only meta-analysis dealing with –141Cins/del polymorphism and schizophrenia, the association was not significant. Such results suggest weak or no participation of the –141C ins/del polymorphism in schizophrenia.

Others *DRD2* gene polymorphisms have been investigated in schizophrenia but the number of studies is still too small to draw conclusion (Sanders et al. 1993; Dubertret et al. 2001; Golimbet et al. 2003; Dubertret et al. 2004). Furthermore, a study examining the sequence of the D_2 receptor gene region that couples to G-proteins found no changes that would alter the protein in schizophrenia (Seeman et al. 1993).

Hence, no consistent findings have emerged from all these genetics studies. However, as the D2 receptor is a common target for all antipsychotics drugs and evidence exists to suggest that D2 action may be necessary for antipsychotic efficacy (Kapur and Mamo 2003); consequently the *DRD2* gene is a prime candidate for pharmacogenetic analysis. Such studies have been conducted to explore the role of *DRD2* gene polymorphisms in the mechanisms of action of the antipsychotics drugs, especially clozapine and other atypical antipsychotics.

Susuki et al (Suzuki et al. 2001) examined the association of the –141C *ins*/*del DRD2* promoter gene polymorphism and response to antipsychotics (bromperidol and nemonapride). After 3 weeks of treatment, while percentage improvement in total BPRS or other subgrouped symptoms (positive, negative, excitement and cognitive symptoms) was similar between the two genotype groups, the patients without *del* allele showed a higher percentage of improvement in anxiety-depression symptoms than those with *del* allele (Suzuki et al. 2001). The same team has investigated the relationship between DRD2 polymorphism at the *Taq*I *A* locus (*A1* and *A2* alleles) and therapeutic response to nemonapride (Suzuki et al. 2000). The patients with one or two *A1* alleles (*n* = 14) showed significantly higher percentage improvement in total BPRS and positive symptoms than those with no *A1* allele after 3-week treatment while the percentage improvement in other subgrouped symptoms (negative, anxiety-depression, excitement and cognitive symptoms) was similar between the two genotype groups. The authors concluded that the data suggested that the *Taq*I *A DRD2* polymorphism is related to early therapeutic response to nemonapride in schizophrenic patients, possibly by modifying the efficiency of DRD2 antagonism of the drug in the central nervous system (Suzuki et al. 2000). In another study, haloperidol non-responders were more frequently homozygous for the *A2* allele than responders (Schafer et al. 2001). Lane and colleagues (Lane et al. 2004) reported that patients with the $Ser^{311}Cys$ genotype had lower PANSS, general psychopathology, and cognitive subscales scores than those with the $Ser^{311}Ser$ genotype. Yamamouchi et al (Yamanouchi et al. 2003) evaluated the haplotype effect of –141C *ins/del* and the *Taq*I *A DRD2* alleles on clinical improvement with risperidone therapy. Interestingly enough, more

symptom reduction was seen among the individuals with *ins-A2*/*del-A1* compared to those with *ins-A2*/*ins-A2* genotype (Yamanouchi et al. 2003).

In conclusion, most findings on this topic have not been confirmed yet and it is premature to draw any conclusion on the role of the *DRD2* in susceptibility to schizophrenia or its treatment.

8.1.2. Gene coding for the D_3 receptor

The most studied polymorphism of the *DRD3* gene in schizophrenia is the Ser^9Gly (*Bal*I). The Ser^9 variant is referred to as allele 1, and the Gly^9 variant is identified as allele 2. A positive association between homozygosity at the *Bal*I polymorphism and/or the 1–1 genotype and schizophrenia has been reported in case-control studies (Crocq et al. 1992; Mant et al. 1994; Kennedy et al. 1995; Asherson et al. 1996; Durany et al. 1996; Spurlock et al. 1998). Some of these also found weaker evidence for association between the 1–1 genotype and schizophrenia. Discordant results from a case-control study indicated an association between homozygotes for either *Bal*I allele and schizophrenia, and an excess of allele 1 in the schizophrenia group when compared to controls (Nimgaonkar et al. 1996). Two independent samples were studied, but results were not consistent between the two groups. One study with a relatively small number of subjects (73 with schizophrenia, and 56 matched controls), found an association between one of the genotypes, in which three of the four single nucleotide polymorphisms in the 5 -leader region differ, and schizophrenia (Sivagnanasundaram et al. 2000). In 133 schizophrenic patients, allele 1 of the *Bal*I polymorphism was reported to be more frequent than in a control group (Shaikh et al. 1996). These authors also performed a meta-analysis of previously published results and concluded that the Ser^9 allele conferred a small increase in susceptibility to schizophrenia.

In contrast to the above studies, many more have reported negative results of associations studies between schizophrenia and the *Bal*I polymorphism or homozygosity at this locus of the dopamine *DRD3* in case-control studies (Jönsson et al. 1993; Nanko et al. 1993; Nöthen et al. 1993; Yang et al. 1993; Di Bella et al. 1994; Saha et al. 1994; Inada et al. 1995; Gaitonde et al. 1996; Tanaka et al. 1996; Chen et al. 1997; Hawi et al. 1998; Malhotra et al. 1998). Negative results in linkage analysis of pedigrees (Wiese et al. 1993, Ambrosio et al. 2004), and in sib-pairs using the transmission disequilibrium test (TDT) (Rothschild et al. 1996, Ambrosio et al. 2004) have also been reported. Negative results of linkage have also been reported for the D3-208 and the D3-Hsac polymorphisms (Sabaté et al. 1994). A recent study examining the contribution of the A-206G transitions in the *DRD3* genes respectively to genetic susceptibility and phenotypic expression of schizophrenia (Baritaki et al. 2004), showed showed marginally nonsignificant differences in the genotypic ($p = 0.054$) and no significance in the allelic ($p = 0.163$) frequencies. However, the A-206/A-206 genotype seems to positively contribute to the disorder appearance, when compared to A-206/G-206 as genotype base line risk ($p = 0.016$, OR = 1.88, 95% CI = 1.09-3.26).

Regarding all the discrepancy between the different analysis meta-analysis of case-control studies have been performed. The first one (Williams et al. 1998), with 2,722 schizophrenic patient and 2,669 controls, showed that the results of the meta-analysis and family-based association study provide independent support for a relationship between schizophrenia and homozygosity at the *BalI* polymorphism of *DRD3*, or between a locus in linkage disequilibrium with it. Next, another independent meta-analysis, with 2717 schizophrenic patient and 2319 controls, found an excess of homozygosity at the *Bal*I polymorphism and of the 1–1 genotype in schizophrenics (Dubertret et al. 1998). Finally, a meta-analysis of all case-control studies comprising 8761 subjects the association between DRD3 Ser9Gly homozygosity and schizophrenia ($\chi^2 = 4.96$, $df = 1$, $p < 0.05$, OR =1.10, 95% CI =1.01-1.20) persisted (Jonsson et al. 2003). The same group with 5430 patients and 5636 controls founded similar results (Jonsson et al. 2004).

Pharmacogenetics studies are less abundant than for the *DRD2*, and focalised on the *BalI* polymorphism. In studies that used clozapine monotherapy, the 1–1 genatype correlated with lack of benefit in some (Shaikh et al. 1996; Scharfetter et al. 1999) but not all, (Malhotra et al. 1998) investigations. Interestingly, Scharfetter and co-workers found that the 1–1 genotype was most frequently represented in non-responder and the 1–2 was more frequent in responder whereas the 2–2 genotype appeared almost exclusively in the responders (Scharfetter et al. 1999; Scharfetter 2004).

Tardive dyskinesia (TD) is the most serious movement disorder induced by long-term treatment with antipsychotic drugs; TD has a high prevalence and is almost irreversible. Following an initial report showing an association of the Ser9Gly polymorphism with TD (Steen et al. 1997), there have been numerous reports, splitting in confirmations and non-confirmations. A meta-analysis of 780 patients (317 with TD and 463 without TD) supports a small but significant contribution of Ser9Gly polymorphisms with TD.

To conclude, these results suggest, at most, a small genetic association of the *DRD3* with the vulnerability to schizophrenia and response to the treatments.

8.1.3. Gene coding for the D_4 receptor

Studies examining the association of the *DRD4* gene is associated with schizophrenia have yielded a number of negative or weak results (Barr et al. 1993; Nanko et al. 1993; Sommer et al. 1993; Shaikh et al. 1994; Petronis et al. 1995; Tanaka et al. 1995; Hong et al. 1997; Kohn et al. 1997; Tani et al. 1997; Hwu et al. 1998; Okuyama et al. 1999; Xing et al. 2003; Serretti et al. 2004), and have largely excluded this gene as a major candidate for susceptibility to schizophrenia (Macciardi et al. 1994). One study that found results suggestive of linkage between the 48-basepair polymorphism and schizophrenia, found no sequence differences in the main ligand binding region (Weiss et al. 1996). Furthermore, there was no genetic interaction found between the *DRD4* exon 3, and the GABA receptor α-1 subunit in the symptoms of major psychoses (Serretti et al. 1999). A study examining polymorphisms at *DRD1*, *DRD2*, *DRD3*, and *DRD4*genes in schizophrenia defined by different diagnostic systems

failed to find an association (Dollfus et al. 1996). Furthermore, the meta-analysis, failed to show any association between *DRD4* gene and schizophrenia (Glatt et al. 2003; Jonsson et al. 2003), excluding definitely this gene as a major candidate for susceptibility to schizophrenia.

Because of the relatively high affinity of clozapine for the dopamine D4 receptor (Van Tol et al. 1991), the relationship between polymorphisms in this gene and response to clozapine treatment have been investigated. Negative reports of the association with clozapine response have included the 48 base-pair repeat, exon 1, and exon 3 polymorphism (Shaikh et al. 1993; Rao et al. 1994; Shaikh et al. 1995; Rietschel et al. 1996; Kohn et al. 1997). One group reported that the $D_{4.7}$ receptor variant was associated with clozapine response when compared to response to typical antipsychotics (Cohen et al. 1999). Another report, however, found an association between homozygotesfor the dopamine $D_{4.7}$ receptor variant and all types of antipsychotics response in schizophrenic patients (Hwu et al. 1998). Despite the differences in pharmacological profile of the dopamine $D_{4.x}$ receptor variants, the clinical impact of these variants on antipsychotics response are unlikely to be the major factor determining antipsychotics response vs. non-response, considering the large range of doses that are commonly used (Marder et al. 1991).

8.1.4. Gene coding for the D_1 and D_5 receptors

The *DRD1* and the *DRD4* gene have been less studying than the genes coding for the dopamine D2-like receptor family. Screening of the 5'-untranslated region polymorphisms showed that this region of the *DRD1* gene does not have a major role in risk for either schizophrenia or bipolar disorder (Cichon et al. 1996). This finding was confirmed by a study on the *Dde*I polymorphism in the 5'-untranslated region of the *DRD1* gene of 148 schizophrenia patients in Japan. When compared with controls, no significant difference in genotypic counts or allele frequencies was found (Kojima et al. 1999). A postmortem study of tissue from schizophrenic patients found several polymorphisms in *DRD1*gene sequence, but none that would alter protein sequence (Ohara et al. 1993). Genome scans for schizophrenia have identified some areas of interest on chromosome 5, but none of these contain the *DRD1* gene (Crowe and Vieland 1999).

From a pharmacogenetic perspective the *DRD1* is currently of limited interest since there is no established relationship between any dopaminergic drug therapy and this receptor. Because the *DRD1* may modulate aspects of cognition (Williams and Goldman-Rakic 1995), these areas could be explored in future research. However, such studies may be futile in the absence of convincing evidence that polymorphisms or mutations in the *DRD1* gene alter the biological activity or pharmacological profile of the receptor.

For the *DRD5* gene, no association with schizophrenia or bipolar disorder was found in a family linkage study (Asherson et al. 1998), or with schizophrenia in multiplex pedigrees (Ravindranathan et al. 1994; Kalsi et al. 1996). Furthermore, no association with schizophrenia was found in a case-control study (Sobell et al. 1995).

As with the D_1 receptor, there are no therapeutic drugs that specifically target the D_5 receptor, and therefore this receptor is of limited interest for pharmacogenetic studies. However, observations of a functional interaction between the D_5 receptor and γ-amino-butyric acid (A), ($GABA_A$) receptors (Liu et al. 2000), may form the basis to study the genetic interaction of these receptors in disease and drug response.

8.2 Mood disorders

Both unipolar and bipolar forms of mood disorder sometimes feature psychotic symptoms. Some evidence from epidemiological research suggests that psychotic forms of mood disorders specifically might be inheritable. Linkage studies of mood disorders in general have also provided some support for that notion, as have associated studies involving dopamine genes and psychotic mood disorder. Furthermore, some research suggests there might be a genetic connection between schizophrenia and bipolar disorder. Finally, bipolar disorders are often treated with antipsychotics, whose clinical potency is primarily related to dopamine D_2/D_3 receptor affinity.

8.2.1. Gene coding for the D_2 receptor

Most studies of the *DRD2* among Caucasians have failed to provide support for an association of mood disorder and *DRD2* alleles (Hallmayer et al. 1994; Craddock et al. 1995; Grassi et al. 1996; Sasaki et al. 1996; Furlong et al. 1998; Serretti and Smeraldi 1999; Serretti et al. 2000). However, one study reports an association between bipolar disorder and *DRD2* alleles (Massat et al. 2002). A study was performed concerning *DRD2* alleles and lithium response among 100 bipolar patients and 25 patients with major depressive disorder (Serretti et al. 1999), but no association was uncovered. Further investigation of *DRD2* genes (and other genetic systems) and psychiatric drug response would therefore appear warranted.

8.2.2. Gene coding for the D_3 receptor

A possible association between bipolar disorder and allele 1 of the *Bal*I polymorphism has been examined. To date, inconsistent results have been published (Rietschel et al. 1993; Shaikh et al. 1993; Parsian et al. 1995; Piccardi Mp et al. 1997; Chiaroni et al. 2000). Furthermore, A meta-analysis of previous association studies (Elvidge et al. 2001) also revealed no difference in allele distributions between bipolar patients and controls for this polymorphism in ethnically homogeneous samples. In view of this evidence, they conclude that variation at the *BalI* is not an important factor influencing the susceptibility to bipolar disorder.

A study was performed concerning *DRD3* alleles and lithium response among 100 bipolar patients and 25 patients with major depressive disorder (Serretti et al. 1998), but no association was uncovered.

8.2.3. Gene coding for the D_4 receptor

As compared to findings with *DRD2* and *DRD3* alleles, studies of the dopamine *DRD4* have yielded more encouraging results. For example, an increased frequency of the $D_{4.7}$ allele was reported among patients with schizophrenia, psychotic unipolar affective disorder or schizoaffective disorder (Weiss et al. 1996), suggesting a possible role of the *DRD4* gene polymorphism in major psychoses (bipolar disorder and schizophrenia) independently of diagnoses (Serretti et al. 1999); this finding was subsequently replicated in subsequently replicated in patients with bipolar disorder, major depressive disorder, schizophrenia, delusional disorder or non specified psychosis (Serretti et al. 2001). Interestingly, an association between *DRD4* has also been found in bipolar and unipolar Japanese patients, in which a stronger association was found for unipolar mood disorder (Manki et al. 1996). More recent family-based studies report a biased transmission of *DRD4* 4- and 2- repeat alleles in bipolar disorder (Muglia et al. 2002). Again, as with other dopamine receptor gene, no association between lithium response and *DRD4* has been discovered (Serretti et al. 1999). It must be noticed, however, that, as with most studies in psychiatric genetics, studies of *DRD4* genes and psychosis have yielded negative findings as well (Di Bella et al. 1996; Oruc et al. 1997; Bocchetta et al. 1999; Serretti et al. 1999; Serretti et al. 2004). Those studies underscore the need for continued research on the relationships between *DRD4* and mood disorders.

8.2.4. Gene coding for the D_1 and D_5 receptors

Several studies have reported negative results of linkage or association of *DRD1* gene with bipolar disorder (Jensen et al. 1992; Nothen et al. 1992) and a lack of single strand conformational polymorphisms in patients with the disorder (Cichon et al. 1994; Shah et al. 1995). Genome scans for schizophrenia and bipolar disorder have identified some areas of interest on chromosome 5, but none of these contain the *DRD1* gene (Crowe and Vieland 1999). However, a recent study, using the TDT suggests that the *DRD1* may play a role in the etiology of bipolar disorder (Ni et al. 2002).

In the case of the *DRD5* gene, no association with bipolar disorder was found in a family linkage study (Asherson et al. 1998). However, the *DRD5* gene is located within the 4p14–16 region where suggestive linkage findings for bipolar disorder have been noted (Kennedy and Macciardi 1998), but no clear results linking the actual gene to bipolar disorder exist (Asherson et al. 1998; Muir et al. 2001). Furthermore, a family-based association study of bipolar disorder with *DRD5*, using TDT failed to show statistically significant differences between transmitted and not transmitted alleles for any polymorphisms studied (Kirov et al. 1999).

8.3. Attention-deficit hyperactivity disorder

Attention-deficit hyper activity disorder (ADHD) is a childhood onset, clinically heterogeneous disorder characterized by excessive motor activity,

impulsiveness and inattention. Roughly 5%-10% of all school-aged children worldwide have ADHD (Wolraich et al. 1998; Scahill and Schwab-Stone 2000; Swanson et al. 2000; Brown et al. 2001), and it is not uncommon for the condition to persist into adulthood. Although the etiology of ADHD is unknown, family, twin and adoption studies have demonstrated high familiality (Faraone and Biederman 1994; Faraone et al. 1994; Hechtman 1996) due mainly to shared gene effects (Faraone and Biederman 1998). It is widely accepted that several genes, each contributing a small fraction of the total genetic variance, are implicated in ADHD (Comings et al. 1996; Fisher et al. 2002).

Several lines of evidences indicated dopamine system dysfunction in the pathogenesis of ADHD. First, methylphenidate, amphetamine and other psychostimulant drugs that inhibit the activity of the dopamine transporter and increased synaptic levels of dopamine effectively control ADHD symptoms. Second, magnetic resonance imaging and single-photon computerized tomography studies (Ernst et al. 1999) demonstrate abnormalities in neuroanatomical areas with rich dopamine innervations in ADHD children. Third, animal studies strongly suggest that abnormalities in dopamine neurotransmission may be pivotal in motor control (Xu et al. 1994; Accili et al. 1996; Giros et al. 1996; Granon et al. 2000) and other neuropsychological (Goldman-Rakic et al. 2000) functions purportedly affected in ADHD.

Hence, dopamine receptor genes are candidates for the susceptibility ADHD and several groups have evaluated the association between ADHD and dopamine receptor polymorphisms.

8.3.1. Gene coding for the D_2 receptor

In 1996, Coming et al (Comings et al. 1996) reported an association between the *A1* allele of the *DRD2* and ADHD as a Tourette's syndrome associated comorbid behavior. In addition a relationship was found between the severity and accuracy of ADHD diagnosis in subjects with Tourette's syndrome and genetic loading for specific alleles of *DRD2*, dopamine transporter gene (*SLC6A3)* and dopamine beta-hydroxylase gene (*DBH)* (in order of relative importance based on correlation [r^2] analysis). In contrast, Rowe et al (Rowe et al. 1999) found that higher counts of ADHD symptoms (based on *DSM IV* criteria) were associated with decreasing frequencies of the *DRD2*A1* allele. Moreover, a positive correlation was found between the *A2* allele and hyperactive-impulsive symptoms, and less so for the inattentive subtype. However, when parental genotypes were used as controls for population heterogeneity, no significant results were found in Rowe's study. Rowe argues this discrepancy from Coming's results may be the effect of multiple haplotypes with either the *A1* or *A2* alleles in linkage disequilibrium with a functional polymorphism. Other possible explanation includes the heterogeneity between the two samples and the possibility that these results are false-negative findings.

Furthermore, association of ADHD and other behavioral phenotypes with *DRD2* genotype may depend to a significant degree on environmental exposure such as history and family stress (Berman and Noble 1997; Madrid et al. 2001). Firm

conclusions cannot be reached because of the small sample in both studies; larger sample with ethnically match unrelated or family member controls are needed validate (or refute) the authors' findings.

8.3.2. Gene coding for the D_3 receptor

A linkage study of two *DRD3* gene polymorphisms and ADHD (Barr et al. 2000) does not support any linkage between the *DRD3* gene and ADHD. Similarly, a TDT analysis more recent studies of cohort of 150 ADHD children by Payton et al (Payton et al. 2001) and 39 children by Muglia et al (Muglia et al. 2002) using did not identify an association or linkage.

8.3.3. Gene coding for the D_4 receptor

Most molecular genetic studies of *DRD4* and ADHD have focused on the polymorphic 48-bp repeat unit. Of particular interest is the 7-repeat allele, given the low frequency of this allele and the similarly low prevalence of ADHD in Asian population (Leung et al. 1996). Thus a considerable number of studies, including both case-control and family-based association studies, have focused on the 7-repeat *DRD4* polymorphism and ADHD (DiMaio et al. 2003). An additional 120-bp duplication polymorphism identified by Seaman et al (Gorelova et al. 2002) has also been the focus of some recent studies (McCracken et al. 2000; Barr et al. 2001). Although many of the studies identified an association between the *DRD4* polymorphism and ADHD, a number of other studies did not (e.g. (Todd et al. 2001). Faraone et al (Faraone et al. 2001) published a meta-analysis of *DRD4* and ADHD. The data were derived from both family-based data (14 studies, 1,665 probands) and case-control studies (8 studies, 1,266 children with ADHD and 3,068 controls). The odds ratio derived from the case-control studies (which indicates the odds of having the 7-repeat allele among individual with ADHD in the relation without the odds for individuals without ADHD) was 1.9 (95% confidence interval = 1.5-2.2, $p < 0.001$). For family-based studies, the odd ratio (an estimate of the haplotype relative risk, the odds of transmission to individual with ADHD of the 7-repeat allele in relation to other alleles) was 1.4 (95% confidence interval = 1.1-1.6, $p = 0.02$). This implicates *DRD4* in ADHD, highlighting the putative importance of dopamine in its etiology. The meta-analysis conducted by Faraone et al. (2001) indicates also that, despite the small risk conferred to individuals by the 7-repeat allele, this allele may play a role at a population level (population attributable risk percent between 9% and 14%) because of its relatively high population frequency.

Molecular genetic association studies have also examined the extent to which individual ADHD traits are affected by certain genes. Novelty seeking and *DRD4* being is a common example (Paterson et al. 1999), but a link has not been firmly established. In a quantitative trait study, the effects of the 7-repeat allele were examined on specific neuropsychological behaviors believed to be trait markers of ADHD (Swanson et al. 2000). The tasks selected were designed to probe the anterior cingulated gyrus, right dorsolateral prefrontal cortex and

other areas proposed by Posner and Raichle as critical loci in the neuroanatomical network theory of attention (Posner and Raichle 1994). No significant differences between those with the 7-repeat allele and those without were found, suggesting the alleles may identify a subgroup of ADHD but not its cognitive components. However, given the small number of patients included in this study, a false-negative result cannot be ruled out.

Recently, a pharmacogenetic study showed that children with ADHD possessing the 7-repeat allele require higher doses of methylphenidate for symptom improvement and normalization (Hamarman et al. 2004), suggesting a role of *DRD4* gene variants in treatment outcomes.

8.3.4. Gene coding for the D_1 and D_5 receptors

The D_1 receptor is involved, in the prefrontal cortex, in working memory (Goldman-Rakic et al. 2000), an executive function that as been studied and previously found to be disturbed in ADHD children (Karatekin and Asarnow 1998). The first published study of *DRD1* in ADHD did not implicate the receptor in increasing risk for ADHD (Comings et al. 1996). A recent study shows a linkage between *DRD1* and ADHD by examining the inheritance of 4 biallelic *DRD1* polymorphisms in the TDT (Misener et al. 2004).

Two groups independently reported associations between *DRD5* polymorphic loci and ADHD (Daly et al. 1999; Barr et al. 2000). Regression analysis showed that *DRD5* accounted for 0.64% of the genetic variance of their ADHD population (Comings et al. 1996). Trends toward linkage, measured by the TDT, were reported (Tahir et al. 2000; Payton et al. 2001). Recently, two additional susceptibility loci at the *DRD5* and *DBH* were identified in a linkage desiquilibrium mapping study (Hawi et al. 2003). For *DRD5*, three markers, covering a region of approximately 68 kb including the single *DRD5* exon were all associated with disease and thus do not provide localizing information. However, correlation between linkage desiquilibrium was observed between markers for distance of about 22 kb, and this segment included the coding region of *DRD5*. More recently, a TDT study replicated an association of a dinucleotide repeat polymorphism near the *DRD5* gene with ADHD (Kustanovich et al. 2004). These studies suggested a possible role for *DRD5* in increasing the risk for ADHD, but the remain difficult to interpret.

8.4. Alcohol dependence and drug addiction

Abused drugs (alcohol, heroin, cocaine, tetrahydrocannabinol and nicotine) elicit a variety of chronically relapsing disorders by interacting with brain reward systems. All of these drugs increase dopamine levels in the shell of nucleus accumbens, which implicates the neurons of this structure in their hedonic and reinforcing properties. Therefore, dopamine receptor genes are candidates for the susceptibility to alcohol dependence and drug addiction and several groups have evaluated the association between drug dependence and dopamine receptor polymorphisms.

8.4.1. Gene coding for the D_2 receptor

In 1990, Blum et al. reported a significant increase of the frequency of the *Taq*I A allele of *DRD2* (Blum et al. 1990). While a number of subsequent studies in ambulatory alcoholics failed to confirm this association (Bolos et al. 1990; Gelernter et al. 1991), others did (Amadeo et al. 1993; Neiswanger et al. 1995). In a meta-analysis of 15 US and international studies of European (non-Hispanic) Caucasians, consisting of 1,015 alcoholics (more severe and less severe) and 898 controls (unassessed and assessed for alcoholism), alcoholics had a higher prevalence ($p < 10^{-7}$) and frequency ($p < 10^{-5}$) of the *A1* allele than controls (Noble 1998). The prevalence of the *A1* allele was 1.5-fold higher in more severe than less severe alcoholics ($p < 10^{-4}$), whereas unassessed controls had a two-fold higher prevalence of the *A1* allele than assessed controls ($p < 10^{-4}$). Whereas more severe alcoholics had a threefold higher *A1* allelic prevalence than assessed controls ($p < 10^{-10}$), *A1* allelic prevalence was virtually identical in less severe alcoholics and in unassessed controls (Noble 1998). Since the study of several different ethnic groups reveal that the frequency of the *A1* allele is highly variable across group population, it has been suggested that the earlier finding may have been related to spurious population association (Goldman et al. 1993; Reich et al. 1999). An analysis of the COGA (Collaborative Study of the Genetics of Alcoholism) data set has been undertaken with the TDT and the affected-family-based association test (AFBAC) to avoid false positives caused by population stratification (Edenberg et al. 1998). This study found no evidence of linkage or association between the *DRD2* locus and alcohol dependence (Edenberg et al. 1998). Similar conflicting results have been obtained with dependence to other drugs of abuse such as psychostimulants, and tobacco (Noble et al. 1993; Singleton et al. 1998; Anokhin et al. 1999; Gelernter et al. 1999; Aoki et al. 2000; Bierut et al. 2000; Hamajima et al. 2002). One likely explanation is that the *A1* allele is associated with a phenotypic trait associated with drug dependence. It has been proposed that this allele is associated with a 'reward deficiency syndrome' (Noble 1998), that may facilitate drug dependence. Another possibility is that this allele may be related to personality traits such as impulsiveness, which constitutes a risk factor for alcohol dependence (Gorwood et al. 2003). It should be noticed that although the *Taq*I polymorphism of *DRD2* does not influence treatment outcome in primary alcohol dependence (Wiesbeck et al. 2003), this polymorphism may influence the response to bromocriptine in alcoholics (Lawford et al. 1995)

8.4.2. Gene coding for the D_3 receptor

Much less genetic studies have been conducted to implicate *DRD3* polymorphisms in drug dependence. The initial studies performed have found no association between the *Bal*I polymorphism and drug dependence. No association has been found with alcohol dependence and *DRD3* (Gorwood et al. 1995; Parsian et al. 1997; Gorwood et al. 2001; Lee and Ryu 2002). Moreover, *DRD3* polymorphism does not affect treatment outcome in primary alcohol dependence (Wiesbeck et al. 2003). Similarly, no significant association has

been initially found between this polymorphism and opiate (Kotler et al. 1999) or cocaine (Freimer et al. 1996) dependence. Nevertheless, more recently, a significant association has been found between the *DRD3* polymorphism and cocaine dependence (Comings et al. 1999). This effect is significant but account only for 1.64% of the variance of cocaine dependence (Comings et al. 1999). Although the *DRD3* was initially involved in rewarding effects of drug of abuse (Depoortere et al. 2000), recent evidence indicates a role for the *DRD3* in reactivity to drug-associated cues and motivation to take drugs (Katz et al. 2003; Le Foll et al. 2003). In agreement with these recent findings, the *Bal*I polymorphism has been significantly associated with opiate dependence in subjects presenting high sensation seeking scores (Duaux et al. 1998). Further genetic studies should focus on specific phenotype such as the reactivity to drugs associated cues or drug-seeking behavior.

8.4.3. Gene coding for the D_4 receptor

The initial association between the 7-repeat allele and opiate dependence has not been replicated (Alderson et al. 2000; Franke et al. 2000). Recently, no association has been found in alcohol- (Asztely et al. 1997; Parsian et al. 1997; Hill et al. 1999) and methamphetamine-dependent subjects (Tsai et al. 2002). Also the results concerning smoking dependence have not been consistent across studies.

One possible explanation is that the long allele may be associated with personality trait such as novelty seeking (Ebstein et al. 1996; Ebstein et al. 1997). It has been proposed that the association found with this polymorphism and drug dependence is related to the enhanced 'novelty seeking' in drug dependent subjects compared to control. But this is subject to controversy (Gelernter et al. 1997; Gebhardt et al. 2000). Another possibility is that this polymorphism is related to a particular phenotype such as the reactivity to smoking-associated cues (Hutchison et al. 2002). It should be noticed that some studies have found an effect only in particular populations: a significant association between this polymorphism and relapse for smoking has been described in an African American, but not in a Caucasian population (Lerman et al. 1998).

8.4.4. Gene coding for D_1 and D_5 receptors

Several polymorphisms for the gene coding for the *DRD1* have been described (Cichon et al. 1994). Although no association with alcohol dependence have been initially described (Sander et al. 1995), recently, a significant association has been found in a sub-group of alcoholics men (Gorwood et al. 2003). It should be noticed that a gender effect has also been described with the *DRD2* polymorphism and alcoholism (Limosin et al. 2002). The association of the *DRD1* and addictive behavior is also strengthened by the significant association found with smokers (Comings et al. 1997). Further studies should evaluate if the *DRD1* is associated with cocaine or opiate dependence.

The polymorphisms identified in the gene coding for the *DRD5* (Sobell et al. 1995) have almost not been studied in the addiction field. A pilot study reported an association between a microsatellite polymorphism at the *DRD5* gene and the liability to substance abuse: (Vanyukov et al. 1998), but this result has not been replicated so far. Moreover, no association has been found between the *DRD5* gene and smoking dependence (Sullivan et al. 2001). The *DRD1* gene appears a better candidate than the *DRD5* gene for further studies.

8.5. Other neuropsychiatric disorders: Tourette's syndrome, obsessive compulsive disorders, etc.

Other neuropsychiatric disorders have been less extensively studied presumably because their etiology seems to be less related to the dopaminergic systems than the disorders seen before.

8.5.1. Gene coding for the D_2 receptor

There is panoply of studies dealing with other phenotypes and *DRD2* polymorphisms. Associations have been found with Parkinson's disease (Plante-Bordeneuve et al. 1997), idiopathic short stature (Miyake et al. 1999), reduced risk of levodopa-induced dyskinesias in Parkinson's disease (Oliveri et al. 1999), prolonged P300 latency in children (Noble et al. 1994) and prolonged P300 latency in a neuropsychiatric population (Blum et al. 1994). Positive results have also been published with schizoid/avoidant behavior (Blum et al. 1997), psychological defense styles (Comings et al. 1995), brain regional glucose metabolism measured by PET (Noble et al. 1997), visuospatial performance in children (Berman and Noble 1995), family stress (Berman and Noble 1997), tardive dyskinesia in female schizophrenics (Chen et al. 1997), and migraine with aura (Peroutka et al. 1998). Others have reported no association with post-traumatic stress disorder (Gelernter et al. 1999), obsessive-compulsive disorder (Novelli et al. 1994), Tourette's syndrome (Gelernter et al. 1990), Parkinson's disease (Pastor et al. 1999), and panic disorder (Crawford et al. 1995). None of these studies has received confirmation.

8.5.2. Gene coding for the D_3 receptor

For the *DRD3* gene, there are negative reports of association between the *Bal*I polymorphism anorexia nervosa (Bruins-Slot et al. 1998), Tourette's syndrome (Devor et al. 1998), and obsessive-compulsive disorder (Catalano et al. 1994).

8.5.3. Gene coding for the D_4 receptor

Other disorders that have an element of impaired impulse control have been studied in relation to polymorphisms in the *DRD4* gene include obsessive-compulsive disorder and Tourette's syndrome. Suggestive findings that the

DRD4 7-repeat variant was associated with tics in patients with obsessive compulsive disorder (Cruz et al. 1997), have not been replicated (Barr et al. 1996; Di Bella et al. 1996). One report, that greater than 6 repeats in the exon 3 polymorphism confers susceptibility to Parkinson's disease (Ricketts et al. 1998), has been contradicted by another study (Kronenberg et al. 1999).

8.5.4. Gene coding for D_1 and D_5 receptors

Studies examining the *DRD1* coding region have looked for association with Tourette's syndrome, with no association (Thompson et al. 1998). Moreover, analysis of a large kindred excluded close linkage of the *DRD1* to Tourette's syndrome (Gelernter et al. 1993).

9. CONCLUSIONS

The discovery that dopamine interacts with five dopamine receptor subtypes has suscited numerous studies aiming at the characterization of these subtypes and of their physiological role(s). Interesting structural features of dopamine receptor genes have been unraveled. The physiological significance of the two splicing variants of the *DRD2* begins to be understood, with each variant playing a distinct role at the presynaptic and postsynaptic levels. In contrast, the significance of polymorphic variations in *DRD3* and *DRD4* genes, as assessed by genetic studies, remain largely unknown. Likewise, results of genetic studies suggestive of a role of any dopamine receptor subtype in the susceptibility to inherited disorders, in which dopamine has been implicated, remain inconsistent across studies or await confirmation.

The dopamine receptor molecules do not function isolated at the plasma membrane, but forms multimeric complexes with various membrane or cytosolic proteins, which intervene in receptor maintenance, subcellular localization and trafficking and ensure a dynamic regulation of intracellular signaling by various extracellular stimuli. Whereas a great deal of data exists regarding the intracellular pathways activated by dopamine receptor subtypes in heterologous expression systems, the physiological pathways in the brain have been unraveled in a limited number of cases only. This represents the next challenge for the understanding of the cellular functions of dopamine receptors.

Initially, the lack of subtype-selective pharmacological agents has hindered the characterization of the function of dopamine receptor subtypes in living animals. To date, subtype-selective agents, notably antagonists, have been identified for the D_3 and D_4 receptor sybtypes only. There is no subtype-selective antagonists able to discriminate the D_1 from the D_5 receptors, of the D_2 receptor from the D_3 and D_4 receptors. It follows that the effects of drugs binding to both D_2 and D_3 receptors, namely antipsychotic drugs, may exert their effects by interacting with either receptor subtypes. Fortunately, the availability of subtype-targeted knockout mice have provided considerable information about the physiological function of dopamine receptors. Nowadays, it can be rather safely stated that the locomotor activity is controled by D_1 and D_2

receptors in the basal conditions, whereas the locomotor activity induced by psychostimulants, NMDA receptor blockade and novelty are controlled by the D_1, D_3 and D_4 receptors, respectively. The D_2 receptor also plays an essential role in mediating the rewarding effects of drugs of abuse, whereas the D_3 receptor specifically mediates the responses of drug-associated cues. The whole set of anatomical, pharmacological data has now provided some hints about the role of the different dopamine receptor subtypes in the aetiology and treatment of psychiatric disorders. This information may have an impact in the design of novel therapeutic tools.

10. REFERENCES

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R.M., Seibyl, J.P., Bowers, M., van Dyck, C.H., Charney, D.S., Innis, R.B. and Laruelle, M., 1998, Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort, *Am. J. Psychiatry.*, **155**: 761-767.

Accili, D., Fishburn, C.S., Drago, J., Steiner, H., Lachowicz, J.E., Park, B.-H., Gauda, E.B., Lee, E.J., Cool, M.H., Sibley, D.R., Gerfen, C.R., Westphal, H. and Fuchs, S., 1996, A targeted mutation of the D_3 receptor gene is associated with hyperactivity in mice, *Proc. Natl. Acad. Sci. USA*, **93**: 1945-1949.

Accili, D., Fishburn, C.S., Drago, J., Steiner, H., Lachowicz, J.E., Park, B.H., Gauda, E.B., Lee, E.J., Cool, M.H., Sibley, D.R., Gerfen, C.R., Westphal, H. and Fuchs, S., 1996, A targeted mutation of the D_3 dopamine receptor gene is associated with hyperactivity in mice, *Proc Natl Acad Sci USA*, **93**: 1945-1949.

Aghajanian, G.K. and Bunney, B.S., 1977, Pharmacological characterization of dopamine "autoreceptors" by microiontophoretic single-cell recording studies, *Adv Biochem Psychopharmacol*, **16**: 433-438.

Alderson, R.F., Curtis, R., Alterman, A.L., Lindsay, R.M. and DiStefano, P.S., 2000, Truncated TrkB mediates the endocytosis and release of BDNF and neurotrophin-4/5 by rat astrocytes and schwann cells in vitro, *Brain Research*, **871**: 210-222.

Amadeo, S., Abbar, M., Fourcade, M.L., Waksman, G., Leroux, M.G., Madec, A., Selin, M., Champiat, J.C., Brethome, A., Leclaire, Y. and et al., 1993, D_2 dopamine receptor gene and alcoholism, *J Psychiatr Res*, **27**: 173-179.

Ambrosio, A.M., Kennedy, J.L., Macciardi, F., Macedo, A., Valente, J., Dourado, A., Oliveira, C.R. and Pato, C., 2004, Family association study between DRD2 and DRD3 gene polymorphisms and schizophrenia in a Portuguese population, *Psychiatry Res*, **125**: 185-191.

Angrist, B., Sathananthan, G., Wilk, S. and Gershon, S., 1974, Amphetamine psychosis: behavioral and biochemical aspects, *J. Psychiatr. Res.*, **11**: 13-23.

Anokhin, A.P., Todorov, A.A., Madden, P.A., Grant, J.D. and Heath, A.C., 1999, Brain event-related potentials, dopamine D_2 receptor gene polymorphism, and smoking, *Genet Epidemiol*, **17 Suppl 1**: S37-42.

Aoki, C., Wu, K., Elste, A., Len, G.W., Lin, S.Y., McAuliffe, G. and Black, I.B., 2000, Localization of brain-derived neurotrophic factor and TrkB receptors to postsynaptic densities of adult rat cerebral cortex, *J Neurosci Res*, **59**: 454-463.

Aoyama, S., Kase, H. and Borrelli, E., 2000, Rescue of locomotor impairment in dopamine D_2 receptor-deficient mice by an adenosine A_{2A} receptor antagonist, *J Neurosci*, **20**: 5848-5852.

Arinami, T., Gao, M., Hamaguchi, H. and Toru, M., 1997, A functional polymorphism in the promoter region of the dopamine D_2 receptor gene is associated with schizophrenia, *Hum Mol Genet*, **6**: 577-582.

Arinami, T., Itokawa, M., Aoki, J., Shibuya, H., Ookubo, Y., Iwawaki, A., Ota, K., Shimizu, H., Hamaguchi, H. and Toru, M., 1996, Further association study on dopamine D_2 receptor variant S311C in schizophrenia and affective disorders, *Am J Med Genet*, **67**: 133-138.

Arinami, T., Itokawa, M., Enguchi, H., Tagaya, H., Yano, S., Shimizu, H., Hamaguchi, H. and Toru, M., 1994, Association of dopamine D_2 receptor molecular variant with schizophrenia, *Lancet*, **343**: 703-704.

Asghari, V., Sanyal, S., Buchwaldt, S., Paterson, A., Jovanovic, V. and Van Tol, H.H., 1995, Modulation of intracellular cyclic AMP levels by different human dopamine D_4 receptor variants, *J Neurochem*, **65**: 1157-1165.

Ashby, C.R.J., Mousumi, P., Gardner, E.L., Heibreder, C.A. and Hagan, J.J., 2003, Acute administration of the selective D_3 receptor antagonist SB-277011A blocks the acquisition and expression of the conditioned place preference response to heroin in male rats, *Synapse*, **48**: 154-156.

Asherson, P., Mant, R., Holmans, P., Williams, J., Cardno, A., Murphy, K., Jones, L., Collier, D., McGuffin, P. and Owen, M.J., 1996, Linkage, association and mutational analysis of the dopamine D_3 receptor gene in schizophrenia, *Mol. Psychiatry*, **1**: 125-132.

Asherson, P., Mant, R., Williams, N., Cardno, A., Jones, L., Murphy, K., Collier, D.A., Nanko, S., Craddock, N., Morris, S., Muir, W., Blackwood, B., McGuffin, P. and Owen, M.J., 1998, A study of chromosome 4p markers and dopamine D_5 receptor gene in schizophrenia and bipolar disorder, *Mol Psychiatry*, **3**: 310-320.

Asztely, F., Erdemli, G. and Kullmann, D.M., 1997, Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake, *Neuron*, **18**: 281-293.

Baik, J.H., Picetti, R., Saiardi, A., Thiriet, G., Dierich, A., Depaulis, A., Le Meur, M. and Borrelli, E., 1995, Parkinsonian-like locomotor impairment in mice lacking dopamine D_2 receptors, *Nature*, **377**: 424-428.

Baritaki, S., Rizos, E., Zafiropoulos, A., Soufla, G., Katsafouros, K., Gourvas, V. and Spandidos, D.A., 2004, Association between schizophrenia and DRD3 or HTR2 receptor gene variants, *Eur J Hum Genet*, **12**: 535-541.

Barr, C.L., Feng, Y., Wigg, K.G., Schachar, R., Tannock, R., Roberts, W., Malone, M. and Kennedy, J.L., 2001, 5'-untranslated region of the dopamine D_4 receptor gene and attention-deficit hyperactivity disorder, *Am J Med Genet*, **105**: 84-90.

Barr, C.L., Kennedy, J.L., Lichter, J.B., Van Tol, H.H., Wetterberg, L., Livak, K.J. and Kidd, K.K., 1993, Alleles at the dopamine D_4 receptor locus do not contribute to the genetic susceptibility to schizophrenia in a large Swedish kindred, *Am J Med Genet*, **48**: 218-222.

Barr, C.L., Wigg, K.G., Feng, Y., Zai, G., Malone, M., Roberts, W., Schachar, R., Tannock, R. and Kennedy, J.L., 2000, Attention-deficit hyperactivity disorder and the gene for the dopamine D_5 receptor, *Mol Psychiatry*, **5**: 548-551.

Barr, C.L., Wigg, K.G., Wu, J., Zai, C., Bloom, S., Tannock, R., Roberts, W., Malone, M., Schachar, R. and Kennedy, J.L., 2000, Linkage study of two polymorphisms at the dopamine D_3 receptor gene and attention-deficit hyperactivity disorder, *Am J Med Genet*, **96**: 114-117.

Barr, C.L., Wigg, K.G., Zovko, E., Sandor, P. and Tsui, L.C., 1996, No evidence for a major gene effect of the dopamine D_4 receptor gene in the susceptibility to Gilles de la Tourette syndrome in five Canadian families, *Am J Med Genet*, **67**: 301-305.

Basso, A.M., Gallagher, K.B., Bratcher, N.A., Brioni, J.D., Moreland, R.B., Hsieh, G.C., Drescher, K., Fox, G.B., Decker, M.W. and Rueter, L.E., 2005, Antidepressant-like effect of $D_{2/3}$ receptor-, but not D_4 receptor-activation in the rat forced swim test, *Neuro-psychopharmacology*, **30**: 1257-1268.

Bergson, C., Levenson, R., Goldman-Rakic, P.S. and Lidow, M.S., 2003, Dopamine receptor-interacting proteins: the Ca^{2+} connection in dopamine signaling, *Trends Pharmacol Sci*, **24**: 486-492.

Bergson, C., Mrzljak, L., Smiley, J.F., Pappy, M., Levenson, R. and Goldman-Rakic, P.S., 1995, Regional, cellular, and subcellular variations in the distribution of D_1 and D_5 dopamine receptors in primate brain, *J. Neurosci.*, **15**: 7821-7836.

Bermak, J.C., Li, M., Bullock, C. and Zhou, Q.Y., 2001, Regulation of transport of the dopamine D_1 receptor by a new membrane-associated ER protein, *Nat Cell Biol*, **3**: 492-498.

Berman, S.M. and Noble, E.P., 1995, Reduced visuospatial performance in children with the D_2 dopamine receptor A1 allele, *Behav Genet*, **25**: 45-58.

Berman, S.M. and Noble, E.P., 1997, The D_2 dopamine receptor (DRD2) gene and family stress; interactive effects on cognitive functions in children, *Behav Genet*, **27**: 33-43.

Betancur, C., Lepee-Lorgeoux, I., Cazillis, M., Accili, D., Fuchs, S. and Rostene, W., 2001, Neurotensin gene expression and behavioral responses following administration of psychostimulants and antipsychotic drugs in dopamine D_3 receptor deficient mice, *Neuropsychopharmacology*, **24**: 170-182.

Bibb, J.A., Chen, J., Taylor, J.R., Svenningsson, P., Nishi, A., Snyder, G.L., Yan, Z., Sagawa, Z.K., Ouimet, C.C., Nairn, A.C., Nestler, E.J. and Greengard, P., 2001, Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5, *Nature*, **410**: 376-380.

Bibb, J.A., Snyder, G.L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A.A., Tsai, L.H., Kwon, Y.T., Girault, J.A., Czernik, A.J., Huganir, R.L., Hemmings, H.C., Jr., Nairn, A.C. and Greengard, P., 1999, Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons, *Nature*, **402**: 669-671.

Bierut, L.J., Rice, J.P., Edenberg, H.J., Goate, A., Foroud, T., Cloninger, C.R., Begleiter, H., Conneally, P.M., Crowe, R.R., Hesselbrock, V., Li, T.K., Nurnberger, J.I., Jr., Porjesz, B., Schuckit, M.A. and Reich, T., 2000, Family-based study of the association of the dopamine D_2 receptor gene (DRD2) with habitual smoking, *Am J Med Genet*, **90**: 299-302.

Björklund, A. and Lindvall, O., 1984, Dopamine-containing systems in the CNS. Handbook of Chemical Neuroanatomy. A. Björklund and T. Hökfelt, Elsevier, Amsterdam. **2**: 55-122.

Blum, K., Braverman, E.R., Dinardo, M.J., Wood, R.C. and Sheridan, P.J., 1994, Prolonged P300 latency in a neuropsychiatric population with the D_2 dopamine receptor A1 allele, *Pharmacogenetics*, **4**: 313-322.

Blum, K., Braverman, E.R., Wu, S., Cull, J.G., Chen, T.J., Gill, J., Wood, R., Eisenberg, A., Sherman, M., Davis, K.R., Matthews, D., Fischer, L., Schnautz, N., Walsh, W., Pontius, A.A., Zedar, M., Kaats, G. and Comings, D.E., 1997, Association of polymorphisms of dopamine D_2 receptor (DRD2), and dopamine transporter (DAT1) genes with schizoid/avoidant behaviors (SAB), *Mol Psychiatry*, **2**: 239-246.

Blum, K., Noble, E.P., Sheridan, P.J., Montgomery, A., Ritchie, T., Jagadeeswaran, P., Nogami, H., Briggs, A.H. and Cohn, J.B., 1990, Allelic association of human dopamine D_2 receptor gene in alcoholism, *Jama*, **263**: 2055-2060.

Bocchetta, A., Piccardi, M.P., Palmas, M.A., Chillotti, C., Oi, A. and Del Zompo, M., 1999, Family-based association study between bipolar disorder and DRD2, DRD4, DAT, and SERT in Sardinia, *Am J Med Genet*, **88**: 522-526.

Bockaert, J. and Pin, J.P., 1999, Molecular tinkering of G protein-coupled receptors: an evolutionary success, *Embo J*, **18**: 1723-1729.

Bolos, A.M., Dean, M., Lucas-Derse, S., Ramsburg, M., Brown, G.L. and Goldman, D., 1990, Population and pedigree studies reveal a lack of association between the dopamine D_2 receptor gene and alcoholism, *Jama*, **264**: 3156-3160.

Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P. and Schwartz, J.-C., 1997, Induction of dopamine D_3 receptor expression as a mechanism of behavioral sensitization to levodopa, *Proc. Natl. Acad. Sci. USA*, **94**: 3363-3367.

Bordet, R., Ridray, S., Schwartz, J.-C. and Sokoloff, P., 2000, Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats, *Eur. J. Neurosci.*, **12**: 2117-2123.

Boulay, D., Depoortere, R., Rostene, W., Perrault, G. and Sanger, D.J., 1999, Dopamine D_3 receptor agonists produce similar decreases in body temperature and locomotor activity in D_3 receptor-knock-out and wild-type mice, *Neuropharmacology*, **38**: 555-565.

Bouthenet, M.-L., Souil, E., Martres, M.-P., Sokoloff, P., Giros, B. and Schwartz, J.-C., 1991, Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with D_2 receptor mRNA, *Brain Res.*, **564**: 203-219.

Braff, D.L., Grillon, C. and Geyer, M.A., 1992, Gating and habituation of the startle reflex in schizophrenic patients, *Arch. Gen. Psychiatry*, **49**: 206-215.

Breen, G., Brown, J., Maude, S., Fox, H., Collier, D., Li, T., Arranz, M., Shaw, D. and StClair, D., 1999, -141 C del/ins polymorphism of the dopamine receptor D_2 gene is associated with schizophrenia in a British population, *Am J Med Genet*, **88**: 407-410.

Breier, A., Su, T.P., Saunders, R., Carson, R.E., Kolachana, B.S., deBartolimeis, A., Weinberger, D.R., Weisenfeld, N., Malhotra, A.K., Eckelman, W.C. and Pickar, D., 1997, Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method, *Proc. Natl. Acad. Sci.*, **94**: 2569-2574.

Brown, R.T., Freeman, W.S., Perrin, J.M., Stein, M.T., Amler, R.W., Feldman, H.M., Pierce, K. and Wolraich, M.L., 2001, Prevalence and assessment of attention-deficit/hyperactivity disorder in primary care settings, *Pediatrics*, **107**: E43.

Bruins-Slot, L., Gorwood, P., Bouvard, M., Blot, P., Ades, J., Feingold, J., Schwartz, J.-C. and Mouren-Simeoni, M.C., 1998, Lack of association between anorexia nervosa and D_3 dopamine receptor gene, *Biol. Psychiatry*, **43**: 76-78.

Bunney, B.S., Aghajanian, G.K. and Roth, R.H., 1973, Comparison of effects of L-dopa, amphetamine and apomorphine on firing rate of rat dopaminergic neurones, *Nat New Biol*, **245**: 123-125.

Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A. and Civelli, O., 1988, Cloning and expression of a rat D_2 receptor cDNA, *Nature*, **336**: 783-787.

Burris, K.D., Filtz, T.M., Chumpradit, S., Kung, M.P., Foulon, C., Hensler, J.G., Kung, H.F. and Molinoff, P.B., 1994, Characterization of [^{125}I](R)-trans-7-hydroxy-2-[N-propyl-N-(3'-iodo-2'-propenyl)amino] tetralin binding to dopamine D_3 receptors in rat olfactory tubercle, *J. Pharmacol. Exp. Ther.*, **268**: 935-942.

Caille, I., Dumartin, B. and Bloch, B., 1996, Ultrastructural localization of D_1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation, *Brain Res*, **730**: 17-31.

Callier, S., Snapyan, M., Le Crom, S., Prou, D., Vincent, J.D. and Vernier, P., 2003, Evolution and cell biology of dopamine receptors in vertebrates, *Biol Cell*, **95**: 489-502.

Cardno, A.G. and Gottesman, II, 2000, Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics, *Am J Med Genet*, **97**: 12-17.

Carey, R.J., 1991, Chronic L-DOPA treatment in the unilateral 6-OHDA rat: evidence for behavioral sensitization and biochemical tolerance, *Brain Res.*, **568**: 205-214.

Carlsson, A., 1988, The current status of the dopamine hypothesis of schizophrenia, *Neuropsychopharmacol.*, **1**: 179-186.

Catalano, M., Sciuto, G., Di Bella, D., Novelli, E., Nobile, M. and Bellodi, L., 1994, Lack of association between obsessive-compulsive disorder and the dopamine D_3 receptor gene: some preliminary considerations, *Am J Med Genet*, **54**: 253-255.

Centonze, D., Grande, C., Saulle, E., Martin, A.B., Gubellini, P., Pavon, N., Pisani, A., Bernardi, G., Moratalla, R. and Calabresi, P., 2003, Distinct roles of D_1 and D_5 dopamine receptors in motor activity and striatal synaptic plasticity, *J Neurosci*, **23**: 8506-8512.

Centonze, D., Usiello, A., Gubellini, P., Pisani, A., Borrelli, E., Bernardi, G. and Calabresi, P., 2002, Dopamine D_2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D_{2L} receptors, *Neuropsychopharmacology*, **27**: 723-726.

Chang, F.M., Kidd, J.R., Livak, K.J., Pakstis, A.J. and Kidd, K.K., 1996, The world-wide distribution of allele frequencies at the human dopamine D_4 receptor locus, *Hum Genet*, **98**: 91-101.

Chen, C.H., Liu, M.Y., Wei, F.C., Koong, F.J., Hwu, H.G. and Hsiao, K.J., 1997, Further evidence of no association between Ser9Gly polymorphism of dopamine D_3 receptor gene and schizophrenia, *Am. J. Med. Genet. [Neuropsychiatric Genetics]*, **74**: 40-43.

Chen, C.H., Wei, F.C., Koong, F.J. and Hsiao, K.J., 1997, Association of TaqI A polymorphism of dopamine D_2 receptor gene and tardive dyskinesia in schizophrenia, *Biol Psychiatry*, **41**: 827-829.

Chiaroni, P., Azorin, J.M., Dassa, D., Henry, J.M., Giudicelli, S., Malthiery, Y. and Planells, R., 2000, Possible involvement of the dopamine D_3 receptor locus in subtypes of bipolar affective disorder, *Psychiatr Genet*, **10**: 43-49.

Cichon, S., Nothen, M.M., Erdmann, J. and Propping, P., 1994, Detection of four polymorphic sites in the human dopamine D_1 receptor gene (DRD1), *Hum Mol Genet*, **3**: 209.

Cichon, S., Nothen, M.M., Rietschel, M., Korner, J. and Propping, P., 1994, Single-strand conformation analysis (SSCA) of the dopamine D_1 receptor gene (DRD1) reveals no significant mutation in patients with schizophrenia and manic depression, *Biol Psychiatry*, **36**: 850-853.

Cichon, S., Nothen, M.M., Stober, G., Schroers, R., Albus, M., Maier, W., Rietschel, M., Korner, J., Weigelt, B., Franzek, E., Wildenauer, D., Fimmers, R. and Propping, P., 1996, Systematic screening for mutations in the 5'-regulatory region of the human dopamine D_1 receptor (DRD1) gene in patients with schizophrenia and bipolar affective disorder, *Am J Med Genet*, **67**: 424-428.

Civelli, O., Bunzow, J. and Grandy, D.K., 1993, Molecular diversity of the dopamine receptors, *Annual Review of Pharmacology & Toxicology*, **33**: 281-307.

Cohen, A.I., Todd, R.D., Harmon, S. and O' Malley, K.L., 1992, Photoreceptors of mouse retinas possess D_4 receptors coupled to adenylate cyclase, *Proc. Natl. Acad. Sci. USA.*, **89**: 12093-12097.

Cohen, B.M., Ennulat, D.J., Centorrino, F., Matthysse, S., Konieczna, H., Chu, H.M. and Cherkerzian, S., 1999, Polymorphisms of the dopamine D_4 receptor and response to antipsychotic drugs, *Psychopharmacology (Berl)*, **141**: 6-10.

Comings, D.E., Gade, R., Wu, S., Chiu, C., Dietz, G., Muhleman, D., Saucier, G., Ferry, L., Rosenthal, R.J., Lesieur, H.R., Rugle, L.J. and MacMurray, P., 1997, Studies of the potential role of the dopamine D_1 receptor gene in addictive behaviors, *Mol Psychiatry*, **2**: 44-56.

Comings, D.E., Gonzalez, N., Wu, S., Gade, R., Muhleman, D., Saucier, G., Johnson, P., Verde, R., Rosenthal, R.J., Lesieur, H.R., Rugle, L.J., Miller, W.B. and MacMurray, J.P., 1999, Studies of the 48 bp repeat polymorphism of the DRD4 gene in impulsive, compulsive, addictive behaviors: Tourette syndrome, ADHD, pathological gambling, and substance abuse, *Am J Med Genet*, **88**: 358-368.

Comings, D.E., MacMurray, J., Johnson, P., Dietz, G. and Muhleman, D., 1995, Dopamine D_2 receptor gene (DRD2) haplotypes and the defense style questionnaire in substance abuse, Tourette syndrome, and controls, *Biol Psychiatry*, **37**: 798-805.

Comings, D.E., Wu, S., Chiu, C., Ring, R.H., Gade, R., Ahn, C., MacMurray, J.P., Dietz, G. and Muhleman, D., 1996, Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: the additive and subtractive effect of the three dopaminergic genes–DRD2, D beta H, and DAT1, *Am J Med Genet*, **67**: 264-288.

Corrigan, M.H., Gallen, C.C., Bonura, M.L. and Merchant, K.M., 2004, Effectiveness of the selective D_4 antagonist sonepiprazole in schizophrenia: a placebo-controlled trial, *Biol Psychiatry*, **55**: 445-451.

Craddock, N., Roberts, Q., Williams, N., McGuffin, P. and Owen, M.J., 1995, Association study of bipolar disorder using a functional polymorphism (Ser311–>Cys) in the dopamine D_2 receptor gene, *Psychiatr Genet*, **5**: 63-65.

Crawford, F., Hoyne, J., Cai, X., Osborne, A., Poston, D., Zaglul, J., Dajani, N., Walsh, S., Bradley, R., Solomon, R. and Mullan, M., 1996, Dopamine DRD2/Cys311 is not associated with chronic schizophrenia, *Am J Med Genet*, **67**: 483-484.

Crawford, F., Hoyne, J., Diaz, P., Osborne, A., Dorotheo, J., Sheehan, D. and Mullan, M., 1995, Occurrence of the Cys311 DRD2 variant in a pedigree multiply affected with panic disorder, *Am J Med Genet*, **60**: 332-334.

Crocq, M.A., Buguet, A., Bisser, S., Burgert, E., Stanghellini, A., Uyanik, G., Dumas, M., Macher, J.P. and Mayerova, A., 1996, BalI and MspI polymorphisms of the dopamine D_3 receptor gene in African Blacks and Caucasians, *Hum. Heredity*, **46**: 58-60.

Crocq, M.A., Mant, R., Asherson, P., Williams, J., Hode, Y., Mayerova, A., Collier, D., Lannfelt, L., Sokoloff, P., Schwartz, J.-C., Gill, M., Macher, J.-P., McGuffin, P. and Owen, M.J., 1992, Association between schizophrenia and homozygosity at the dopamine D_3 receptor gene, *J. Med. Genet.*, **29**: 858-860.

Crowe, R.R. and Vieland, V., 1999, Report of the Chromosome 5 Workshop of the Sixth World Congress on Psychiatric Genetics, *Am J Med Genet*, **88**: 229-232.

Cruz, C., Camarena, B., King, N., Paez, F., Sidenberg, D., de la Fuente, J.R. and Nicolini, H., 1997, Increased prevalence of the seven-repeat variant of the dopamine D_4 receptor gene in patients with obsessive-compulsive disorder with tics, *Neurosci Lett*, **231**: 1-4.

Cunningham, C.L., Howard, M.A., Gill, S.J., Rubinstein, M., Low, M.J. and Grandy, D.K., 2000, Ethanol-conditioned place preference is reduced in dopamine D_2 receptor-deficient mice, *Pharmacol Biochem Behav*, **67**: 693-699.

Dal Toso, R., Somme, B., Ewert, M., Herb, A., Prichett, D.B., Bach, A., Shivers, B.D. and Seeburg, P.H., 1989, The dopamine D_2 receptor: two molecular forms generated by alternative splicing, *EMBO J.*, **8**: 4025-4034.

Daly, G., Hawi, Z., Fitzgerald, M. and Gill, M., 1999, Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children, *Mol Psychiatry*, **4**: 192-196.

Dao-Castellana, M.H., Paillere-Martinot, M.L., Hantraye, P., Attar-Levy, D., Remy, P., Crouzel, C., Artiges, E., Feline, A., Syrota, A. and Martinot, J.L., 1997, Presynaptic dopaminergic function in the striatum of schizophrenic patients, *Schizophr Res*, **23**: 167-174.

Dearry, A., Gringrich, J.A., Falardeau, P., Fremeau, R.T., Bates, M.D. and Caron, M.G., 1990, Molecular cloning and expression of the gene for a human D_1 dopamine receptor, *Nature*, **347**: 72-76.

Depoortere, R., Perrault, G. and Sanger, D.J., 2000, The D_3 antagonist PNU-99194A potentiates the discriminative cue produced by the D_3 agonist 7-OH-DPAT, *Pharmacology, Biochemistry & Behavior*, **65**: 31-34.

Devor, E.J., Dill-Devor, R.M. and Magee, H.J., 1998, The Bal I and Msp I polymorphisms in the dopamine D_3 receptor gene display, linkage disequilibrium with each other but no association with Tourette syndrome, *Psychiatr Genet*, **8**: 49-52.

Di Bella, D., Catalano, M., Cichon, S. and Nothen, M.M., 1996, Association study of a null mutation in the dopamine D_4 receptor gene in Italian patients with obsessive-compulsive disorder, bipolar mood disorder and schizophrenia, *Psychiatr Genet*, **6**: 119-121.

Di Bella, D., Catalano, M., Strukel, A., Nobile, M., Novelli, E. and Smeraldi, E., 1994, Distribution of the MscI polymorphism of the dopamine D_3 receptor in an Italian psychotic population, *Psychiatr Genet*, **4**: 39-42.

Di Ciano, P., Understood, R.J., Hagan, J.J. and Everitt, B.J., 2003, Attenuation of cue-controlled cocaine-seeking by a selective D_3 receptor antagonist SB-277011-A, *Neuropsychopharmacology*, **28**: 329-338.

Diaz, J., Lévesque, D., Lammers, C.H., Griffon, N., Martres, M.-P., Schwartz, J.-C. and Sokoloff, P., 1995, Phenotypical characterization of neurons expressing the dopamine D_3 receptor, *Neuroscience*, **65**: 731-745.

Diaz, J., Pilon, C., Le Foll, B., Gros, C., Triller, A., Schwartz, J.-C. and Sokoloff, P., 2000, Dopamine D_3 receptors expressed by all mesencephalic dopamine neurons, *J. Neurosci.*, **20**: 8677-8684.

Dickinson, S.D., Sabeti, J., Larson, G.A., Giardina, K., Rubinstein, M., Kelly, M.A., Grandy, D.K., Low, M.J., Gerhardt, G.A. and Zahniser, N.R., 1999, Dopamine D_2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum, *J Neurochem*, **72**: 148-156.

DiMaio, S., Grizenko, N. and Joober, R., 2003, Dopamine genes and attention-deficit hyperactivity disorder: a review, *J Psychiatry Neurosci*, **28**: 27-38.

Dockstader, C.L., Rubinstein, M., Grandy, D.K., Low, M.J. and van der Kooy, D., 2001, The dopamine D_2 receptor is critical in mediating opiate motivation only in opiate-dependent and withdrawn mice, *Eur J Neurosci*, **13**: 995-1001.

Dollfus, S., Campion, D., Vasse, T., Preterre, P., Laurent, C., d'Amato, T., Thibaut, F., Mallet, J. and Petit, M., 1996, Association study between dopamine D_1, D_2, D_3, and D_4 receptor genes and schizophrenia defined by several diagnostic systems, *Biological Psychiatry*, **40**: 419-421.

Drago, J., Gerfen, C.R., Westphal, H. and Steiner, H., 1996, D_1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the striatum, *Neuroscience*, **74**: 813-823.

Duarte, C., Alonso, R., Bichet, N., Cohen, C., Soubrie, P. and Thiebot, M.H., 2003, Blockade by the Cannabinoid CB_1 Receptor Antagonist, Rimonabant (SR141716), of the Potentiation by Quinelorane of Food-Primed Reinstatement of Food-Seeking Behavior, *Neuropsychopharmacology*, **29**: 911-920.

Duaux, E., Gorwood, P., Griffon, N., Bourdel, M.C., Sautel, F., Sokoloff, P., Schwartz, J.C., Ades, J., Loo, H. and Poirier, M.F., 1998, Homozygosity at the dopamine D_3 receptor gene is associated with opiate dependence, *Mol Psychiatry*, **3**: 333-336.

Dubertret, C., Gorwod, P., Ades, J., Feingold, J., Schwartz, J.-C. and Sokoloff, P., 1998, A meta-analysis of DRD3 gene and schizophrenia: ethnic heterogeneity and significant association in caucasians, *Am. J. Med. Genet. [Neuropsychiatric Genetics]*, **81**: 318-322.

Dubertret, C., Gorwood, P., Gouya, L., Deybach, J.C. and Ades, J., 2001, Association and excess of transmission of a DRD2 haplotype in a sample of French schizophrenic patients, *Schizophr Res*, **49**: 203-212.

Dubertret, C., Gouya, L., Hanoun, N., Deybach, J.C., Ades, J., Hamon, M. and Gorwood, P., 2004, The 3' region of the DRD2 gene is involved in genetic susceptibility to schizophrenia, *Schizophr Res*, **67**: 75-85.

Dulawa, S.C., Grandy, D.K., Low, M.J., Paulus, M.P. and Geyer, M.A., 1999, Dopamine D_4 receptor-knock-out mice exhibit reduced exploration of novel stimuli, *J Neurosci*, **19**: 9550-9556.

Dulubova, I., Horiuchi, A., Snyder, G.L., Girault, J.A., Czernik, A.J., Shao, L., Ramabhadran, R., Greengard, P. and Nairn, A.C., 2001, ARPP-16/ARPP-19: a highly conserved family of cAMP-regulated phosphoproteins, *J Neurochem*, **77**: 229-238.

Dumartin, B., Caille, I., Gonon, F. and Bloch, B., 1998, Internalization of D_1 dopamine receptor in striatal neurons in vivo as evidence of activation by dopamine agonists, *J Neurosci*, **18**: 1650-1661.

Durany, N., Thome, J., Palomo, A., Foley, P., Riederer, P. and Cruz-Sanchez, F.F., 1996, Homozygosity at the dopamine D_3 receptor gene in schizophrenic patients, *Neurosci Lett*, **220**: 151-154.

Ebstein, R.P., Macciardi, F., Heresco-Levi, U., Serretti, A., Blaine, D., Verga, M., Nebamov, L., Gur, E., Belmaker, R.H., Avnon, M. and Lerer, B., 1997, Evidence for an association between the dopamine D_3 receptor gene DRD3 and schizophrenia, *Hum. Hered.*, **47**: 6-16.

Ebstein, R.P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., Bennett, E.R., Nemanov, L., Katz, M. and Belmaker, R.H., 1996, Dopamine D_4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking, *Nat Genet*, **12**: 78-80.

Edenberg, H.J., Foroud, T., Koller, D.L., Goate, A., Rice, J., Van Eerdewegh, P., Reich, T., Cloninger, C.R., Nurnberger, J.I., Jr., Kowalczuk, M., Wu, B., Li, T.K., Conneally, P.M., Tischfield, J.A., Wu, W., Shears, S., Crowe, R., Hesselbrock, V., Schuckit, M., Porjesz, B. and Begleiter, H., 1998, A family-based analysis of the association of the dopamine D_2 receptor (DRD2) with alcoholism, *Alcohol Clin Exp Res*, **22**: 505-512.

El-Ghundi, M., George, S.R., Drago, J., Fletcher, P.J., Fan, T., Nguyen, T., Liu, C., Sibley, D.R., Westphal, H. and O'Dowd, B.F., 1998, Disruption of dopamine D_1 receptor gene expression attenuates alcohol-seeking behavior, *Eur J Pharmacol*, **353**: 149-158.

El-Ghundi, M., O'Dowd, B.F., Erclik, M. and George, S.R., 2003, Attenuation of sucrose reinforcement in dopamine D_1 receptor deficient mice, *Eur J Neurosci*, **17**: 851-862.

Elliot, E.E., Sibley, D.R. and Katz, J.L., 2003, Locomotor and discriminative-stimulus effects of cocaine in dopamine D_5 receptor knockout mice, *Psychopharmacology (Berl)*, **169**: 161-168.

Elmer, G.I., Pieper, J.O., Rubinstein, M., Low, M.J., Grandy, D.K. and Wise, R.A., 2002, Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D_2 receptor knock-out mice, *J. Neurosci.*, **15**: RC224.

Elvidge, G., Jones, I., McCandless, F., Asherson, P., Owen, M.J. and Craddock, N., 2001, Allelic variation of a BalI polymorphism in the DRD3 gene does not influence susceptibility to bipolar disorder: results of analysis and meta-analysis, *Am J Med Genet*, **105**: 307-311.

Emamian, E.S., Karayiorgou, M. and Gogos, J.A., 2004, Decreased phosphorylation of NMDA receptor type 1 at serine 897 in brains of patients with Schizophrenia, *J Neurosci*, **24**: 1561-1564.

Engber, T.M., Susel, Z., Juncos, J.L. and Chase, T.N., 1989, Continuous and intermittent levodopa differentially affect rotation induced by D_1 and D_2 dopamine agonists, *Brain Res.*, **552**: 113-118.

Enjalbert, A. and Bockaert, J., 1983, Pharmacological characterization of the D_2 dopamine receptor negatively coupled with adenylate cyclase in rat anterior pituitary, *Mol Pharmacol*, **23**: 576-584.

Ernst, M., Zametkin, A.J., Matochik, J.A., Pascualvaca, D., Jons, P.H. and Cohen, R.M., 1999, High midbrain [18F]DOPA accumulation in children with attention deficit hyperactivity disorder, *Am J Psychiatry*, **156**: 1209-1215.

Falardeau, P., Bedard, P.J. and Di Paolo, T., 1988, Relation between brain dopamine loss and D_2 dopamine receptor density in MPTP monkeys, *Neurosci Lett*, **86**: 225-229.

Fallon, J.H., Loughlin, S.E. and Ribak, C.E., 1983, The islands of Calleja complex of rat basal forebrain. III. Histochemical evidence for a striatopallidal system, *J. Comp. Neurol.*, **218**: 91-120.

Falzone, T.L., Gelman, D.M., Young, J.I., Grandy, D.K., Low, M.J. and Rubinstein, M., 2002, Absence of dopamine D_4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear, *Eur J Neurosci*, **15**: 158-164.

Faraone, S.V. and Biederman, J., 1994, Is attention deficit hyperactivity disorder familial?, *Harv Rev Psychiatry*, **1**: 271-287.

Faraone, S.V. and Biederman, J., 1998, Neurobiology of attention-deficit hyperactivity disorder, *Biol Psychiatry*, **44**: 951-958.

Faraone, S.V., Biederman, J. and Milberger, S., 1994, An exploratory study of ADHD among second-degree relatives of ADHD children, *Biol Psychiatry*, **35**: 398-402.

Faraone, S.V., Doyle, A.E., Mick, E. and Biederman, J., 2001, Meta-analysis of the association between the 7-repeat allele of the dopamine D_4 receptor gene and attention deficit hyperactivity disorder, *Am J Psychiatry*, **158**: 1052-1057.

Farnebo, L.O. and Hamberger, B., 1971, Drug-induced changes in the release of ^{3}H-monoamines from field stimulated rat brain slices, *Acta Physiol Scand Suppl*, **371**: 35-44.

Fiorentini, C., Gardoni, F., Spano, P., Di Luca, M. and Missale, C., 2003, Regulation of dopamine D_1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors, *J Biol Chem*, **278**: 20196-20202.

Fishburn, C., Bellili, D., David, C., Carmon, S. and Fuchs, S., 1993, A novel short isoform of the D_3 dopamine receptor generated by alternative splicing in the third cytoplasmic loop, *J Biol Chem*, **268**: 5872-5878.

Fisher, S.E., Francks, C., McCracken, J.T., McGough, J.J., Marlow, A.J., MacPhie, I.L., Newbury, D.F., Crawford, L.R., Palmer, C.G., Woodward, J.A., Del'Homme, M., Cantwell, D.P., Nelson, S.F., Monaco, A.P. and Smalley, S.L., 2002, A genomewide scan for loci involved in attention-deficit/hyperactivity disorder, *Am J Hum Genet*, **70**: 1183-1196.

Frances, H., Le Foll, B., Diaz, J., Smirnova, M. and Sokoloff, P., 2004a, Role of DRD3 in morphine-induced conditioned place preference using drd3-knockout mice, *Neuroreport*, **15**: 2245-2249.

Frances, H., Smirnova, M., Leriche, L. and Sokoloff, P., 2004b, Dopamine D_3 receptor ligands modulate the acquisition of morphine-conditioned place preference, *Psychopharmacology (Berl)*, **175**: 127-133.

Franke, P., Nothen, M.M., Wang, T., Knapp, M., Lichtermann, D., Neidt, H., Sander, T., Propping, P. and Maier, W., 2000, DRD4 exon III VNTR polymorphism-susceptibility factor for heroin dependence? Results of a case-control and a family-based association approach, *Mol Psychiatry*, **5**: 101-104.

Freedman, S.B., Patel, S., Marwood, R., Emms, F., Seabrook, G.R., Knowles, M.R. and McAllister, G., 1994, Expression and pharmacological characterization of the human D_3 dopamine receptor, *J. Pharmacol. Exp. Ther.*, **268**: 417-426.

Freimer, M., Kranzler, H., Satel, S., Lacobelle, J., Skipsey, K., Charney, D. and Gelernter, J., 1996, No association between D_3 dopamine receptor (DRD3) alleles and cocaine dependence, *Addiction Biology*, **1**: 281-287.

Fremeau, R.T., Duncan, G.E., Fornaretto, M.G., Dearry, A., Gingrich, J.A., Brees, G.R. and Caron, M.G., 1991, Localization of D_1 dopamine receptor mRNA in brain supports a role in cognitive, affective and neuroendocrine aspects of dopaminergic neurotransmission, *Proc. Natl. Acad. Sci. USA*, **88**: 3772-3776.

Furlong, R.A., Coleman, T.A., Ho, L., Rubinsztein, J.S., Walsh, C., Paykel, E.S. and Rubinsztein, D.C., 1998, No association of a functional polymorphism in the dopamine D_2 receptor promoter region with bipolar or unipolar affective disorders, *Am J Med Genet*, **81**: 385-387.

Gaitonde, E.J., Morris, A., Sivagnanasundaram, S., McKenna, P.J., Hunt, D.M. and Mollon, J.D., 1996, Assessment of association of D_3 dopamine receptor MscI polymorphism with schizophrenia: analysis of symptom ratings, family history, age at onset, and movement disorders, *Am J Med Genet*, **67**: 455-458.

Gebhardt, C., Leisch, F., Schussler, P., Fuchs, K., Stompe, T., Sieghart, W., Hornik, K., Kasper, S. and Aschauer, H.N., 2000, Non-association of dopamine D_4 and D_2 receptor genes with personality in healthy individuals, *Psychiatr Genet*, **10**: 131-137.

Gefvert, O., Lindstrom, L.H., Waters, N., Waters, S., Carlsson, A. and Tedroff, J., 2003, Different corticostriatal patterns of L-DOPA utilization in patients with untreated schizophrenia and patients treated with classical antipsychotics or clozapine, *Scand J Psychol*, **44**: 289-292.

Gelernter, J., Kennedy, J.L., Grandy, D.K., Zhou, Q.Y., Civelli, O., Pauls, D.L., Pakstis, A., Kurlan, R., Sunahara, R.K., Niznik, H.B. and et al., 1993, Exclusion of close linkage of Tourette's syndrome to D_1 dopamine receptor, *Am. J. Psychiatry*, **150**: 449-453.

Gelernter, J., Kranzler, H., Coccaro, E., Siever, L., New, A. and Mulgrew, C.L., 1997, D_4 dopamine-receptor (DRD4) alleles and novelty seeking in substance-dependent, personality-disorder, and control subjects, *Am J Hum Genet*, **61**: 1144-1152.

Gelernter, J., Kranzler, H. and Satel, S.L., 1999, No association between D_2 dopamine receptor (DRD2) alleles or haplotypes and cocaine dependence or severity of cocaine dependence in European- and African-Americans, *Biol Psychiatry*, **45**: 340-345.

Gelernter, J., O'Malley, S., Risch, N., Kranzler, H.R., Krystal, J., Merikangas, K., Kennedy, J.L. and Kidd, K.K., 1991, No association between an allele at the D_2 dopamine receptor gene (DRD2) and alcoholism, *Jama*, **266**: 1801-1807.

Gelernter, J., Pakstis, A.J., Pauls, D.L., Kurlan, R., Gancher, S.T., Civelli, O., Grandy, D. and Kidd, K.K., 1990, Gilles de la Tourette syndrome is not linked to D_2-dopamine receptor, *Arch Gen Psychiatry*, **47**: 1073-1077.

Gelernter, J., Southwick, S., Goodson, S., Morgan, A., Nagy, L. and Charney, D.S., 1999, No association between D_2 dopamine receptor (DRD2) "A" system alleles, or DRD2 haplotypes, and posttraumatic stress disorder, *Biol Psychiatry*, **45**: 620-625.

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma, F.J. and Sibley, D.R., 1990, D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons, *Science*, **250**: 1429-1432.

Gines, S., Hillion, J., Torvinen, M., Le Crom, S., Casado, V., Canela, E.I., Rondin, S., Lew, J.Y., Watson, S., Zoli, M., Agnati, L.F., Verniera, P., Lluis, C., Ferre, S., Fuxe, K. and Franco, R., 2000, Dopamine D_1 and adenosine A_1 receptors form functionally interacting heteromeric complexes, *Proc Natl Acad Sci USA*, **97**: 8606-8611.

Giros, B., Jaber, M., Jones, S.R., Wightman, R.M. and Caron, M.G., 1996, Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter, *Nature*, **379**: 606-612.

Giros, B., Martres, M.-P., Pilon, C., Sokoloff, P. and Schwartz, J.-C., 1991, Shorter variants of the D_3 dopamine receptor produced through various patterns of alternative splicing, *Bioch. Biophys. Res. Commun.*, **176**: 1584-1592.

Giros, B., Sokoloff, P., Martres, M.-P., Riou, J.-F., Emorine, L.J. and Schwartz, J.-C., 1989, Alternative splicing directs the expression of two D_2 dopamine receptor isoforms, *Nature*, **342**: 923-926.

Glatt, S.J., Faraone, S.V. and Tsuang, M.T., 2003, Meta-analysis identifies an association between the dopamine D_2 receptor gene and schizophrenia, *Mol Psychiatry*, **8**: 911-915.

Glatt, S.J., Faraone, S.V. and Tsuang, M.T., 2003, Schizophrenia is not associated with DRD4 48-base-pair-repeat length or individual alleles: results of a meta-analysis, *Biol Psychiatry*, **54**: 629-635.

Glickstein, S.B. and Schmauss, C., 2001, Dopamine receptor functions: lessons from knockout mice, *Pharmacol Ther*, **91**: 63-83.

Goldman-Rakic, P.S., Muly, E.C.R. and Williams, G.V., 2000, D_1 receptors in prefrontal cells and circuits, *Brain Res. Rev.*, **31**: 295-301.

Goldman, D., Brown, G.L., Albaugh, B., Robin, R., Goodson, S., Trunzo, M., Akhtar, L., Lucas-Derse, S., Long, J., Linnoila, M. and et al., 1993, DRD2 dopamine receptor genotype, linkage disequilibrium, and alcoholism in American Indians and other populations, *Alcohol Clin Exp Res*, **17**: 199-204.

Golimbet, V.E., Aksenova, M.G., Nosikov, V.V., Orlova, V.A. and Kaleda, V.G., 2003, Analysis of the linkage of the Taq1A and Taq1B loci of the dopamine D_2 receptor gene with schizophrenia in patients and their siblings, *Neurosci Behav Physiol*, **33**: 223-225.

Gorelova, N., Seamans, J.K. and Yang, C.R., 2002, Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex, *J Neurophysiol*, **88**: 3150-3166.

Gorwood, P., Limosin, F., Batel, P., Duaux, E., Gouya, L. and Ades, J., 2001, The genetics of addiction: alcohol-dependence and D_3 dopamine receptor gene, *Pathol Biol (Paris)*, **49**: 710-717.

Gorwood, P., Limosin, F., Batel, P., Hamon, M., Ades, J. and Boni, C., 2003, The A9 allele of the dopamine transporter gene is associated with delirium tremens and alcohol-withdrawal seizure, *Biol Psychiatry*, **53**: 85-92.

Gorwood, P., Martres, M.P., Ades, J., Sokoloff, P., Noble, E.P., Geijer, T., Blum, K., Neiman, J., Jonsson, E., Feingold, J. and et al., 1995, Lack of association between alcohol-

dependence and D_3 dopamine receptor gene in three independent samples, *Am J Med Genet*, **60**: 529-531.

Granon, S., Passetti, F., Thomas, K.L., Dalley, J.W., Everitt, B.J. and Robbins, T.W., 2000, Enhanced and impaired attentional performance after infusion of D_1 dopaminergic receptor agents into rat prefrontal cortex, *J Neurosci*, **20**: 1208-1215.

Grassi, E., Mortilla, M., Amaducci, L., Pallanti, S., Pazzagli, A., Galassi, F., Guarnieri, B.M., Petruzzi, C., Bolino, F., Ortenzi, L., Nistico, R., De Cataldo, S., Rossi, A. and Sorbi, S., 1996, No evidence of linkage between schizophrenia and D_2 dopamine receptor gene locus in Italian pedigrees, *Neurosci Lett*, **206**: 196-198.

Griffon, N. C. P., Schwartz, J.-C. and Sokoloff, P., 1995, The preferential dopamine D_3 receptor ligand (+)UH 232 is a partial agonist, *Eur. J. Pharmacol.*, **282**: R3-R4.

Griffon, N., Crocq, M.A., Pilon, C., Martres, M.-P., Mayerova, A., Uyanik, G., Burgert, E., Duval, F., Macher, J.-P., Javoy-Agid, F., Tamminga, C.A., Schwartz, J.-C. and Sokoloff, P., 1996, Dopamine D_3 receptor gene: organization, transcript variants and polymorphism associated with schizophrenia, *Am. J. Med. Genet. [Neuropsychiatric Genetics]*, **67**: 58-70.

Griffon, N., Pilon, C., Sautel, F., Schwartz, J.-C. and Sokoloff, P., 1996, Antipsychotics with inverse agonist activity at the dopamine D_3 receptor, *J. Neural. Transm.*, **103**: 1166-1175.

Griffon, N., Pilon, C., Sautel, F., Schwartz, J.C. and Sokoloff, P., 1997, Two intracellular pathways for the dopamine D_3 receptor: opposite and synergistic interactions with cyclic AMP, *J Neurochem*, **68**: 1-9.

Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.-C. and Sokoloff, P., 2001, BDNF controls dopamine D_3 receptor expression and triggers behavioural sensitization, *Nature*, **411**: 86-89.

Gurevich, E.V., Bordelon, Y., Shapiro, R.M., Arnold, S.E., Gur, R.E. and Joyce, J.N., 1997, Mesolimbic dopamine D_3 receptors and use of antipsychotics in patients with schizophrenia, *Arch. Gen. Psychiatry*, **54**: 225-232.

Hall, H., Halldin, C., Dijkstra, D., Wikstrom, H., Wise, L.D., Pugsley, T.A., Sokoloff, P., Pauli, S., Farde, L. and Sedvall, G., 1996, Autoradiographic localisation of D_3 dopamine receptors in human brain using the selective receptor agonist (+)-[^{3}H]PD 128907, *Psychopharmacology*, **128**: 240-247.

Hallmayer, J., Maier, W., Schwab, S., Ertl, M.A., Minges, J., Ackenheil, M., Lichtermann, D. and Wildenauer, D.B., 1994, No evidence of linkage between the dopamine D_2 receptor gene and schizophrenia, *Psychiatry Res*, **53**: 203-215.

Hamajima, N., Ito, H., Matsuo, K., Saito, T., Tajima, K., Ando, M., Yoshida, K. and Takahashi, T., 2002, Association between smoking habits and dopamine receptor D_2 taqI A A2 allele in Japanese males: a confirmatory study, *J Epidemiol*, **12**: 297-304.

Hamarman, S., Fossella, J., Ulger, C., Brimacombe, M. and Dermody, J., 2004, Dopamine receptor 4 (DRD4) 7-repeat allele predicts methylphenidate dose response in children with attention deficit hyperactivity disorder: a pharmacogenetic study, *J Child Adolesc Psychopharmacol*, **14**: 564-574.

Harano, M., 1997, Ser-311-Cys polymorphism of the dopamine D_2 receptor gene and schizophrenia–an analysis of schizophrenic patients in Fukuoka, *Kurume Med J*, **44**: 201-208.

Harrison, P.J. and Owen, M.J., 2003, Genes for schizophrenia? Recent findings and their pathophysiological implications, *Lancet*, **361**: 417-419.

Hattori, M., Nanko, S., Dai, X.Y., Fukuda, R. and Kazamatsuri, H., 1994, Mismatch PCR RFLP detection of DRD2 Ser311Cys polymorphism and schizophrenia, *Biochem Biophys Res Commun*, **202**: 757-763.

Hawi, Z., Lowe, N., Kirley, A., Gruenhage, F., Nothen, M., Greenwood, T., Kelsoe, J., Fitzgerald, M. and Gill, M., 2003, Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci, *Mol Psychiatry*, **8**: 299-308.

Hawi, Z., McCabe, U., Straub, R.E., O'Neill, A., Kendler, K.S., Walsh, D. and Gill, M., 1998, Examination of new and reported data of the DRD3/MscI polymorphism: no support for the proposed association with schizophrenia, *Mol Psychiatry*, **3**: 150-155.

Hechtman, L., 1996, Families of children with attention deficit hyperactivity disorder: a review, *Can J Psychiatry*, **41**: 350-360.

Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H. and Surmeier, D.J., 2000, D_2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+

currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade, *J Neurosci*, **20**: 8987-8995.

Herroelen, L., De Backer, J.-P., Wilczak, N., Flamez, A., Vauquelin, G. and De Keyser, J., 1994, Autoradiographic distribution of D_3-type dopamine receptors in human brain using [^{3}H]7-hydroxy-N,N-di-*n*-propyl-2-aminotetralin, *Brain Res.*: 222-228.

Hersch, S.M., Ciliax, B.J., Gutekunst, C.A., Rees, H.D., Heilman, C.J., Yung, K.K., Bolam, J.P., Ince, E., Yi, H. and Levey, A.I., 1995, Electron microscopic analysis of D_1 and D_2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents, *J Neurosci*, **15**: 5222-5237.

Herve, D., Levi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J.P., Glowinski, J. and Girault, J.A., 1993, G(olf) and Gs in rat basal ganglia: possible involvement of G(olf) in the coupling of dopamine D_1 receptor with adenylyl cyclase, *J Neurosci*, **13**: 2237-2248.

Hietala, J., Syvalahti, E., Vuorio, K., Nagren, K., Lehikoinen, P., Ruotsalainen, U., Rakkolainen, V., Lehtinen, V. and Wegelius, U., 1994, Striatal D_2 dopamine receptor characteristics in neuroleptic-naive schizophrenic patients studied with positron emission tomography, *Arch Gen Psychiatry*, **51**: 116-123.

Hill, S.Y., Zezza, N., Wipprecht, G., Locke, J. and Neiswanger, K., 1999, Personality traits and dopamine receptors (D_2 and D_4): linkage studies in families of alcoholics, *Am J Med Genet*, **88**: 634-641.

Hillion, J., Canals, M., Torvinen, M., Casado, V., Scott, R., Terasmaa, A., Hansson, A., Watson, S., Olah, M.E., Mallol, J., Canela, E.I., Zoli, M., Agnati, L.F., Ibanez, C.F., Lluis, C., Franco, R., Ferre, S. and Fuxe, K., 2002, Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D_2 receptors, *J Biol Chem*, **277**: 18091-18097.

Himei, A., Koh, J., Sakai, J., Inada, Y., Akabame, K. and Yoneda, H., 2002, The influence on the schizophrenic symptoms by the DRD2Ser/Cys311 and -141C Ins/Del polymorphisms, *Psychiatry Clin Neurosci*, **56**: 97-102.

Holmes, A., Hollon, T.R., Gleason, T.C., Liu, Z., Dreiling, J., Sibley, D.R. and Crawley, J.N., 2001, Behavioral characterization of dopamine D_5 receptor null mutant mice, *Behav Neurosci*, **115**: 1129-1144.

Holmes, A., Lachowicz, J.E. and Sibley, D.R., 2004, Phenotypic analysis of dopamine receptor knockout mice; recent insights into the functional specificity of dopamine receptor subtypes, *Neuropharmacology*, **47**: 1117-1134.

Hong, C.J., Lee, Y.L., Sim, C.B. and Hwu, H.G., 1997, Dopamine D_4 receptor variants in Chinese sporadic and familial schizophrenics, *Am J Med Genet*, **74**: 412-415.

Hori, H., Ohmori, O., Shinkai, T., Kojima, H. and Nakamura, J., 2001, Association analysis between two functional dopamine D_2 receptor gene polymorphisms and schizophrenia, *Am J Med Genet*, **105**: 176-178.

Huang, Q., Zhou, D., Chase, K., Gusella, J.F., Aronin, N. and Difiglia, M., 1992, Immunohistochemical localization of the D_1 dopamine receptor in rat brain reveals its axonal transport, pre- and post-synaptic localization, and prevalence in the basal ganglia, limbic system and thalamic reticular nucleus, *Proc. Natl. Acad. Sci. USA*, **89**: 11988-11992.

Hutchison, K.E., LaChance, H., Niaura, R., Bryan, A. and Smolen, A., 2002, The DRD4 VNTR polymorphism influences reactivity to smoking cues, *J Abnorm Psychol*, **111**: 134-143.

Hwu, H.G., Hong, C.J., Lee, Y.L., Lee, P.C. and Lee, S.F., 1998, Dopamine D_4 receptor gene polymorphisms and neuroleptic response in schizophrenia, *Biol Psychiatry*, **44**: 483-487.

Inada, T., Sugita, T., Dobashi, I., Inagaki, A., Kitao, Y., Matsuda, G., Kato, S., Takano, T., Yagi, G. and Asai, M., 1995, Dopamine D_3 receptor gene polymorphism and the psychiatric symptoms seen in first-break schizophrenic patients, *Psychiatr Genet*, **5**: 113-116.

Jeanneteau, F., Diaz, J., Sokoloff, P. and Griffon, N., 2004, Interactions of GIPC with Dopamine D_2, D_3 but not D_4 Receptors Define a Novel Mode of Regulation of G Protein-coupled Receptors, *Mol Biol Cell*, **15**: 696-705.

Jeanneteau, F., Guillin, O., Diaz, J., Griffon, N. and Sokoloff, P., 2004, GIPC recruits GAIP (RGS19) to attenuate dopamine D_2 receptor signaling, *Mol. Biol. Cell.*, **15**: 4926-4937.

Jensen, S., Plaetke, R., Holik, J., Hoff, M., O'Connell, P., Reimherr, F., Wender, P., Zhou, Q.Y., Civelli, O., Litt, M. and et al., 1992, Linkage analysis of the D_1 dopamine receptor gene and manic depression in six families, *Hum Hered*, **42**: 269-275.

Jentsch, J. and Roth, R., 1999, The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia, *Neuropsychopharmacology*, **20**: 201-225.

Jönsson, E., Lannfelt, L., Sokoloff, P., Schwartz, J.C. and Sedvall, G., 1993, Lack of association in a Bal I polymorphism in the dopamine D_3 receptor gene in schizophrenia, *Acta Psychiatrica Scandinavica*, **87**: 345-347.

Jonsson, E.G., Flyckt, L., Burgert, E., Crocq, M.A., Forslund, K., Mattila-Evenden, M., Rylander, G., Asberg, M., Nimgaonkar, V.L., Edman, G., Bjerkenstedt, L., Wiesel, F.A. and Sedvall, G.C., 2003, Dopamine D_3 receptor gene Ser9Gly variant and schizophrenia: association study and meta-analysis, *Psychiatr Genet*, **13**: 1-12.

Jonsson, E.G., Kaiser, R., Brockmoller, J., Nimgaonkar, V.L. and Crocq, M.A., 2004, Meta-analysis of the dopamine D_3 receptor gene (DRD3) Ser9Gly variant and schizophrenia, *Psychiatr Genet*, **14**: 9-12.

Jonsson, E.G., Nothen, M.M., Neidt, H., Forslund, K., Rylander, G., Mattila-Evenden, M., Asberg, M., Propping, P. and Sedvall, G.C., 1999, Association between a promoter polymorphism in the dopamine D_2 receptor gene and schizophrenia, *Schizophr Res*, **40**: 31-36.

Jonsson, E.G., Sedvall, G.C., Nothen, M.M. and Cichon, S., 2003, Dopamine D_4 receptor gene (DRD4) variants and schizophrenia: meta-analyses, *Schizophr Res*, **61**: 111-119.

Jonsson, E.G., Sillen, A., Vares, M., Ekholm, B., Terenius, L. and Sedvall, G.C., 2003, Dopamine D_2 receptor gene Ser311Cys variant and schizophrenia: association study and meta-analysis, *Am J Med Genet B Neuropsychiatr Genet*, **119**: 28-34.

Joseph, J.D., Wang, Y.-M., Miles, P.R., Budygin, E.A., Picetti, R., Gainetdinov, R.R., Caron, M.G. and Wightman, R.M., 2002, Dopamine autoreceptor regulation of release and uptake in mouse slices in the absence of D_3 receptors, *Neuroscience*, **112**: 39-49.

Jung, M.Y., Skryabin, B.V., Arai, M., Abbondanzo, S., Fu, D., Brosius, J., Robakis, N.K., Polites, H.G., Pintar, J.E. and Schmauss, C., 1999, Potentiation of the D_2 mutant motor phenotype in mice lacking dopamine D_2 and D_3 receptors, *Neuroscience*, **91**: 911-924.

Kalivas, P.W. and Stewart, J., 1991, Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity, *Brain Res. Rev.*, **16**: 223-244.

Kalsi, G., Sherrington, R., Mankoo, B.S., Brynjolfsson, J., Sigmundsson, T., Curtis, D., Read, T., Murphy, P., Butler, R., Petursson, H. and Gurling, H.M., 1996, Linkage study of the D_5 dopamine receptor gene (DRD5) in multiplex Icelandic and English schizophrenia pedigrees, *Am J Psychiatry*, **153**: 107-109.

Kampman, O., Anttila, S., Illi, A., Lehtimaki, T., Mattila, K.M., Roivas, M. and Leinonen, E., 2003, Dopamine receptor D_2 -141C Insertion/Deletion polymorphism in a Finnish population with schizophrenia, *Psychiatry Res*, **121**: 89-92.

Kaneshima, M., Higa, T., Nakamoto, H. and Nagamine, M., 1997, An association study between the Cys311 variant of dopamine D_2 receptor gene and schizophrenia in the Okinawan population, *Psychiatry Clin Neurosci*, **51**: 379-381.

Kapelski, P., Czerski, P.M., Godlewski, S., Dmitrzak-Weglarz, M. and Hauser, J., 2002, [Lack of association between 141C Ins/Del promoter polymorphism of DRD2 gene and schizophrenia], *Psychiatr Pol*, **36**: 413-419.

Kapur, S. and Mamo, D., 2003, Half a century of antipsychotics and still a central role for dopamine D_2 receptors, *Prog Neuropsychopharmacol Biol Psychiatry*, **27**: 1081-1090.

Karatekin, C. and Asarnow, R.F., 1998, Working memory in childhood-onset schizophrenia and attention-deficit/hyperactivity disorder, *Psychiatry Res*, **80**: 165-176.

Katz, J.L., Chausmer, A.L., Elmer, G.I., Rubinstein, M., Low, M.J. and Grandy, D.K., 2003, Cocaine-induced locomotor activity and cocaine discrimination in dopamine D_4 receptor mutant mice, *Psychopharmacology (Berl)*, **170**: 108-114.

Kebabian, J.W. and Calne, D.B., 1979, Multiple receptors for dopamine, *Nature*, **277**: 93-96.

Kehr, W., Carlsson, A., Lindqvist, M., Magnusson, T. and Atack, C., 1972, Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity, *J Pharm Pharmacol*, **24**: 744-747.

Kelly, M.A., Rubinstein, M., Phillips, T.J., Lessov, C.N., Burkhart-Kasch, S., Zhang, G., Bunzow, J.R., Fang, Y., Gerhardt, G.A., Grandy, D.K. and Low, M.J., 1998, Locomotor activity in D_2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations, *J Neurosci*, **18**: 3470-3479.

Kennedy, J.L., Billett, E.A., Macciardi, F.M., Verga, M., Parsons, T.J., Meltzer, H.Y., Lieberman, J. and Buchanan, J.A., 1995, Association study of dopamine D_3 receptor gene and schizophrenia, *Am J Med Genet*, **60**: 558-562.

Kennedy, J.L. and Macciardi, F.M., 1998, Chromosome 4 workshop, *Psychiatr Genet*, **8**: 67-71.

Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A. and de la Calle, A., 2000, Dopamine D_5 receptors of rat and human brain, *Neuroscience*, **100**: 689-699.

Khan, Z.U., Mrzljak, L., Gutierrez, A., de la Calle, A. and Goldman-Rakic, P.S., 1998, Prominence of the dopamine D_2 short isoform in dopaminergic pathways, *Proc. Natl. Acad. Sci. U S A*, **95**: 7731-7736.

Kirov, G., Jones, I., McCandless, F., Craddock, N. and Owen, M.J., 1999, Family-based association studies of bipolar disorder with candidate genes involved in dopamine neurotransmission: DBH, DAT1, COMT, DRD2, DRD3 and DRD5, *Mol Psychiatry*, **4**: 558-565.

Kobayashi, M., Iaccarino, C., Saiardi, A., Heidt, V., Bozzi, Y., Picetti, R., Vitale, C., Westphal, H., Drago, J. and Borrelli, E., 2004, Simultaneous absence of dopamine D_1 and D_2 receptor-mediated signaling is lethal in mice, *Proc Natl Acad Sci U S A*, **101**: 11465-11470.

Koeltzow, T.E., Xu, M., Cooper, D.C., Hu, X.T., Tonegawa, S., Wolf, M.E. and White, F.J., 1998, Alterations in dopamine release but not dopamine autoreceptor function in dopamine D_3 receptor mutant mice, *J. Neurosci.*, **18**: 2231-2238.

Kohn, Y., Ebstein, R.P., Heresco-Levy, U., Shapira, B., Nemanov, L., Gritsenko, I., Avnon, M. and Lerer, B., 1997, Dopamine D_4 receptor gene polymorphisms: relation to ethnicity, no association with schizophrenia and response to clozapine in Israeli subjects, *Eur Neuropsychopharmacol*, **7**: 39-43.

Kojima, S., Nakayama, T., Kuwajima, G., Suzuki, H. and Sakata, T., 1999, TrkB mutant lacking the amino-terminal half of the extracellular portion acts as a functional brain-derived neurotrophic factor receptor, *Biochimica et Biophysica Acta*, **1420**: 104-110.

Koob, G.F., 1992, Dopamine, addiction and reward, *Sem. Neurosci.*, **4**: 139-148.

Kotler, M., Cohen, H., Kremer, I., Mel, H., Horowitz, R., Ohel, N., Gritsenko, I., Nemanov, L., Katz, M. and Ebstein, R., 1999, No association between the serotonin transporter promoter region (5-HTTLPR) and the dopamine D_3 receptor (BalI D3DR) polymorphisms and heroin addiction, *Mol Psychiatry*, **4**: 313-314.

Kramer, M.S., Last, B., Getson, A. and Reines, S.A., 1997, The effects of a selective D_4 dopamine receptor antagonist (L-745,870) in acutely psychotic inpatients with schizophrenia, *Arch. Gen. Psychiatry*, **54**: 567-572.

Kronenberg, M.F., Menzel, H.J., Ebersbach, G., Wenning, G.K., Luginger, E., Gollner, M., Ransmayr, G., Utermann, G., Poewe, W. and Kronenberg, F., 1999, Dopamine D_4 receptor polymorphism and idiopathic Parkinson's disease, *Eur J Hum Genet*, **7**: 397-400.

Kruzich, P.J., Suchland, K.L. and Grandy, D.K., 2004, Dopamine D_4 receptor-deficient mice, congenic on the C57BL/6J background, are hypersensitive to amphetamine, *Synapse*, **53**: 131-139.

Kustanovich, V., Ishii, J., Crawford, L., Yang, M., McGough, J.J., McCracken, J.T., Smalley, S.L. and Nelson, S.F., 2004, Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5, *Mol Psychiatry*, **9**: 711-717.

L'hirondel, M., Cheramy, A., Godeheu, G., Artaud, F., Saiardi, A., Borrelli, E. and Glowinski, J., 1998, Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D_2 receptor-deficient mice., *Brain Res.*, **792**: 253-262.

Lahti, R.A., Roberts, R.C., Cochrane, E.V., Primus, R.J., Gallager, D.W., Conley, R.R. and Tamminga, C.A., 1998, Direct determination of dopamine D_4 receptors in normal and schizophrenic postmortem brain tissue: a [3H]NGD-94-1 study, *Mol Psychiatry*, **3**: 528-533.

Lahti, R.A., Roberts, R.C., Conley, R.R., Cochrane, E.V., Mutin, A. and Tamminga, C.A., 1996, D2-type dopamine receptors in postmortem human brain sections from normal and schizophrenic subjects, *Neuroreport*, **7**: 1945-1948.

Lajiness, M.E., Chio, C.L. and Huff, R.M., 1995, Signaling mechanisms of D_2, D_3 and D_4 dopamine receptors determined in transfected cells, *Clin. Pharmacol.*, **18**: S25-S33.

Lammers, C.H., Diaz, J., Schwartz, J.-C. and Sokoloff, P., 2000, Selective increase of dopamine D_3 receptor gene expression as a common effect of chronic antidepressant treatments, *Mol. Psychiatry*, **5**: 378-388.

Lane, H.Y., Lee, C.C., Chang, Y.C., Lu, C.T., Huang, C.H. and Chang, W.H., 2004, Effects of dopamine D_2 receptor Ser311Cys polymorphism and clinical factors on risperidone efficacy for positive and negative symptoms and social function, *Int J Neuropsychopharmacol*, **7**: 461-470.

Lannfelt, L., Sokoloff, P., Martres, M.P., Pilon, C., Giros, B., Jönsson, E., Sedvall, G. and Schwartz, J.C., 1992, Amino acid substitution in the dopamine D_3 receptor as a useful polymorphism for investigating psychiatric disorders, *Psychiatric Genetics*, **2**: 249-256.

Laruelle, M., Abi-Dargham, A., Gil, R., Kegeles, L. and Innis, R., 1999, Increased dopamine transmission in schizophrenia: relationship to illness phases, *Biol Psychiatry*, **46**: 56-72.

Laruelle, M., Abi-Dargham, A., van Dyck, C.H., Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., Baldwin, R.M., Seibyl, J.P., Krystal, J.H., Charney, D.S. and Innis, R.B., 1996, Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects, *Proc. Natl. Acad. Sci. USA*, **93**: 9235-9240.

Lattanzi, L., Dell'Osso, L., Cassano, P., Pini, S., Rucci, P., Houck, P.R., Gemignani, A., Battistini, G., Bassi, A., Abelli, M. and Cassano, G.B., 2002, Pramipexole in treatment-resistant depression: a 16-week naturalistic study, *Bipolar Disord*, **4**: 307-314.

Laurent, C., Bodeau-Pean, S., Campion, D., d'Amato, T., Jay, M., Dollfus, S., Thibault, F., Petit, M., Samolyk, D., Martinez, M. and et al., 1994, No major role for the dopamine D_2 receptor Ser–>Cys311 mutation in schizophrenia, *Psychiatr Genet*, **4**: 229-230.

Lawford, B.R., Young, R.M., Rowell, J.A., Qualichefski, J., Fletcher, B.H., Syndulko, K., Ritchie, T. and Noble, E.P., 1995, Bromocriptine in the treatment of alcoholics with the D_2 dopamine receptor A1 allele, *Nat Med*, **1**: 337-341.

Le Foll, B., Schwartz, J.-C. and Sokoloff, P., 2000, Dopamine D_3 receptor agents as potential new medications for drug addiction, *Eur. Psychiatry*, **15**: 140-146.

Le Foll, B., Francès, H., Diaz, J., Schwartz, J.-C. and Sokoloff, P., 2002, Role of the dopamine D_3 receptor in reactivity to cocaine-associated cues in mice, *Eur. J. Neurosci.*, **15**: 2016-2026.

Le Foll, B., Diaz, J. and Sokoloff, P., 2003a, Increased dopamine D_3 receptor expression accompanying behavioural sensitization to nicotine in rats, *Synapse*, **47**: 176-183.

Le Foll, B., Schwartz, J.-C. and Sokoloff, P., 2003b, Disruption of nicotine conditioning by dopamine D_3 receptor ligands, *Mol. Psychiatry*, **8**: 225-230.

Le Foll, B., Diaz, J. and Sokoloff, P., 2005a, Neuroadaptations to hyperdopaminergia indopamine D_3 receptor-deficient mice, *Life Sci.*, **76**: 1281-1296.

Le Foll, B., Goldberg, S.R. and Sokoloff, P., 2005b, Dopamine D_3 receptor and drug dependence: effect on reward or beyond?, *Neuropharmacology,* in press.

Le Foll, B., Sokoloff, P., Stark, H. and Goldberg, S.R., 2005c, Dopamine D_3 receptor ligands block nicotine-induced conditioned place preferences through a mechanism that does not involve discriminative-stimulus or antidepressant-like effects, *Neuropsychopharmacology*, **30**: 720-730.

Le Moine, C. and Bloch, B., 1995, D_1 and D_2 dopamine receptor gene expression in the striatum: sensitive cRNA probes demonstrate prominent segregation of D_1 and D_2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum, *J. Comp. Neurol.*, **355**: 418-426.

Lecrubier, Y., 2003, A partial D_3 receptor agonist in schizophrenia, *Eur. Neuropsychopharmacol.*, **13 Suppl. 4**: S167-S168.

Lee, F.J., Xue, S., Pei, L., Vukusic, B., Chery, N., Wang, Y., Wang, Y.T., Niznik, H.B., Yu, X.M. and Liu, F., 2002, Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D_1 receptor, *Cell*, **111**: 219-230.

Lee, M.S. and Ryu, S.H., 2002, No association between the dopamine D_3 receptor gene and Korean alcohol dependence, *Psychiatr Genet*, **12**: 173-176.

Lerer, B., Segman, R.H., Fangerau, H., Daly, A.K., Basile, V.S., Cavallaro, R., Aschauer, H.N., McCreadie, R.G., Ohlraun, S., Ferrier, N., Masellis, M., Verga, M., Scharfetter, J., Rietschel, M., Lovlie, R., Levy, U.H., Meltzer, H.Y., Kennedy, J.L., Steen, V.M. and Macciardi, F., 2002, Pharmacogenetics of tardive dyskinesia: combined analysis of 780 patients supports association with dopamine D_3 receptor gene Ser9Gly polymorphism, *Neuropsychopharmacology*, **27**: 105-119.

Leriche, L., Schwartz, J.C. and Sokoloff, P., 2003, The dopamine D_3 receptor mediates locomotor hyperactivity induced by NMDA receptor blockade, *Neuropharmacology*, **45**: 174-181.

Lerman, C., Caporaso, N., Main, D., Audrain, J., Boyd, N.R., Bowman, E.D. and Shields, P.G., 1998, Depression and self-medication with nicotine: the modifying influence of the dopamine D_4 receptor gene, *Health Psychol*, **17**: 56-62.

Leung, P.W., Luk, S.L., Ho, T.P., Taylor, E., Mak, F.L. and Bacon-Shone, J., 1996, The diagnosis and prevalence of hyperactivity in Chinese schoolboys, *Br J Psychiatry*, **168**: 486-496.

Lévesque, D., Diaz, J., Pilon, C., Martres, M.-P., Giros, B., Souil, E., Schott, D., Morgat, J.-L., Schwartz, J.-C. and Sokoloff, P., 1992, Identification, characterization and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]-hydroxy-N,N di-n-propyl-2-aminotetralin, *Proc. Natl. Acad. Sci. USA*, **89**: 8155-8159.

Lévesque, D., Martres, M.-P., Diaz, J., Griffon, N., Lammers, C.H., Sokoloff, P. and Schwartz, J.-C., 1995, A paradoxical regulation of the dopamine D_3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons, *Proc. Natl. Acad. Sci. USA*, **92**: 1719-1723.

Levey, A.I., Hersch, S.M., Rye, D.B., Sunahara, R.K., Niznik, H.B., Kitt, C.A., Price, D.L., Maggid, R., Brann, M.R. and Ciliax, B.J., 1993, Localization of D_1 and D_2 dopamine receptors in brain with subtype-specific antibodies, *Proc. Natl. Acad. Sci. USA*, **90**: 8861-8865.

Lezcano, N., Mrzljak, L., Eubanks, S., Levenson, R., Goldman-Rakic, P. and Bergson, C., 2000, Dual signaling regulated by calcyon, a D_1 dopamine receptor interacting protein, *Science*, **287**: 1660-1664.

Li, T., Arranz, M., Aitchison, K.J., Bryant, C., Liu, X., Kerwin, R.W., Murray, R., Sham, P. and Collier, D.A., 1998, Case-control, haplotype relative risk and transmission disequilibrium analysis of a dopamine D_2 receptor functional promoter polymorphism in schizophrenia, *Schizophr Res*, **32**: 87-92.

Lichter, J.B., Barr, C.L., Kenney, J.L., Van Tol, H.H.M., Kidd, K.K. and Livak, K.J., 1993, A hypervariable segment in the human dopamine D_4 (DRD4) gene, *Hum. Mol. Genet.*, **2**: 767-773.

Limosin, F., Gorwood, P., Loze, J.Y., Dubertret, C., Gouya, L., Deybach, J.C. and Ades, J., 2002, Male limited association of the dopamine receptor D_2 gene TaqI a polymorphism and alcohol dependence, *Am J Med Genet*, **112**: 343-346.

Lindstrom, L.H., Gefvert, O., Hagberg, G., Lundberg, T., Bergstrom, M., Hartvig, P. and Langstrom, B., 1999, Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(beta-11C) DOPA and PET, *Biol Psychiatry*, **46**: 681-688.

Liu, F., Wan, Q., Pristupa, Z.B., Yu, X.M., Wang, Y.T. and Niznik, H.B., 2000, Direct protein-protein coupling enables cross-talk between dopamine D_5 and gamma-aminobutyric acid A receptors, *Nature*, **403**: 274-280.

Liu, K., Bergson, C., Levenson, R. and Schmauss, C., 1994, On the origin of mRNA encoding the truncated dopamine D_3-type receptor D_{3nf} and detection of D_{3nf}-like immunoreactivity in human brain, *J. Biol. Chem.*, **269**: 29220-29226.

Liu, Y.F., Civelli, O., Zhou, Q.Y. and Albert, P.R., 1992, Cholera toxin-sensitive 3',5'-cyclic adenosine monophosphate and calcium signals of the human dopamine-D_1 receptor: selective potentiation by protein kinase A, *Mol Endocrinol*, **6**: 1815-1824.

Lundstrom, K. and Turpin, M.P., 1996, Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D_3 receptor with the Semliki virus system, *Bioch. Biophys. Res. Comm.*, **225**: 1068-1072.

Macciardi, F., Verga, M., Kennedy, J.L., Petronis, A., Bersani, G., Pancheri, P. and Smeraldi, E., 1994, An association study between schizophrenia and the dopamine receptor genes DRD3 and DRD4 using haplotype relative risk, *Hum. Hered.*, **44**: 328-336.

Madrid, G.A., MacMurray, J., Lee, J.W., Anderson, B.A. and Comings, D.E., 2001, Stress as a mediating factor in the association between the DRD2 TaqI polymorphism and alcoholism, *Alcohol*, **23**: 117-122.

Maj, J., Dziedzicka-Wasylewska, M., Rogoz, E. and Rogoz, Z., 1998, Effect of antidepressant drugs administered repeatedly on the dopamine D_3 receptors in the rat brain, *Eur. J. Pharmacol.*, **351**: 31-37.

Maldonado, R., Saiardi, A., Valverde, O., Samad, T.A., Roques, B.P. and Borrelli, E., 1997, Absence of opiate rewarding effects in mice lacking dopamine D_2 receptors, *Nature*, **388**: 586-589.

Malhotra, A.K., Goldman, D., Buchanan, R.W., Rooney, W., Clifton, A., Kosmidis, M.H., Breier, A. and Pickar, D., 1998, The dopamine D_3 receptor (DRD3) Ser9Gly polymorphism and

schizophrenia: a haplotype relative risk study and association with clozapine response, *Mol Psychiatry*, **3**: 72-75.

Manki, H., Kanba, S., Muramatsu, T., Higuchi, S., Suzuki, E., Matsushita, S., Ono, Y., Chiba, H., Shintani, F., Nakamura, M., Yagi, G. and Asai, M., 1996, Dopamine D_2, D_3 and D_4 receptor and transporter gene polymorphisms and mood disorders, *J Affect Disord*, **40**: 7-13.

Mant, R., Williams, J., Asherson, P., Parfitt, E., McGuffin, P. and Owen, M.J., 1994, The relationship between homozygosity at the dopamine D_3 receptor gene and schizophrenia, *Am. J. Med. Genet.*, **54**: 21-26.

Marder, S.R., Wirshing, W.C. and Van Putten, T., 1991, Drug treatment of schizophrenia. Overview of recent research, *Schizophr Res*, **4**: 81-90.

Martres, M.P., Sokoloff, P., Giros, B. and Schwartz, J.C., 1992, Effects of dopamine transmission interruption on the D_2 receptor isoforms in various cerebral tissues, *J. Neurochem.*, **58**: 673-679.

Massat, I., Souery, D., Del-Favero, J., Van Gestel, S., Serretti, A., Macciardi, F., Smeraldi, E., Kaneva, R., Adolfsson, R., Nylander, P.O., Blackwood, D., Muir, W., Papadimitriou, G.N., Dikeos, D., Oruc, L., Segman, R.H., Ivezic, S., Aschauer, H., Ackenheil, M., Fuchshuber, S., Dam, H., Jakovljevic, M., Peltonen, L., Hilger, C., Hentges, F., Staner, L., Milanova, V., Jazin, E., Lerer, B., Van Broeckhoven, C. and Mendlewicz, J., 2002, Positive association of dopamine D_2 receptor polymorphism with bipolar affective disorder in a European Multicenter Association Study of affective disorders, *Am J Med Genet*, **114**: 177-185.

McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., Liu, A. and Nelson, S.F., 2000, Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D_4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD), *Mol Psychiatry*, **5**: 531-536.

McDonald, W.M., Sibley, D.R., Kilpatrick, B.F. and Caron, M.G., 1984, Dopaminergic inhibition of adenylate cyclase correlates with high affinity agonist binding to anterior pituitary D_2 dopamine receptors, *Mol Cell Endocrinol*, **36**: 201-209.

McGuffin, P., Owen, M.J. and Farmer, A.E., 1995, Genetic basis of schizophrenia, *Lancet*, **346**: 678-682.

McNamara, F.N., Clifford, J.J., Tighe, O., Kinsella, A., Drago, J., Croke, D.T. and Waddington, J.L., 2003, Congenic D_{1A} dopamine receptor mutants: ethologically based resolution of behavioural topography indicates genetic background as a determinant of knockout phenotype, *Neuropsychopharmacology*, **28**: 86-99.

Meador-Woodruff, J.H., Grandy, D.K., Van Tol, H.H.M., Damask, S.P., Little, K.Y., Civelli, O. and Watson, S.J., 1994, Dopamine receptor gene expression in the human medial temporal lobe, *Neuropsychopharmacol.*, **10**: 239-248.

Meador-Woodruff, J.H., Haroutunian, V., Powchik, P., Davidson, M., Davis, K.L. and Watson, S.J., 1997, Dopamine receptor transcript expression in striatum and prefrontal and occipital cortex, *Arch. Gen. Psychiatry*, **54**: 1089-1095.

Meador-Woodruff, J.H., Mansour, A., Bunzow, J.R., Van Tol, H.H.M., Watson, S.J. and Civelli, O., 1989, Distribution of D_2 dopamine receptor mRNA in rat brain, *Proc. Natl. Acad. Sci.*, **86**: 7625-7628.

Meador-Woodruff, J.H., Mansour, A., Grandy, D.K., Damask, S.P., Civelli, O. and Watson Jr, S.J., 1992, Distribution of D_5 dopamine receptor mRNA in the rat brain, *Neurosci. Lett.*, **145**: 209-212.

Mei, Y.A., Griffon, N., Buquet, C., Martres, M.-P., Vaudry, H., Schwartz, J.-C., Sokoloff, P. and Cazin, L., 1995, Activation of dopamine D_4 receptor inhibits an L-type calcium current in cerebellar granule cells, *Neuroscience*, **68**: 107-116.

Mercuri, N.B., Saiardi, A., Picetti, R., Calabresi, P., Bernardi, G. and Borelli, E., 1997, Loss of autoreceptor function in dopaminergic neurons from dopamine D_2 receptor deficient mice, *Neuroscience*, **79**: 323-327.

Millan, M.J., Gobert, A., Newman-Tancredi, A., Lejeune, F., Cussac, D., Rivet, J.-M., Audinot, V., Dubuffet, T. and Lavielle, G., 2000, S33084, a novel potent, selective, and competitive antagonist at dopamine D_3-receptors: I receptorial, electrophysiological and neurochemical profile compared with GR218,231 and L741,626, *J. Pharmacol. Exp. Ther.*, **293**: 1048-1062.

Millan, M.J., Peglion, J.-L., Vian, J., Rivet, J.-M., Brocco, M., Gobert, A., A., N.-T., Dacquet, C., Bervoets, K., Giradon, S., Jacques, V., Chaput, C. and Audinot, V., 1995, Functional correlates of dopamine D_3 receptor activation in the rat *in vivo* and their modulation by the selective antagonist, (+) S-14297 : I. activation of postsynaptic D_3 receptors mediates hypothermia, whereas blockade of D_2 receptors elicits prolactin secretion and catalepsy, *J. Pharmacol. Exp. Ther*, **275**: 885-898.

Miner, L.L., Drago, J., Chamberlain, P.M., Donovan, D. and Uhl, G.R., 1995, Retained cocaine conditioned place preference in D_1 receptor deficient mice, *Neuroreport*, **6**: 2314-2316.

Misener, V.L., Luca, P., Azeke, O., Crosbie, J., Waldman, I., Tannock, R., Roberts, W., Malone, M., Schachar, R., Ickowicz, A., Kennedy, J.L. and Barr, C.L., 2004, Linkage of the dopamine receptor D_1 gene to attention-deficit/hyperactivity disorder, *Mol Psychiatry*, **9**: 500-509.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M. and Caron, M.G., 1998, Dopamine receptors: from structure to function, *Physiol Rev*, **78**: 189-225.

Miyake, H., Nagashima, K., Onigata, K., Nagashima, T., Takano, Y. and Morikawa, A., 1999, Allelic variations of the D_2 dopamine receptor gene in children with idiopathic short stature, *J Hum Genet*, **44**: 26-29.

Mohn, A.R., Gainetdinov, R.R., Caron, M.G. and Koller, B.H., 1999, Mice with reduced NMDA receptor expression display behaviors related to schizophrenia, *Cell*, **98**: 427-436.

Moises, H.W., Gelernter, J., Giuffra, L.A., Zarcone, V., Wetterberg, L., Civelli, O., Kidd, K.K., Cavalli-Sforza, L.L., Grandy, D.K., Kennedy, J.L. and et al., 1991, No linkage between D_2 dopamine receptor gene region and schizophrenia, *Arch Gen Psychiatry*, **48**: 643-647.

Monsma, F.J., Mahan, L.C., McVittie, L.D., Gerfen, C.R. and Sibley, D.R., 1991, Molecular cloning and expression of a D_1 dopamine receptor linked to adenylyl cyclase activation, *Proc. Natl. Acad. Sci.*, **87**: 6723-6727.

Morissette, M., Goulet, M., Grondin, R., Blanchet, P., Bedard, P.J., Di Paolo, T. and Levesque, D., 1998, Associative and limbic regions of monkey striatum express high levels of dopamine D_3 receptors: effects of MPTP and dopamine agonist replacement therapies, *Eur. J. Neurosci.*, **10**: 2565-2573.

Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R. and Goldman-Rakic, P.S., 1996, Localization of dopamine D_4 receptors in GABAergic neurons of the primate brain, *Nature*, **381**: 245-248.

Muglia, P., Jain, U. and Kennedy, J.L., 2002, A transmission disequilibrium test of the Ser9/Gly dopamine D_3 receptor gene polymorphism in adult attention-deficit hyperactivity disorder, *Behav Brain Res*, **130**: 91-95.

Muglia, P., Petronis, A., Mundo, E., Lander, S., Cate, T. and Kennedy, J.L., 2002, Dopamine D_4 receptor and tyrosine hydroxylase genes in bipolar disorder: evidence for a role of DRD4, *Mol Psychiatry*, **7**: 860-866.

Muir, W.J., Thomson, M.L., McKeon, P., Mynett-Johnson, L., Whitton, C., Evans, K.L., Porteous, D.J. and Blackwood, D.H., 2001, Markers close to the dopamine D_5 receptor gene (DRD5) show significant association with schizophrenia but not bipolar disorder, *Am J Med Genet*, **105**: 152-158.

Muriel, M.P., Bernard, V., Levey, A.I., Laribi, O., Abrous, D.N., Agid, Y., Bloch, B. and Hirsch, E.C., 1999, Levodopa induces a cytoplasmic localization of D_1 dopamine receptors in striatal neurons in Parkinson's disease, *Ann Neurol*, **46**: 103-111.

Nanko, S., Hattori, M., Dai, X.Y., Fukuda, R. and Kazamatsuri, H., 1994, DRD2 Ser311/Cys311 polymorphism in schizophrenia, *Lancet*, **343**: 1044.

Nanko, S., Hattori, M., Ikeda, K., Sasaki, T., Kazamatsuri, H. and Kuwata, S., 1993, Dopamine D_4 receptor polymorphism and schizophrenia, *Lancet*, **341**: 689-690.

Nanko, S., Sasaki, T., Fukuda, R., Hattori, M., Dai, X.Y., Kazamatsuri, H., Kuwata, S., Juji, T. and Gill, M., 1993, A study of the association between schizophrenia and the dopamine D_3 receptor gene, *Hum Genet*, **92**: 336-338.

Narita, M., Mizuo, K., Mizoguchi, H., Sakata, M., Tseng, L.F. and Suzuki, T., 2003, Molecular evidence for the functional role of dopamine D_3 receptor in the morphine-induced rewarding effect and hyperlocomotion, *J Neurosci*, **23**: 1006-1012.

Neiswanger, K., Hill, S.Y. and Kaplan, B.B., 1995, Association and linkage studies of the TAQI A1 allele at the dopamine D_2 receptor gene in samples of female and male alcoholics, *Am J Med Genet*, **60**: 267-271.

Neve, K.A., DuRand, C.J. and Teeter, M.M., 2003, Structural analysis of the mammalian D_2, D_3 and D_4 dopamine receptors. Dopamine Receptors and Transporters. Function, Imaging and

Clinical Implication (Second edition). A. Sidhu, M. Laruelle and P. Vernier. New York, Marcel Dekker, Inc.**:** 77-144.

Ng, G.Y., O'Dowd, B.F., Caron, M., Dennis, M., Brann, M.R. and George, S.R., 1994, Phosphorylation and palmitoylation of the human D_{2L} dopamine receptor in Sf9 cells, *J Neurochem*, **63**: 1589-1595.

Ni, X., Trakalo, J.M., Mundo, E., Macciardi, F.M., Parikh, S., Lee, L. and Kennedy, J.L., 2002, Linkage disequilibrium between dopamine D_1 receptor gene (DRD1) and bipolar disorder, *Biol Psychiatry*, **52**: 1144-1150.

Nimchinsky, E.A., Hof, P.R., Janssen, W.G., Morrison, J.H. and Schmauss, C., 1997, Expression of dopamine D_3 receptor dimers and tetramers in brain and in transfected cells, *J Biol Chem*, **272**: 29229-29237.

Nimgaonkar, V.L., Sanders, A.R., Ganguli, R., Zhang, X.R., Brar, J., Hogge, W., Fann, W.E., Patel, P.I. and Chakravarti, A., 1996, Association of schizophrenia and the dopamine D_3 receptor gene locus in two independent samples, *Am. J. Med. Genet. [Neuropsychiatric Genetics]*, **67**: 505-514.

Noble, E.P., 1998, The D_2 dopamine receptor gene: a review of association studies in alcoholism and phenotypes, *Alcohol*, **16**: 33-45.

Noble, E.P., Berman, S.M., Ozkaragoz, T.Z. and Ritchie, T., 1994, Prolonged P300 latency in children with the D_2 dopamine receptor A1 allele, *Am J Hum Genet*, **54**: 658-668.

Noble, E.P., Blum, K., Khalsa, M.E., Ritchie, T., Montgomery, A., Wood, R.C., Fitch, R.J., Ozkaragoz, T., Sheridan, P.J., Anglin, M.D. and et al., 1993, Allelic association of the D_2 dopamine receptor gene with cocaine dependence, *Drug Alcohol Depend*, **33**: 271-285.

Noble, E.P., Gottschalk, L.A., Fallon, J.H., Ritchie, T.L. and Wu, J.C., 1997, D_2 dopamine receptor polymorphism and brain regional glucose metabolism, *Am J Med Genet*, **74**: 162-166.

Nöthen, M., Körner, J., Lannfelt, L., Sokoloff, P., Schwartz, J.-C., Lanczik, M., Rietchel, M., Cichon, S., Kramer, R., Fimmers, R., Möller, H.J., Beckman, H. and Propping, P., 1993, Lack of association between schizophrenia and alleles of the dopamine D_1, D_2, D_3 and D_4 receptor loci, *Psychiatr. Genet.*, **3**: 89-94.

Nöthen, M.M., Erdmann, J., Korner, J., Lanczik, M., Fritze, J., Fimmers, R., Grandy, D.K., O'Dowd, B. and Propping, P., 1992, Lack of association between dopamine D_1 and D_2 receptor genes and bipolar affective disorder, *Am J Psychiatry*, **149**: 199-201.

Nöthen, M.M., Wildenauer, D., Cichon, S., Albus, M., Maier, W., Minges, J., Lichtermann, D., Bondy, B., Rietschel, M., Korner, J. and et al., 1994, Dopamine D_2 receptor molecular variant and schizophrenia, *Lancet*, **343**: 1301-1302.

Novelli, E., Nobile, M., Diaferia, G., Sciuto, G. and Catalano, M., 1994, A molecular investigation suggests no relationship between obsessive-compulsive disorder and the dopamine D_2 receptor, *Neuropsychobiology*, **29**: 61-63.

O'Malley, K.L., Harmon, S., Tang, L. and Todd, R.D., 1992, The rat dopamine D_4 receptor sequence, gene structure, and demonstration of expression in the cardiovascular system, *The New Biologist*, **2**: 137-146.

Oak, J.N., Oldenhof, J. and Van Tol, H.H., 2000, The dopamine D(4) receptor: one decade of research, *Eur J Pharmacol*, **405**: 303-327.

Ohara, K., Nagai, M., Tani, K., Nakamura, Y. and Ino, A., 1998, Functional polymorphism of -141C Ins/Del in the dopamine D_2 receptor gene promoter and schizophrenia, *Psychiatry Res*, **81**: 117-123.

Ohara, K., Nakamura, Y., Xie, D.-W., Ishigaki, T., Deng, Z.-L., Tani, K., Zhang, H.-Y., Kondo, N., Liu, J.-C., Miyasato, K. and Ohara, K., 1996, Polymorphisms of dopamine D2-like (D_2, D_3 and D_4) receptors in schizophrenia, *Biol. Psychiatry*, **40**: 1209-1217.

Ohara, K., Ulpian, C., Seeman, P., Sunahara, R.K., Van Tol, H.H. and Niznik, H.B., 1993, Schizophrenia: dopamine D_1 receptor sequence is normal, but has DNA polymorphisms, *Neuropsychopharmacology*, **8**: 131-135.

Okuyama, Y., Ishiguro, H., Toru, M. and Arinami, T., 1999, A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia, *Biochem Biophys Res Commun*, **258**: 292-295.

Oliveri, R.L., Annesi, G., Zappia, M., Civitelli, D., Montesanti, R., Branca, D., Nicoletti, G., Spadafora, P., Pasqua, A.A., Cittadella, R., Andreoli, V., Gambardella, A., Aguglia, U. and Quattrone, A., 1999, Dopamine D_2 receptor gene polymorphism and the risk of levodopa-induced dyskinesias in PD, *Neurology*, **53**: 1425-1430.

Onali, P. and Schwartz, J.P., 1983, Inhibition of VIP-sensitive adenylate cyclase by dopamine in rat anterior pituitary., *Adv. Biochem. Psychopharmacol.*, **36**: 199-207.

Oruc, L., Verheyen, G.R., Furac, I., Jakovljevic, M., Ivezic, S., Raeymaekers, P. and Van Broeckhoven, C., 1997, Analysis of the tyrosine hydroxylase and dopamine D_4 receptor genes in a Croatian sample of bipolar I and unipolar patients, *Am J Med Genet*, **74**: 176-178.

Ostow, M., 2002, Pramipexole for depression, *Am J Psychiatry*, **159**: 320-321.

Parsian, A., Chakraverti, S. and Todd, R.D., 1995, Possible association between the D_3 receptor gene and bipolar affective disorder, *Am. J. Med. Genet. [Neuropsychiatric Genetics]*, **60**: 234-237.

Parsian, A., Chakraverty, S., Fisher, L. and Cloninger, C.R., 1997, No association between polymorphisms in the human dopamine D_3 and D_4 receptors genes and alcoholism, *Am J Med Genet*, **74**: 281-285.

Pastor, P., Munoz, E., Obach, V., Marti, M.J., Blesa, R., Oliva, R. and Tolosa, E., 1999, Dopamine receptor D_2 intronic polymorphism in patients with Parkinson's disease, *Neurosci Lett*, **273**: 151-154.

Paterson, A.D., Sunohara, G.A. and Kennedy, J.L., 1999, Dopamine D_4 receptor gene: novelty or nonsense?, *Neuropsychopharmacology*, **21**: 3-16.

Payton, A., Holmes, J., Barrett, J.H., Hever, T., Fitzpatrick, H., Trumper, A.L., Harrington, R., McGuffin, P., O'Donovan, M., Owen, M., Ollier, W., Worthington, J. and Thapar, A., 2001, Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: a family-based study, *Am J Med Genet*, **105**: 464-470.

Pedersen, U.B., Norby, B., Jensen, A.A., Schiodt, M., Hansen, A., Suhr Jenssen, P., Scheideler, M., Thastrup, O. and Ansersen, P.H., 1994, Characteristics of stably expressed human D1a and D1b receptors: atypical behavior of the dopamine D1b receptor, *Eur. J. Pharmacol.*, **267**: 85-93.

Perachon, S., Betancur, C., Pilon, C., Rostene, W., Schwartz, J.-C. and Sokoloff, P., 2000, Role of dopamine D_3 receptors in thermoregulation: a reappraisal, *Neuroreport*, **11**: 221-225.

Peroutka, S.J., Price, S.C., Wilhoit, T.L. and Jones, K.W., 1998, Comorbid migraine with aura, anxiety, and depression is associated with dopamine D_2 receptor (DRD2) NcoI alleles, *Mol Med*, **4**: 14-21.

Petronis, A., Macciardi, F., Athanassiades, A., Paterson, A.D., Verga, M., Meltzer, H.Y., Cola, P., Buchanan, J.A., Van Tol, H.H. and Kennedy, J.L., 1995, Association study between the dopamine D_4 receptor gene and schizophrenia, *Am J Med Genet*, **60**: 452-455.

Phillips, T.J., Brown, K.J., Burkhart-Kasch, S., Wenger, C.D., Kelly, M.A., Rubinstein, M., Grandy, D.K. and Low, M.J., 1998, Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D_2 receptors, *Nat Neurosci*, **1**: 610-615.

Piccardi Mp, Severino G, Bocchetta A, Palmas Ma, Ruiu S and Del Zompo M, 1997, No evidence of association between dopamine D_3 receptor gene and bipolar affective disorder, *American Journal of Medical Genetics*, **74**: 137-139.

Pilla, M., Perachon, S., Sautel, F., Garrido, F., Mann, A., Wermuth, C.G., Schwartz, J.-C., Everitt, B.J. and Sokoloff, P., 1999, Selective inhibition of cocaine-seeking behaviour by a partial dopamine D_3 receptor agonist, *Nature*, **400**: 371-375.

Pilon, C., Lévesque, D., Dimitriadou, V., Griffon, N., Martres, M.P., Schwartz, J.-C. and Sokoloff, P., 1994, Functional coupling of the human dopamine D_3 receptor in a transfected NG 108-15 neuroblastoma-glioma hybrid cell line, *Eur. J. Pharmacol. [Mol. Pharmacol. Sect.]*, **268**: 129-139.

Plante-Bordeneuve, V., Taussig, D., Thomas, F., Said, G., Wood, N.W., Marsden, C.D. and Harding, A.E., 1997, Evaluation of four candidate genes encoding proteins of the dopamine pathway in familial and sporadic Parkinson's disease: evidence for association of a DRD2 allele, *Neurology*, **48**: 1589-1593.

Porsolt, R.D., Anton, G., Blavet, N. and Jalfre, M., 1978, Behavioral despair in rats: a new model sensitive to antidepressant treatments, *Eur. J. Pharmacol.*, **47**: 379-391.

Posner, M.I. and Raichle, M.E., 1994, Images of mind. New York, Sci Am Library.

Primus, R.J., Thurkauf, A., Xu, J., Yevich, E., McInerney, S., Shaw, K., Tallman, J.F. and Gallagher, D.W., 1997, Localization and characterization of dopamine D_4 binding sites in rat and human brain by use of the novel, D_4 receptor-selective ligand [^{3}H]NGD 94-1, *J. Pharmacol. Exp. Ther.*, **282**: 1020-1027.

Ralph-Williams, R.J., Lehmann-Masten, V. and Geyer, M.A., 2003, Dopamine D_1 rather than D_2 receptor agonists disrupt prepulse inhibition of startle in mice, *Neuropsychopharmacology*, **28**: 108-118.

Ralph, R.J., Varty, G.B., Kelly, M.A., Wang, Y.-M., Caron, M.G., Rubinstein, M., Grandy, D.K., Low, M.J. and Geyer, M.A., 1999, The dopamine D_2, but not D_3 or D_4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice, *J. Neurosci*, **19**: 4627-4633.

Rao, P.A., Pickar, D., Gejman, P.V., Ram, A., Gershon, E.S. and Gelernter, J., 1994, Allelic variation in the D_4 dopamine receptor (DRD4) gene does not predict response to clozapine, *Arch Gen Psychiatry*, **51**: 912-917.

Ravindranathan, A., Coon, H., DeLisi, L., Holik, J., Hoff, M., Brown, A., Shields, G., Crow, T. and Byerley, W., 1994, Linkage analysis between schizophrenia and a microsatellite polymorphism for the D_5 dopamine receptor gene, *Psychiatr Genet*, **4**: 77-80.

Reavill, C., Taylor, S.G., Wood, M.D., Ashmeade, T., Austin, N.E., Avenell, K.Y., Boyfield, I., Branch, C.L., Cilia, J., Coldwell, M.C., Hadley, M.S., Hunter, A.J., Jeffrey, P., Jewitt, F., Johnson, C.N., Jones, D.N.C., Medhurst, A.D., Middlemiss, D.N., Nash, D.J., Riley, G.J., Routledge, C., Stemp, G., Thewlis, K.M., Trail, B., Vong, A.K.K. and Hagan, J.J., 2000, Pharmacological actions of a novel high-affinity, and selective human dopamine D_3 receptor antagonist, SB-277011-A, *J. Pharmacol. Exp. Ther.*, **294**: 1154-1165.

Regier, D.A., Farmer, M.E. and Rae, D.S., 1990, Comorbidity of mental disorders with alcohol and other drug abuse- Results from the epidemiologic catchment area (ECA) study, *JAMA*, **264**: 2511-2518.

Reich, T., Hinrichs, A., Culverhouse, R. and Bierut, L., 1999, Genetic studies of alcoholism and substance dependence, *Am J Hum Genet*, **65**: 599-605.

Reynolds, G.P. and Mason, S.L., 1994, Are striatal dopamine D_4 receptors increased in schizophrenia ?, *J. Neurochim.*, **63**: 1576-1577.

Ricketts, M.H., Hamer, R.M., Manowitz, P., Feng, F., Sage, J.I., Di Paola, R. and Menza, M.A., 1998, Association of long variants of the dopamine D_4 receptor exon 3 repeat polymorphism with Parkinson's disease, *Clin Genet*, **54**: 33-38.

Rietschel, M., Naber, D., Oberlander, H., Holzbach, R., Fimmers, R., Eggermann, K., Moller, H.J., Propping, P. and Nothen, M.M., 1996, Efficacy and side-effects of clozapine: testing for association with allelic variation in the dopamine D_4 receptor gene, *Neuropsychopharmacology*, **15**: 491-496.

Rietschel, M., Nöthen, M.M., Lannfelt, L., Sokoloff, P., Schwartz, J.-C., Lanczik, M., Pritze, J., Cichon, S., Fimmers, R., Körner, J., Möller, H.J. and Propping, P., 1993, A serine to glycine substitution at position 9 in the extracellular N-terminal part of the dopamine D_3 receptor protein. No role in the genetic predisposition to bipolar affective disorder, *Psychiatric Res.*, **46**: 253-259.

Risinger, F.O., Freeman, P.A., Rubinstein, M., Low, M.J. and Grandy, D.K., 2000, Lack of operant ethanol self-administration in dopamine D_2 receptor knockout mice, *Psychopharmacology (Berl)*, **152**: 343-350.

Robinson, S.W. and Caron, M.G., 1997, Selective inhibition of adenylyl cyclase type V by the dopamine D_3 receptor, *Mol Pharmacol*, **52**: 508-514.

Rohrmeier, T., Putzhammer, A., Sartor, H., Knapp, M., Albus, M., Borrmann-Hassenbach, M., Lichtermann, D., Wildenauer, D., Schwab, S., Maier, W., Klein, H.E. and Eichhammer, P., 2003, [No association of 141C-ins/del polymorphism in the D_2 dopamine receptor gene in schizophrenia], *Psychiatr Prax*, **30 Suppl 2**: S212-215.

Rothschild, L.G., Badner, J., Cravchik, A., Gershon, E.S. and Gejman, P.V., 1996, No association detected between a D_3 receptor gene-expressed variant and schizophrenia, *Am. J. Med. Genet.*, **67**: 232-234.

Rowe, D.C., Van den Oord, E.J., Stever, C., Giedinghagen, L.N., Gard, J.M., Cleveland, H.H., Gilson, M., Terris, S.T., Mohr, J.H., Sherman, S., Abramowitz, A. and Waldman, I.D., 1999, The DRD2 TaqI polymorphism and symptoms of attention deficit hyperactivity disorder, *Mol Psychiatry*, **4**: 580-586.

Rubinstein, M., Phillips, T.J., Bunzow, J.R., Falzone, T.L., Dziewczapolski, G., Zhang, G., Fang, Y., Larson, J.L., McDougall, J.A., Chester, J.A., Saez, C., Pugsley, T.A., Gershanik, O., Low, M.J. and Grandy, D.K., 1997, Mice lacking dopamine D_4 receptors are supersensitive to ethanol, cocaine, and methamphetamine, *Cell*, **90**: 991-1001.

Ryoo, H.L., Pierrotti, D. and Joyce, J.N., 1998, Dopamine D_3 receptor is decreased and D_2 receptor is elevated in the striatum of Parkinson's disease, *Mov. Disord.*, **13**: 788-797.

Sabaté, O., Campion, D., d'Amato, T., Martres, M.-P., Sokoloff, P., Giros, B., Leboyer, M., Jay, M., Guedj, F., Thibaut, F., Dollfus, S., Preterre, P., Petit, M., Babron, M.C., Waksman, G., Mallet, J. and Schwartz, J.-C., 1994, Failure to find evidence for linkage or association between the dopamine D_3 receptor gene and schizophrenia, *Am. J. Psychiat.*, **151**: 107-111.

Saha, N., Tsoi, W.F., Low, P.S., Basair, J. and Tay, J.S., 1994, Lack of association of the dopamine D_3 receptor gene polymorphism (BalI) in Chinese schizophrenic males, *Psychiatr Genet*, **4**: 201-204.

Salamone, J.D., 1994, The involvement of nucleus accumbens dopamine in appetitive and aversive motivation, *Behav. Brain Res.*, **61**: 117-133.

Salamone, J.D., Correa, M., Mingote, S.M. and Weber, S.M., 2005, Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine, *Curr Opin Pharmacol*, **5**: 34-41.

Sander, T., Harms, H., Podschus, J., Finckh, U., Nickel, B., Rolfs, A., Rommelspacher, H. and Schmidt, L.G., 1995, Dopamine D_1, D_2 and D_3 receptor genes in alcohol dependence, *Psychiatr Genet*, **5**: 171-176.

Sanders, A.R., Rincon-Limas, D.E., Chakraborty, R., Grandchamp, B., Hamilton, J.D., Fann, W.E. and Patel, P.I., 1993, Association between genetic variation at the porphobilinogen deaminase gene and schizophrenia, *Schizophr Res*, **8**: 211-221.

Sasaki, T., Macciardi, F.M., Badri, F., Verga, M., Meltzer, H.Y., Lieberman, J., Howard, A., Bean, G., Joffe, R.T., Hudson, C.J. and Kennedy, J.L., 1996, No evidence for association of dopamine D_2 receptor variant (Ser311/Cys311) with major psychosis, *Am J Med Genet*, **67**: 415-417.

Sautel, F., Griffon, N., Lévesque, D., Pilon, C., Schwartz, J.-C. and Sokoloff, P., 1995, A functional test identifies dopamine agonists selective for D_3 versus D_2 receptors, *Neuroreport*, **6**: 329-332.

Sautel, F., Griffon, N., Sokoloff, P., Schwartz, J.-C., Launay, C., Simon, P., Costentin, J., Schoenfelder, A., Garrido, F., Mann, A. and Wermuth, C.G., 1995, Nafadotride, a potent preferential dopamine D_3 receptor antagonist, activates locomotion in rodents, *J. Pharmacol. Exp. Ther.*, **275**: 1239-1246.

Scahill, L. and Schwab-Stone, M., 2000, Epidemiology of ADHD in school-age children, *Child Adolesc Psychiatr Clin N Am*, **9**: 541-555, vii.

Scarselli, M., Novi, F., Schallmach, E., Lin, R., Baragli, A., Colzi, A., Griffon, N., Corsini, G.U., Sokoloff, P., Levenson, R., Vogel, Z. and Maggio, R., 2001, D_2/D_3 dopamine receptor heterodimers exhibit unique functional properties, *J Biol Chem*, **276**: 30308-30314.

Schafer, M., Rujescu, D., Giegling, I., Guntermann, A., Erfurth, A., Bondy, B. and Moller, H.J., 2001, Association of short-term response to haloperidol treatment with a polymorphism in the dopamine D(2) receptor gene, *Am J Psychiatry*, **158**: 802-804.

Scharfetter, J., 2004, Pharmacogenetics of dopamine receptors and response to antipsychotic drugs in schizophrenia–an update, *Pharmacogenomics*, **5**: 691-698.

Scharfetter, J., Chaudhry, H.R., Hornik, K., Fuchs, K., Sieghart, W., Kasper, S. and Aschauer, H.N., 1999, Dopamine D_3 receptor gene polymorphism and response to clozapine in schizophrenic Pakastani patients, *European Neuropsychopharmacology*, **10**: 17-20.

Schindler, K.M., Pato, M.T., Dourado, A., Macedo, A., Azevedo, M.H., Kennedy, J.L. and Pato, C.N., 2002, Association and linkage disequilibrium between a functional polymorphism of the dopamine-2 receptor gene and schizophrenia in a genetically homogeneous Portuguese population, *Mol Psychiatry*, **7**: 1002-1005.

Schmauss, C., Haroutunian, V., Davis, K.L. and Davidson, M., 1993, Selective loss of dopamine D_3-type receptor mRNA expression in parietal and motor cortices of patients with chronic schizophrenia, *Proc. Natl. Acad. Sci. USA*, **90**: 8942-8946.

Schoemaker, H., Claustre, Y., Fage, D., Rouquier, L., Chergui, K., Curet, O., Oblin, A., Gonon, F., Carter, C., Benavides, J. and Scatton, B., 1997, Neurochemical characteristics of amisulpride, an atypical dopamine D_2/D_3 receptor antagonist with both presynaptic and limbic selectivity, *J Pharmacol Exp Ther*, **280**: 83-97.

Schoots, O. and Van Tol, H.H., 2003, The human dopamine D_4 receptor repeat sequences modulate expression, *Pharmacogenomics J*, **3**: 343-348.

Seeman, P., Guan, H.C., Nobrega, J., Jiwa, D., Markstein, R., Balk, J.H., Picetti, R., Borrelli, E. and Van Tol, H.H., 1997, Dopamine D2-like sites in schizophrenia, but not in Alzheimer's, Huntington's, or control brains, for [3H]benzquinoline, *Synapse*, **25**: 137-146.

Seeman, P., Guan, H.C. and Van Tol, H.H.M., 1993, Dopamine D_4 receptors elevated in schizophrenia, *Nature*, **365**: 441-445.

Seeman, P., Ohara, K., Ulpian, C., Seeman, M.V., Jellinger, K., Van Tol, H.H. and Niznik, H.B., 1993, Schizophrenia: normal sequence in the dopamine D_2 receptor region that couples to G-proteins, *Neuropsychopharmacology*, **8**: 137-142.

Segal, D.M., Moraes, C.T. and Mash, D.C., 1997, Up-regulation of D_3 dopamine receptor mRNA in the nucleus accumbens of human cocaine fatalities, *Mol. Brain Res.*, **45**: 335-339.

Senogles, S.E., 1994, The D_2 dopamine receptor isoforms signal through distinct Giα proteins to inhibit adenylyl cyclase, *J. Biol. Chem.*, **269**: 23120-23127.

Senogles, S.E., Heimert, T.L., Odife, E.R. and Quasney, M.W., 2004, A region of the third intracellular loop of the short form of the D_2 dopamine receptor dictates Gi coupling specificity, *J Biol Chem*, **279**: 1601-1606.

Serretti, A., Cusin, C., Lattuada, E., Lilli, R., Lorenzi, C., Di Bella, D., Catalano, M. and Smeraldi, E., 1999, No interaction between serotonin transporter gene and dopamine receptor D_4 gene in symptomatology of major psychoses, *Am J Med Genet*, **88**: 481-485.

Serretti, A., Lattuada, E., Lorenzi, C., Lilli, R. and Smeraldi, E., 2000, Dopamine receptor D_2 Ser/Cys 311 variant is associated with delusion and disorganization symptomatology in major psychoses, *Mol Psychiatry*, **5**: 270-274.

Serretti, A., Lilli, R., Di Bella, D., Bertelli, S., Nobile, M., Novelli, E., Catalano, M. and Smeraldi, E., 1999, Dopamine receptor D_4 gene is not associated with major psychoses, *Am J Med Genet*, **88**: 486-491.

Serretti, A., Lilli, R., Lorenzi, C., Franchini, L., Di Bella, D., Catalano, M. and Smeraldi, E., 1999, Dopamine receptor D_2 and D_4 genes, $GABA_A$ alpha-1 subunit genes and response to lithium prophylaxis in mood disorders, *Psychiatry Res*, **87**: 7-19.

Serretti, A., Lilli, R., Lorenzi, C., Franchini, L. and Smeraldi, E., 1998, Dopamine receptor D_3 gene and response to lithium prophylaxis in mood disorders, *Int J Neuropsychopharmcol*, **1**: 125-129.

Serretti, A., Lilli, R., Lorenzi, C., Lattuada, E. and Smeraldi, E., 2001, DRD4 exon 3 variants associated with delusional symptomatology in major psychoses: a study on 2,011 affected subjects, *Am J Med Genet*, **105**: 283-290.

Serretti, A., Lorenzi, C., Mandelli, L., Cichon, S., Schumacher, J., Nothen, M.M., Rietschel, M., Tullius, M. and Ohlraun, S., 2004, DRD4 exon 3 variants are not associated with symptomatology of major psychoses in a German population, *Neurosci Lett*, **368**: 269-273.

Serretti, A., Macciardi, F., Catalano, M., Bellodi, L. and Smeraldi, E., 1999, Genetic variants of dopamine receptor D_4 and psychopathology, *Schizophr Bull*, **25**: 609-618.

Serretti, A., Macciardi, F., Cusin, C., Lattuada, E., Lilli, R., Di Bella, D., Catalano, M. and Smeraldi, E., 1999, No interaction of GABA(A) alpha-1 subunit and dopamine receptor D_4 exon 3 genes in symptomatology of major psychoses, *Am J Med Genet*, **88**: 44-49.

Serretti, A., Macciardi, F., Cusin, C., Lattuada, E., Souery, D., Lipp, O., Mahieu, B., Van Broeckhoven, C., Blackwood, D., Muir, W., Aschauer, H.N., Heiden, A.M., Ackenheil, M., Fuchshuber, S., Raeymaekers, P., Verheyen, G., Kaneva, R., Jablensky, A., Papadimitriou, G.N., Dikeos, D.G., Stefanis, C.N., Smeraldi, E. and Mendlewicz, J., 2000, Linkage of mood disorders with D_2, D_3 and TH genes: a multicenter study, *J Affect Disord*, **58**: 51-61.

Serretti, A. and Smeraldi, E., 1999, Dopamine D_2 receptor gene not associated with symptomatology of mood disorders, *Am J Med Genet*, **88**: 294-297.

Shah, M., Coon, H., Holik, J., Hoff, M., Helmer, V., Panos, P. and Byerley, W., 1995, Mutation scan of the D_1 dopamine receptor gene in 22 cases of bipolar I disorder, *Am J Med Genet*, **60**: 150-153.

Shaikh, S., Ball, D., Craddock, N., Castle, D., Hunt, N., Mant, R., Owen, M., Collier, D. and Gill, M., 1993, The dopamine D_3 receptor gene: no association with bipolar affective disorder, *J Med Genet*, **30**: 308-309.

Shaikh, S., Collier, D., Arranz, M., Ball, D., Gill, M. and Kerwin, R., 1994, DRD2 Ser311/Cys311 polymorphism in schizophrenia, *Lancet*, **343**: 1045-1046.

Shaikh, S., Collier, D., Kerwin, R.W., Pilowsky, L.S., Gill, M., Xu, W.M. and Thornton, A., 1993, Dopamine D_4 receptor subtypes and response to clozapine, *Lancet*, **341**: 116.

Shaikh, S., Collier, D.A., Sham, P., Pilowsky, L., Sharma, T., Lin, L.K., Crocq, M.A., Gill, M. and Kerwin, R., 1995, Analysis of clozapine response and polymorphisms of the dopamine D_4 receptor gene (DRD4) in schizophrenic patients, *Am J Med Genet*, **60**: 541-545.

Shaikh, S., Collier, D.A., Sham, P.C., Ball, D., Aitchison, K., Vallada, H., Smith, I., Gill, M. and Kerwin, R.W., 1996, Allelic association between a Ser-9-Gly polymorphism in the dopamine D_3 receptor gene and schizophrenia, *Hum Genet*, **97**: 714-719.

Shaikh, S., Gill, M., Owen, M., Asherson, P., McGuffin, P., Nanko, S., Murray, R.M. and Collier, D.A., 1994, Failure to find linkage between a functional polymorphism in the dopamine D_4 receptor gene and schizophrenia, *Am J Med Genet*, **54**: 8-11.

Singleton, A.B., Thomson, J.H., Morris, C.M., Court, J.A., Lloyd, S. and Cholerton, S., 1998, Lack of association between the dopamine D_2 receptor gene allele DRD2*A1 and cigarette smoking in a United Kingdom population, *Pharmacogenetics*, **8**: 125-128.

Siuciak, J.A. and Fujiwara, R.A., 2004, The activity of pramipexole in the mouse forced swim test is mediated by D_2 rather than D_3 receptors, *Psychopharmacology (Berl)*, **175**: 163-169.

Sivagnanasundaram, S., Morris, A.G., Gaitonde, E.J., McKenna, P.J., Mollon, J.D. and Hunt, D.M., 2000, A cluster of single nucleotide polymorphisms in the 5'-leader of the human dopamine D_3 receptor gene (DRD3) and its relationship to schizophrenia, *Neurosci Lett*, **279**: 13-16.

Smith, D.R., Striplin, C.D., Geller, A.M., Mailman, R.B., Drago, J., Lawler, C.P. and Gallagher, M., 1998, Behavioural assessment of mice lacking D_{1A} dopamine receptors, *Neuroscience*, **86**: 135-146.

Smith, J.W., Fetsko, L.A., Xu, R. and Wang, Y., 2002, Dopamine D_{2L} receptor knockout mice display deficits in positive and negative reinforcing properties of morphine and in avoidance learning, *Neuroscience*, **113**: 755-765.

Sobell, J.L., Lind, T.J., Sigurdson, D.C., Zald, D.H., Snitz, B.E., Grove, W.M., Heston, L.L. and Sommer, S.S., 1995, The D_5 dopamine receptor gene in schizophrenia: identification of a nonsense change and multiple missense changes but lack of association with disease, *Hum Mol Genet*, **4**: 507-514.

Sokoloff, P., Andrieux, M., Besançon, R., Pilon, C., Martres, M.-P., Giros, B. and Schwartz, J.-C., 1992, Pharmacology of human D_3 dopamine receptor expressed in a mammalian cell line: comparison with D_2 receptor, *Eur. J. Pharmacol. Mol. Pharmacol. Sect.*, **225**: 331-337.

Sokoloff, P., Diaz, J., Le Foll, B., Guillin, O., Leriche, L., Bezard, E. and Gross, C. (2005). The dopamine D_3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. Current Drug Targets - CNS and Neurological Disorders, Bentham Science Publishers Ltd.

Sokoloff, P., Diaz, J., Levesque, D., Pilon, C., Dimitriadou, V., Griffon, N., Lammers, C.H., Martres, M.P. and Schwartz, J.C., 1995, Novel dopamine receptor subtypes as targets for antipsychotic drugs, *Annals New York Academy of Sciences*, **757**: 278-292.

Sokoloff, P., Giros, B., Martres, M.-P., Bouthenet, M.-L. and Schwartz, J.-C., 1990, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics, *Nature*, **347**: 146-151.

Sommer, S.S., Lind, T.J., Heston, L.L. and Sobell, J.L., 1993, Dopamine D_4 receptor variants in unrelated schizophrenic cases and controls, *Am J Med Genet*, **48**: 90-93.

Spano, P.F., Govoni, S. and Trabucchi, M., 1978, Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system, *Adv. Biochem. Psychopharmacol.*, **19**: 155-165.

Spurlock, G., Williams, J., McGuffin, P., Aschauer, H.N., Lenzinger, E., Fuchs, K., Sieghart, W.C., Meszaros, K., Fathi, N., Laurent, C., Mallet, J., Macciardi, F., Pedrini, S., Gill, M., Hawi, Z., Gibson, S., Jazin, E.E., Yang, H.T., Adolfsson, R., Pato, C.N., Dourado, A.M. and Owen, M.J., 1998, European Multicentre Association Study of Schizophrenia: a study of the DRD2 Ser311Cys and DRD3 Ser9Gly polymorphisms, *Am J Med Genet*, **81**: 24-28.

Staley, J.K. and Mash, D.C., 1996, Adaptive increase in D_3 dopamine receptors in the brain reward circuits of human cocaine fatalities, *J. Neurosci.*, **16**: 6100-6106.

Steen, V.M., Lovlie, R., McEwan, T. and McCreadie, R.G., 1997, Dopamine D_3 receptor gene variant and susceptibility to tardive dyskinesia in schizophrenic patients, *Mol. Psychiatry*, **2**: 139-145.

Steiner, H., Fuchs, S. and Accili, D., 1997, D_3 dopamine receptor-deficient mouse: evidence for reduced anxiety, *Physiol. Behav.*, **63**: 137-141.

Stober, G., Jatzke, S., Heils, A., Jungkunz, G., Knapp, M., Mossner, R., Riederer, P. and Lesch, K.P., 1998, Insertion/deletion variant (-141C Ins/Del) in the 5' regulatory region of the dopamine D_2 receptor gene: lack of association with schizophrenia and bipolar affective disorder. Short communication, *J Neural Transm*, **105**: 101-109.

Sullivan, P.F., Neale, M.C., Silverman, M.A., Harris-Kerr, C., Myakishev, M.V., Wormley, B., Webb, B.T., Ma, Y., Kendler, K.S. and Straub, R.E., 2001, An association study of DRD5 with smoking initiation and progression to nicotine dependence, *Am J Med Genet*, **105**: 259-265.

Sunahara, R.K., Guan, H.C., O'Dowd, B.F., Seeman, P., Laurier, L.G., Ng, G., George, S.R., Torchia, J., Van Tol, H.H.M. and Niznik, H.B., 1991, Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1, *Nature*, **350**: 614-619.

Sunahara, R.K., Niznik, H.B., Weiner, D.M., Stormann, T.M., Brann, M.R., Kennedy, J.L., Gelernter, J.E., Rozmahel, R., Yang, Y., Israel, Y., Seeman, P. and O'Dowd, B.F., 1990, Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5, *Nature*, **347**: 80-83.

Surmeier, D.J., Song, W.-J. and Yan, Z., 1996, Coordinated expression of dopamine receptors in neostriatal medium spiny neurons, *J. Neurosci.*, **16**: 6579-6591.

Suzuki, A., Kondo, T., Mihara, K., Yasui-Furukori, N., Ishida, M., Furukori, H., Kaneko, S., Inoue, Y. and Otani, K., 2001, The -141C Ins/Del polymorphism in the dopamine D_2 receptor gene promoter region is associated with anxiolytic and antidepressive effects during treatment with dopamine antagonists in schizophrenic patients, *Pharmacogenetics*, **11**: 545-550.

Suzuki, A., Mihara, K., Kondo, T., Tanaka, O., Nagashima, U., Otani, K. and Kaneko, S., 2000, The relationship between dopamine D_2 receptor polymorphism at the Taq1 A locus and therapeutic response to nemonapride, a selective dopamine antagonist, in schizophrenic patients, *Pharmacogenetics*, **10**: 335-341.

Suzuki, M., Hurd, Y.l., Sokoloff, P., Schwartz, J.C. and Sedvall, G., 1998, D_3 dopamine receptor mRNA is widely expressed in the human brain, *Brain Res*, **779**: 58-74.

Svenningsson, P., Nishi, A., Fisone, G., Girault, J.A., Nairn, A.C. and Greengard, P., 2004, DARPP-32: an integrator of neurotransmission, *Annu Rev Pharmacol Toxicol*, **44**: 269-296.

Svenningsson, P., Tzavara, E.T., Carruthers, R., Rachleff, I., Wattler, S., Nehls, M., McKinzie, D.L., Fienberg, A.A., Nomikos, G.G. and Greengard, P., 2003, Diverse psychotomimetics act through a common signaling pathway, *Science*, **302**: 1412-1415.

Svensson, K., Carlsson, M., Carlsson, A., Hjorth, S., Johansson, A.M. and Eriksson, E., 1986, The putative selective dopamine autoreceptor antagonists (+)-AJ 76 and (+)-UH 232 stimulate prolactine release in rats, *Eur. J. Pharmacol.*, **130**: 237-242.

Swanson, J., Oosterlaan, J., Murias, M., Schuck, S., Flodman, P., Spence, M.A., Wasdell, M., Ding, Y., Chi, H.C., Smith, M., Mann, M., Carlson, C., Kennedy, J.L., Sergeant, J.A., Leung, P., Zhang, Y.P., Sadeh, A., Chen, C., Whalen, C.K., Babb, K.A., Moyzis, R. and Posner, M.I., 2000, Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D_4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention, *Proc Natl Acad Sci USA*, **97**: 4754-4759.

Tahir, E., Yazgan, Y., Cirakoglu, B., Ozbay, F., Waldman, I. and Asherson, P.J., 2000, Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children, *Mol Psychiatry*, **5**: 396-404.

Tallerico, T., Ulpian, C. and Liu, I.S., 1999, Dopamine D_2 receptor promoter polymorphism: no association with schizophrenia, *Psychiatry Res*, **85**: 215-219.

Tanaka, T., Igarashi, S., Onodera, O., Tanaka, H., Kameda, K., Takahashi, K., Tsuji, S. and Ihda, S., 1995, Lack of association between dopamine D_4 receptor gene and schizophrenia, *Am J Med Genet*, **60**: 580-582.

Tanaka, T., Igarashi, S., Onodera, O., Tanaka, H., Takahashi, M., Maeda, M., Kameda, K., Tsuji, S. and Ihda, S., 1996, Association study between schizophrenia and dopamine D_3 receptor gene polymorphism, *Am. J. Med. Genet. [Neuropsychiatric Genetics]*, **67**: 366-368.

Tang, L., Todd, R.D., Heller, A. and O'Malley, K.L., 1994, Pharmacological and functional characterization of D_2, D_3 and D_4 dopamine receptors in fibroblasts and dopaminergic cell lines, *J. Pharmacol. Exp. Ther.*, **268**: 495-502.

Tani, M., Koide, S., Onishi, H., Nakamura, Y. and Yamagami, S., 1997, Association analysis of the SmaI polymorphism of the dopamine D_4 receptor in Japanese schizophrenics, *Psychiatr Genet*, **7**: 137-139.

Thompson, M., Comings, D.E., Feder, L., George, S.R. and O'Dowd, B.F., 1998, Mutation screening of the dopamine D_1 receptor gene in Tourette's syndrome and alcohol dependent patients, *Am J Med Genet*, **81**: 241-244.

Tiberi, M. and Caron, M.G., 1994, High agonist-independent activity is a distinguishing feature of the dopamine D_{1B} receptor subtype, *J. Biol. Chem.*, **269**: 27925-27931.

Tiberi, M., Jarvie, K.R., Silvia, C., Falardeau, P., Gingrich, J.A., Godinot, N., Bertrand, L., Yang-Feng, T.L., Fremeau Jr., R.T. and Caron, M.G., 1991, Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D_1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D_{1A} receptor, *Proc. Natl. Acad. Sci.USA*, **88**: 7491-7495.

Todd, R.D., Neuman, R.J., Lobos, E.A., Jong, Y.J., Reich, W. and Heath, A.C., 2001, Lack of association of dopamine D_4 receptor gene polymorphisms with ADHD subtypes in a population sample of twins, *Am J Med Genet*, **105**: 432-438.

Tomiyama, K., McNamara, F.N., Clifford, J.J., Kinsella, A., Drago, J., Tighe, O., Croke, D.T., Koshikawa, N. and Waddington, J.L., 2002, Phenotypic resolution of spontaneous and D1-like agonist-induced orofacial movement topographies in congenic dopamine D_{1A} receptor 'knockout' mice, *Neuropharmacology*, **42**: 644-652.

Tran, A.H., Tamura, R., Uwano, T., Kobayashi, T., Katsuki, M. and Ono, T., 2005, Dopamine D_1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place, *Proc Natl Acad Sci USA*, **102**: 2117-2122.

Truffinet, P., Tamminga, C.A., Fabre, L.F., Meltzer, H.Y., Riviere, M.E. and Papillon-Downey, C., 1999, Placebo-controlled study of the D_4/5-HT_{2A} antagonist fananserin in the treatment of schizophrenia, *Am J Psychiatry*, **156**: 419-425.

Tsai, S.J., Cheng, C.Y., Shu, L.R., Yang, C.Y., Pan, C.W., Liou, Y.J. and Hong, C.J., 2002, No association for D_2 and D_4 dopamine receptor polymorphisms and methamphetamine abuse in Chinese males, *Psychiatr Genet*, **12**: 29-33.

Undie, A.S. and Friedman, E., 1990, Stimulation of a dopamine D_1 receptor enhances inositol phosphates formation in rat brain, *J Pharmacol Exp Ther*, **253**: 987-992.

Usiello, A., Baik, J.H., Rouge-Pont, F., Picetti, R., Dierich, A., LeMeur, M., Piazza, P.V. and Borrelli, E., 2000, Distinct functions of the two isoforms of dopamine D_2 receptors, *Nature*, **408**: 199-203.

Valerio, A., Belloni, M., Gorno, M.L., Tinti, C., Memo, M. and Spano, P., 1994, Dopamine D_2, D_3, and D_4 receptor mRNA levels in rat brain and pituitary during aging, *Neurobiol Aging*, **15**: 713-719.

Van Tol, H.H.M., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B. and Civelli, O., 1991, Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine, *Nature*, **350**: 610-614.

Van Tol, H.H.M., Wu, C.M., Guan, H.C., O'Hara, K., Bunzow, J.R., Civelli, O., Kennedy, J., Seeman, P., Niznik, H.B. and Janovic, V., 1992, Multiple dopamine D_4 receptor variants in the human population, *Nature*, **358**: 149-152.

Vanyukov, M.M., Moss, H.B., Gioio, A.E., Hughes, H.B., Kaplan, B.B. and Tarter, R.E., 1998, An association between a microsatellite polymorphism at the DRD5 gene and the liability to substance abuse: pilot study, *Behav Genet*, **28**: 75-82.

Vorel, S.R., Ashby, C.R.J., Paul, M., Liu, X., Hayes, R., Hagan, J.J., Middlemiss, D.N., Stemp, G. and Gardner, E.L., 2002, Dopamine D_3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats, *J Neurosci.*, **22**: 9595-9603.

Waddington, J.L., Clifford, J.J., McNamara, F.N., Tomiyama, K., Koshikawa, N. and Croke, D.T., 2001, The psychopharmacology-molecular biology interface: exploring the behavioural roles of dopamine receptor subtypes using targeted gene deletion ('knockout'), *Prog Neuropsychopharmacol Biol Psychiatry*, **25**: 925-964.

Wang, Y., Xu, R., Sasaoka, T., Tonegawa, S., Kung, M.P. and Sankoorikal, E.B., 2000, Dopamine D_2 long receptor-deficient mice display alterations in striatum-dependent functions, *J Neurosci*, **20**: 8305-8314.

Waters, N., Svensson, K., Haadsma-Svensson, S.R., Smith, M.W. and Carlsson, A., 1993, The dopamine D_3 receptor: a postsynaptic receptor inhibitory on rat locomotor activity, *J. Neural Transm. [Gen. Sect.]*, **94**: 11-19.

Weiss, J., Magert, H.J., Cieslak, A. and Forssmann, W.G., 1996, Association between different psychotic disorders and the DRD4 polymorphism, but no differences in the main ligand binding region of the DRD4 receptor protein compared to controls, *Eur J Med Res*, **1**: 439-445.

Werge, T., Elbaek, Z., Andersen, M.B., Lundbaek, J.A. and Rasmussen, H.B., 2003, Cebus apella, a nonhuman primate highly susceptible to neuroleptic side effects, carries the GLY^9 dopamine receptor D_3 associated with tardive dyskinesia in humans, *Pharmacogenomics J*, **3**: 97-100.

Wersinger, C., White, B.H., Sidhu, A. and Senogles, S.E., 2003, Physiology, pharmacology, and physiopathology of the D1-like family of dopamine receptors in the central nervous system. Dopamine Receptors and Transporters. Function, Imaging, and Clinical Implication. A. Sidhu, M. Laruelle and P. Vernier, Marcel Dekker, Inc. **Second Edition:** 145-210.

Wiesbeck, G.A., Weijers, H.G., Wodarz, N., Herrmann, M.J., Johann, M., Keller, H.K., Michel, T.M. and Boning, J., 2003, Dopamine D_2 (DAD2) and dopamine D_3 (DAD3) receptor gene polymorphisms and treatment outcome in alcohol dependence, *J Neural Transm*, **110**: 813-820.

Wiese, C., Lannfelt, L., Kristbjarnarson, H., Yang, L., Zoega, T., Sokoloff, P., Ivarsson, O., Schwartz, J.C., Moises, H.W. and Helgason, T., 1993, No evidence of linkage between schizophrenia and D_3 dopamine receptor gene locus in Icelandic pedigrees, *Psychiatry Res*, **46**: 69-78.

Williams, G.V. and Goldman-Rakic, P.S., 1995, Modulation of memory fields by dopamine D_1 receptors in prefrontal cortex, *Nature*, **376**: 572-575.

Williams, J., Spurlock, G., Holmans, P., Mant, R., Murphy, K., Jones, L., Cardno, A., Asherson, P., Blackwood, D., Muir, W., Meszaros, K., Aschauer, H., Mallet, J., Laurent, C., Pekkarinen, P., Seppala, J., Stefanis, C.N., Papadimitriou, G.N., Macciardi, F., Verga, M., Pato, C., Azevedo, H., Crocq, M.-A., Gurling, H., Kalsi, G., Curtis, D., McGuffin, P. and Owen, M.J., 1998, A meta-analysis and transmission disequilibrium study of association between the dopamine D_3 receptor gene and schizophrenia, *Mol. Psychiatry*, **3**: 141-149.

Willner, P., 1997, The mesolimbic dopamine system as a target for rapid antidepressant action, *Int. Clin. Psychopharmacology*, **12**: S7-S14.

Wise, R.A., 2004, Dopamine, learning and motivation, *Nat Rev Neurosci*, **5**: 483-494.

Wise, R.A. and Bozarth, M.A., 1987, A psychomotor stimulant theory of addiction, *Psychol. Rev.*, **94**: 469-492.

Wolf, M.E. and Roth, R.H., 1990, Autoreceptor regulation of dopamine synthesis, *Ann N Y Acad Sci*, **604**: 323-343.

Wolraich, M.L., Hannah, J.N., Baumgaertel, A. and Feurer, I.D., 1998, Examination of DSM-IV criteria for attention deficit/hyperactivity disorder in a county-wide sample, *J Dev Behav Pediatr*, **19**: 162-168.

Xing, Q.H., Wu, S.N., Lin, Z.G., Li, H.F., Yang, J.D., Feng, G.Y., Wang, M.T., Yang, W.W. and He, L., 2003, Association analysis of polymorphisms in the upstream region of the human dopamine D_4 receptor gene in schizophrenia, *Schizophr Res*, **65**: 9-14.

Xu, M., Koeltzow, T.E., Tirado Santiago, G., Moratella, R., Cooper, D.C., Hu, X.-T., White, N.M., Graybiel, A.M., White, F.J. and Tonegawa, S., 1997, Dopamine D_3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D_1 and D_2 receptors, *Neuron*, **19**: 837-848.

Xu, M., Moratalla, R., Gold, L.H., Hiroi, N., Koob, G.F., Graybiel, A.M. and Tonegawa, S., 1994, Dopamine D_1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses, *Cell*, **79**: 729-742.

Xu, M., Moratalla, R., Gold, L.H., Hiroi, N., Koob, G.F., Graybiel, A.M. and Tonegawa, S., 1994, Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D_1 receptor mutant mice., *Cell*, **79**: 729-742.

Yamanouchi, Y., Iwata, N., Suzuki, T., Kitajima, T., Ikeda, M. and Ozaki, N., 2003, Effect of DRD2, 5-HT2A, and COMT genes on antipsychotic response to risperidone, *Pharmacogenomics J*, **3**: 356-361.

Yang, L., Li, T., Wiese, C., Lannfelt, L., Sokoloff, P., Xu, C.T., Zeng, Z., Schwartz, J.-C., Liu, X. and Moises, H.W., 1993, No association between schizophrenia and homozygosity at the D_3 dopamine receptor gene, *Am. J. Med. Genet.[Neuropsychiatr. Genet.]*, **48**: 83-86.

Zapata, A., Witkins, J.M. and Shippenberg, T.S., 2001, Selective D_3 receptor agonist effects of (+)-PD 128907 on dialysate dopamine at low doses, *Neuropharmacology*, **41**: 351-359.

Zhou, Q.Z., Grandy, D.K., Thambi, L., Kushner, J.A., Van Tol H.H.M., Cone, R., Pribnow, D., Salon, J., Bunzow, J.R. and O., C., 1990, Cloning and expression of human and rat D_1 dopamine receptors, *Nature*, **347**: 76-86.

Zoli, M., Torri, C., Ferrari, R., Jansson, A., Zini, I., Fuxe, K. and Agnati, L.F., 1998, The emergence of the volume transmission concept, *Brain Res Brain Res Rev*, **26**: 136-147.

15. THE NEUROBIOLOGY OF GABA RECEPTORS

Enrico Sanna, Paolo Follesa, and Giovanni Biggio*

Gamma-aminobutyric acid (GABA) was first identified in 1950 (Roberts 1950; Awapara 1950) in brain extracts of various animal species and was subsequently found to be the principal inhibitory neurotransmitter in the central nervous system (CNS). GABA is synthesized as a result of decarboxylation of glutamic acid and is released by neurons into the synaptic cleft in order to transmit inhibitory signals to other nerve cells. Given the wide distribution of GABA throughout the brain (more than 30% of all central synapses in mammals are GABAergic), it is not surprising that GABA-mediated neurotransmission plays an important role not only in the control of various brain functions but also, as reviewed in this chapter, in the pathophysiology of several mental and neurological conditions including anxiety, sleep disorders, epilepsy, and Huntington's chorea.

Recent insights into the structure, function, and pharmacology of GABA receptors have contributed substantially to the identification of key neurobiological and neurochemical mechanisms that underlie the regulation of neuronal excitability and of emotional and behavioral states. They have also facilitated the development as well as characterization of the mechanisms of action of therapeutic agents used in the treatment of GABA-related pathologies.

1. $GABA_A$ RECEPTORS

The inhibitory actions of GABA are mediated by the activation of two main types of cell surface receptor: ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. This portion of the review will focus on the $GABA_A$ receptor.

* Enrico Sanna, Department of Experimental Biology, Section of Neuroscience, and Center of Excellence for the "Neurobiology of Dependence", University of Cagliari, Cagliari, Italy.
Paolo Follesa, Department of Experimental Biology, Section of Neuroscience, and Center of Excellence for the "Neurobiology of Dependence", University of Cagliari, Cagliari, Italy.
Giovanni Biggio, Department of Experimental Biology, Section of Neuroscience, Center of Excellence for the "Neurobiology of Dependence", University of Cagliari, Cagliari, C.N.R. Institute of Neuroscience, Cagliari, Italy.

1.1. Molecular structure, assembly, and distribution

The $GABA_A$ receptor belongs to the superfamily of ligand-gated ion channels, which also includes the nicotinic acetylcholine, glycine, and 5-HT_3 serotonin receptors (Barnard 1998). It is a macromolecular complex that includes an integral ion channel permeable predominantly to chloride ions, and it mediates the fast component of synaptic inhibition. $GABA_A$ receptors form as a result of the assembly of five subunits from among 21 gene products that have been identified to date, including α_1 to α_6, β_1 to β_4, γ_1 to γ_4, δ, ε, π, ρ_1 to ρ_3, and θ (Barnard 1998). The pentameric combination of these subunits, with certain constraints, thus generates a potentially large number of different $GABA_A$ receptor subtypes. The receptor subunits are polypeptides with a molecular size of ~50 kDa and share substantial sequence similarity. Their common tertiary structure is characterized by an extracellular amino terminus, four hydrophobic transmembrane domains (TM1 to TM4), a large second intracellular loop, and an extracellular carboxyl terminus. The potential for receptor diversity is further increased by the fact that some subunits exist in different forms that result from alternative mRNA splicing; the γ_2 subunit, for example, exists in short (S) and long (L) isoforms that differ by the presence in γ_2L of an additional eight–amino acid sequence that is located in the second intracellular loop and which contains a consensus phosphorylation site for protein kinase C (Whiting 1990).

In the mammalian CNS, most $GABA_A$ receptors are formed from a combination of α and β subunits together with a γ, δ, or ε subunit. $GABA_A$ receptors present predominantly in the retina are an exception in that they comprise homomeric assemblies of ρ subunits (Barnard 1998). These receptors were initially designated $GABA_C$ receptors because of their unusual pharmacology (they are insensitive to bicuculline and benzodiazepines); they were recently reclassified by the International Union of Pharmacology Subcommittee on Nomenclature as $GABA_A$ receptors on the basis of their structural and functional homology with other receptors of this class (Barnard 1998).

The most abundant $GABA_A$ receptor subtype comprises α, β, and γ subunits, with the δ or ε subunit substituting for the γ subunit in certain brain regions (Sieghart 1995; McKernan 1996). The γ subunit interacts with gephyrin, a cytoskeletal protein that contributes to the clustering of $GABA_A$ receptors in the synaptic membrane (Essrich 1998; Nusser 1998). In contrast, most $GABA_A$ receptors that contain the δ subunit are localized extrasynaptically, where they mediate the tonic component of GABAergic inhibition (Semyanov 2004).

The various $GABA_A$ receptor subunits are differentially expressed both with regard to brain region and developmentally (Barnard 1998; McKernan 1996). Certain subunits, such as α_1, are expressed almost ubiquitously, whereas others, such as α_6, are present only in restricted cell populations. In addition, whereas some neuronal populations express almost the entire repertoire of $GABA_A$ receptor subunits, others express only a restricted number. The cerebral cortex, caudate, putamen, and hippocampus exhibit a complex pattern of subunit expression, suggestive of the presence of receptor heterogeneity. These brain regions manifest a high level of expression of α_1, β_2, and γ_2 subunits, with receptors that contain these three subunits constituting the most abundant

$GABA_A$ receptor subtype (~45% of all such receptors) in the CNS (McKernan 1996).

The subunit composition of $GABA_A$ receptors determines their functional properties. The kinetics of activation and deactivation of the Cl^- channel as well as the sensitivity of the receptors to various allosteric modulators, including benzodiazepines, neurosteroids, barbiturates, anesthetics, and zinc, are thus dependent on subunit composition.

1.2. Physiological considerations

$GABA_A$ receptors are responsible for the fast component of the inhibitory postsynaptic current. The $GABA_A$ receptor–operated ion channel is permeable primarily to Cl^-, and the effect of receptor activation depends on the electrochemical gradient of Cl^-. In the CNS of adult animals, the presence of a K^+/Cl^- cotransporter in the neuronal membrane (Rivera 1999) ensures that the concentration of Cl^- in the extracellular compartment is higher than that in the cell and that the equilibrium potential for Cl^- is more negative than is the resting membrane potential. The activation of $GABA_A$ receptors thus results in the influx of Cl^- into the cell and in the hyperpolarization of the neuronal membrane that is responsible for the increase in the threshold of neuronal excitability. In contrast, the K^+/Cl^- cotransporter is not expressed in the cell membrane of immature neurons, which results in a higher concentration of Cl^- inside the cell than out and a more positive Cl^- equilibrium potential. The activation of $GABA_A$ receptors in immature neurons thus leads to membrane depolarization, and such $GABA_A$ receptor–mediated neuronal excitation is thought to be physiologically important for brain development (Ben-Ari 1994). It may contribute, for example, to the ability of GABA to promote both neuronal survival and differentiation during development (Belhage 1998).

GABAergic inhibition has been shown to involve both phasic and tonic components. Whereas phasic inhibition is mediated by postsynaptic $GABA_A$ receptors that are activated by GABA released into the synaptic cleft as a result of the passage of an action potential, tonic inhibition is mediated by a more continuous and weaker activation of $GABA_A$ receptors that are localized extrasynaptically (Semyanov 2004). Tonic GABAergic currents are generated in response to the lower concentrations of GABA that are achieved in the extra-synaptic region as a result either of diffusion of the neurotransmitter away from the synaptic cleft (spillover) or of its release from astrocytes (Liu 2000). Extrasynaptic $GABA_A$ receptors differ from the synaptic receptors in terms of both functional and pharmacological properties. Indeed, consistent with their functional role, they possess a higher affinity for GABA and a reduced rate of desensitization. In addition, extrasynaptic $GABA_A$ receptors manifest a reduced sensitivity to the competitive antagonist SR95531. Structurally, the extrasynaptic receptors are characterized by the presence of the δ subunit. In cerebellar granule cells, $GABA_A$ receptors that contain the δ subunit also contain the α_6 subunit (Nusser 1998), whereas in granule cells of the dentate gyrus of the hippocampus they also contain the α_4 subunit (Sur 1999). The role of tonic inhibition mediated by

extrasynaptic $GABA_A$ receptors is to regulate the membrane potential and thereby to modulate neuronal excitability.

1.3. Pharmacological aspects

The $GABA_A$ receptor is an important target of many drugs. In addition to the agonist binding site, $GABA_A$ receptors contain recognition sites for various endogenous and exogenous molecules that allosterically modulate, either positively or negatively, the inhibitory action of GABA. These recognition sites include those for benzodiazepines, barbiturates, neurosteroids, and picrotoxin. In addition, other drugs such as general anesthetics and alcohol influence the function of these receptors (Barnard 1998; Sieghart 1995; Whiting 2003).

Among these various modulatory sites, that for benzodiazepines is by far the best characterized, in part because of the clinical relevance of this class of drugs. Benzodiazepines, as well as many other related compounds, possess anxiolytic, sedative, hypnotic, anticonvulsant, and muscle-relaxant properties, and the effects of these drugs are mediated by selective facilitation of GABA action at the $GABA_A$ receptor. Molecular studies have revealed that the benzodiazepine recognition site is distinct from but functionally coupled to the GABA binding site, and that it comprises one amino acid residue in the α_1 subunit (histidine-101) (Wieland 1992). The affinity and modulatory efficacy of these drugs at $GABA_A$ receptors are thus greatly influenced by which α and γ subunit isoforms are present. $GABA_A$ receptors that contain the α_1, α_2, α_3, or α_5 subunit thus possess a high affinity for classical benzodiazepines such as diazepam and lorazepam, whereas those containing the α_4 or α_6 subunit have virtually no affinity for these drugs (Barnard 1998; Whiting 2003). Other benzodiazepine-like compounds, such as the imidazopyridine zolpidem and the pyrazolopyrimidine zaleplon, possess selectivity for receptors that contain the α_1 subunit (Barnard 1998; Sanna 2002).

Barbiturates include drugs that are used in anesthesiology and in the treatment of epilepsy. Although the binding site for barbiturates on the $GABA_A$ receptor has not been identified, it is thought to be localized inside the Cl^- channel in the proximity of the TM2 and TM3 domains. Barbiturates appear to potentiate $GABA_A$ receptor–mediated synaptic inhibition by increasing the mean open time of the Cl^- channel, which results in an increase in the Cl^- conductance of the cell membrane (Eghbali 2000). In contrast to benzodiazepines, barbiturates also promote opening of the Cl^- channel in the absence of GABA, although this effect is only apparent at concentrations higher than those effective in modulation of the action of GABA.

Picrotoxin, pentylenetetrazol, and *t*-butylbicyclophosphorothionate act as blockers of the $GABA_A$ receptor–operated Cl^- channel and exhibit a marked convulsant action in vivo. The action of picrotoxin is use dependent and results in a decrease in the mean open time of the channel. The antibiotic penicillin is also able to block the activity of the receptor-associated Cl^- channel; the net negative charge of the antibiotic allows it to interact with positively charged amino acids within the channel and thereby to block channel conductance.

Neurosteroids, including 5α-reduced progesterone metabolites such as allopregnanolone and tetrahydrodeoxycorticosterone (THDOC), as well as synthetic steroids such as alphaxalone and ganaxolone are potent and efficacious positive modulators of $GABA_A$ receptor function. These compounds induce effects similar to those of barbiturates, potentiating the action of GABA as well as increasing Cl^- channel activity in the absence of agonist (Lambert 2003).

1.4. Animal models (transgenic mice)

The generation and characterization of genetically engineered mice have provided important insight into the roles of specific $GABA_A$ receptor subtypes in mediating some of the pharmacological effects of benzodiazepines. Knock-in mice with a point mutation that results in the replacement of histidine-101 of the α_1 subunit of the $GABA_A$ receptor with an arginine residue express α_1-containing receptors with no affinity for benzodiazepines (Rudolph 1999; McKernan 2000). These mutant animals are resistant to the sedative and amnesic effects of diazepam, whereas they exhibit a normal responsiveness to its anxiolytic effect and a partial sensitivity to its anticonvulsant action. These observations thus demonstrated that the sedative and amnesic effects as well as, in part, the anticonvulsant effect of diazepam are mediated by $GABA_A$ receptor subtypes that contain the α_1 subunit . The generation of mice with a homologous mutation in the α_2, α_3, or α_5 subunit has revealed that the anxiolytic effect of diazepam is mediated by receptors that contain the α_2 subunit, whereas all of these three α subunits contribute to the muscle-relaxant effect of the drug (Low 2000; Collinson 2002). With the exception of this latter effect of diazepam, knock-in mice that express the mutant α_5 subunit do not show an altered sensitivity to this benzodiazepine; other studies with these animals have implicated α_5-containing receptors in cognitive and memory processes, however.

1.5. $GABA_A$ Receptor Plasticity

1.5.1. Physiological conditions: pregnancy and the role of steroids

An important feature of $GABA_A$ receptors is their plasticity that is manifest on exposure to brief or long-lasting physiological stimuli or to long-term pharmacological treatment. The kinetic characteristics of the various binding sites associated with the $GABA_A$ receptor, as well as the expression of receptor subunit genes in different regions of the brain, are thus affected by various stimuli or by long-term exposure to a variety of allosteric receptor modulators, including endogenous neurosteroids. Indeed, the neuroactive steroid allopregnanolone plays an important role in the physiological modulation of $GABA_A$ receptor function, expression, and plasticity.

The concentrations of certain neuroactive steroids, including that of allopregnanolone, increase markedly in the plasma and brain and exert a tonic modulatory action on the activity of $GABA_A$ receptors in the cerebral cortex of rats during pregnancy. In contrast, a rapid and substantial decrease in the concentrations of these compounds immediately before parturition and their low

levels during lactation may represent a withdrawal-like phenomenon (Follesa 1998; Concas 1998). The increase in the concentration of allopregnanolone during pregnancy is accompanied by changes in the density and drug sensitivity of $GABA_A$ receptors in the brain (Follesa 1998; Concas 1998 and 1999; Herbison 2001), and these changes are in turn associated with alterations in the expression of $GABA_A$ receptor subunit genes (Follesa 1998; Concas 1998 and 1999). In particular, the amounts of the γ_2 subunit mRNA and protein in the rat cerebral cortex and hippocampus decrease progressively during pregnancy (Follesa 1998). Treatment of dams with finasteride, an inhibitor of the synthesis of neuroactive steroids, prevents this decrease in the amounts of the γ_2 subunit in both the cerebral cortex and the hippocampus (Concas 1998), suggesting that these changes are induced by the increase in neuroactive steroid concentrations during pregnancy. These molecular changes might be expected to affect the pharmacology of $GABA_A$ receptor–mediated neurotransmission. The down-regulation of the γ_2 subunit might thus explain the reduction in the activity of the $GABA_A$ receptor–associated Cl^- channel as well as the reduced ability of diazepam to potentiate GABA action that are apparent during pregnancy (Follesa 1998, Concas 1998).

The amount of the mRNA for the α_5 subunit of the $GABA_A$ receptor also decreases in the cerebral cortex during pregnancy in the rat (Follesa 1998), whereas the abundance of the α_4 subunit mRNA increases in the hippocampus, but not in the cortex, after delivery (Concas 1999). This latter effect on the α_4 subunit mRNA appears consistent with the observation that progesterone withdrawal in rats results in an increase in the amounts of this mRNA and the encoded protein in the hippocampus (Smith 1998). In contrast, the amounts of α_1, α_2, α_3, β_1, β_2, and β_3 subunit mRNAs in the rat cerebral cortex or hippocampus do not change during pregnancy or after delivery, suggesting that the time- and region-dependent changes in the abundance of the γ_2, α_5, and α_4 subunit mRNAs are specific (Follesa 1998; Concas 1998; Fenelon 1996). The amount of the α_1 subunit mRNA does increase in hypothalamic magnocellular neurons during pregnancy (Fenelon 1996; Brussaard 1997), whereas the abundance of α_2 and β_2 subunit mRNAs does not change and that of the γ_2 subunit mRNA decreases in the supraoptic nucleus (Fenelon 1996). These observations suggest that the ratio of α_1-containing to α_2-containing receptors in hypothalamic neurons increases markedly around the time of delivery (Brussaard 2000), a conclusion supported by the detection of changes in $GABA_A$ receptor function and sensitivity to allopregnanolone in these hypothalamic neurons during late pregnancy (Brussaard 1997). It is therefore possible that only select neurons containing specific populations of $GABA_A$ receptors contribute to the changes in receptor function and gene expression observed during pregnancy and after delivery, and these changes may be achieved by different mechanisms. Fluctuations in the concentrations of neuroactive steroids during the reproductive cycle thus induce a $GABA_A$ receptor plasticity in the hypothalamus that differs from that observed in the cerebral cortex or hippocampus (Follesa 1998; Concas 1998 and 1999), and they regulate $GABA_A$ receptor gene expression in a subunit- and neuron-specific manner.

1.5.2. Pharmacological conditions: chronic ethanol treatment and withdrawal

Chronic treatment with positive allosteric modulators that act at different sites of the $GABA_A$ receptor results in changes in the biochemical and functional properties of the receptor that are accompanied by changes in the abundance of specific receptor subunits (Follesa 2003 and 2004; Holt 1996 and 1997; Impagnatiello 1996; Mhatre 1992 and 1993; Montpied 1991; Morrow 1990; Roca 1990; Yu 1996). Long-term ethanol administration and withdrawal also elicit neurochemical and molecular effects in the brain of rats similar to those induced by drugs that potentiate $GABA_A$ receptor function (Follesa 2003 and 2004; Mhatre 1993; Morrow 1990; Cagetti 2003; Devaud 1997; Mahmoudi 1997; Majchrowicz 1975; Schweizer 1998; Tseng 1993). In contrast, $GABA_B$ receptors do not appear to contribute to the pharmacological effects of ethanol (Mehta 1990).

Long-term ethanol treatment increases the binding of Ro 15-4513 in rat brain (Mahtre 1988) and mouse cerebellum (Becker 1996) as well as alters the behavioral effects of this drug (Becker 1989; Mehta 1989), a partial inverse agonist of the benzodiazepine site that antagonizes the pharmacological effects of low doses of ethanol mediated through $GABA_A$ receptors (Mehta 1988; Ticku 1988). Ethanol sensitivity is thought to correlate with the presence of α, β, and γ subunits in $GABA_A$ receptors (Criswell 1993; Mehta 1999).

Effects of chronic ethanol administration and its abrupt withdrawal on $GABA_A$ receptor gene expression and plasticity in several brain regions have been investigated in an attempt to characterize the molecular mechanisms that underlie the phenomena of ethanol dependence and tolerance. Several in vivo studies performed with rats have shown that long-term ethanol treatment reduces the levels of α_1, α_2, and α_5 subunit mRNAs and proteins in the cerebellum and cerebral cortex (Mahtre 1992 and 1993; Morrow 1990; Devaud 1997; Buck 1991; Charlton 1997; Montpied 1991) but does not alter those of γ_2L, γ_3, and δ subunit mRNAs (Devaud 1997) or that of the γ_2 subunit protein in the cerebral cortex. Furthermore, the abundance of the α_4, β_2 or β_3, and γ_1 subunits of the $GABA_A$ receptor is increased in the cerebral cortex of ethanol-dependent rats and of rats subjected to ethanol withdrawal (Devaud 1997; Mahtre1994). In the rat hippocampus, the expression of the α_1 subunit at both the mRNA and protein levels is reduced by chronic ethanol administration, whereas the amounts of α_5 (Charlton 1997), α_4, γ_1, and γ_2S (Devaud 1997) in the hippocampus as well as that of the α_6 subunit mRNA in the cerebellum (Mahtre 1992) are increased in ethanol-dependent animals.

Detailed studies on the effects of chronic ethanol treatment and subsequent ethanol withdrawal have also been performed with cultured neurons (Follesa 2003 and 2004; Sanna 2003) in order to determine whether changes in $GABA_A$ receptor gene expression are accompanied by changes in receptor function. These studies have indeed found a correlation between changes in the expression of specific subunit genes and alterations in the modulatory action of subunit-selective drugs (Follesa 2003 and 2004; Sanna 2003). For example, the amount of the α_4 subunit mRNA increases in cerebellar granule cells and hippocampal

neurons in culture in response to ethanol withdrawal. The functional consequences of this change in α_4 subunit gene expression were examined by determining the ability of flumazenil to modulate receptor function. Electrophysiological recording from individual neurons revealed that flumazenil had virtually no modulatory effect on $GABA_A$ receptor function in either type of neuron either under control conditions or after long-term treatment with ethanol (Follesa 2003; Sanna 2003). In contrast, flumazenil markedly potentiated GABA-evoked Cl^- currents in cells subjected to ethanol withdrawal (Follesa 2003; Sanna 2003). These findings are consistent with the observation that recombinant $GABA_A$ receptors that contain the α_4 subunit manifest a reduced sensitivity to classical benzodiazepine agonists and to zolpidem as well as a distinct pattern of regulation (positive rather than no allosteric modulation) by flumazenil.

Long-term ethanol exposure also reduced the modulatory efficacy of the benzodiazepine receptor agonists lorazepam, zolpidem, and zaleplon as well as that of the inverse agonists Ro 15-4513 and FG 7142 in cultured hippocampal neurons (Sanna 2003), effects that were associated with a reduced abundance of mRNAs encoding the receptor subunits α_1, α_3, and γ_2. Ethanol withdrawal restored the efficacy of lorazepam, but not that of low concentrations of zolpidem or zaleplon, to control values. These effects of ethanol withdrawal were accompanied by up-regulation of the α_2 and α_3 subunit mRNAs and by a persistent down-regulation of the α_1 subunit mRNA.

The changes in $GABA_A$ receptor gene expression induced by ethanol withdrawal are similar to those elicited by withdrawal of benzodiazepines (Follesa 2001), imidazopyridines or pyrazolopyrimidines (Follesa 2002), or neurosteroids (Follesa 2000). These molecular changes might thus reflect a common mechanism by which these various drugs trigger plastic changes in receptor function that underlie the development of withdrawal symptoms in vivo. The increase in the abundance of the α_4 subunit mRNA induced by withdrawal of ethanol, diazepam, or neuroactive steroids might therefore contribute to changes in the sensitivity of $GABA_A$ receptors to drugs and endogenous modulators.

1.6. $GABA_A$ Receptors and Neurological Disorders

The widespread distribution of $GABA_A$ receptors in the brain and their role in the regulation of anxiety, sleep, epileptogenic activity, and muscle tension suggest the possibility that various neurological or mental disorders might be linked to mutations in the genes for the many $GABA_A$ receptor subunits. Tentative associations of human diseases with naturally occurring mutations in these genes have been proposed, and target mutations have been introduced in experimental systems to identify the specific roles of individual $GABA_A$ receptor subunits. The analysis of naturally occurring mutations in animals as well as the experimental disruption of specific $GABA_A$ receptor genes may thus provide models with which to gain insight into the pathophysiology of human

disorders linked to defects in GABAergic transmission. Characterization of the genetic contribution to disease will be easier for single-gene disorders, in which the mutation is inherited in a Mendelian manner, than for complex diseases such as schizophrenia, alcoholism, and epilepsy, in which the inherited component is likely to be multigenic.

We will now review several disorders that have been associated with $GABA_A$ receptor gene mutations, although it remains unclear whether these genetic alterations are fundamental to the etiology of disease. We will also discuss Huntington's disease, with which no alteration of $GABA_A$ receptor genes has been associated to date but in which GABAergic transmission is highly compromised.

1.6.1. Angelman or happy puppet syndrome

Angelman syndrome (AS) is an inherited disorder with a prevalence of 1 in 12,000 to 20,000 (Clayton-Smith 1992; Steffenburg 1996). Its features include severe motor and intellectual retardation, ataxia, hypotonia, epilepsy, absence of speech, bouts of inappropriate laughter, and unusual facies characterized by a large mandible and open-mouthed expression revealing the tongue, prompting Bower and Jeavons to suggest the designation "happy puppet syndrome" (Bower 1967). Given that Angelman had previously described three "puppet children" (Angelman 1965), Williams and Frias suggested the eponym Angelman syndrome to avoid the potentially derisive and derogatory connotations of "happy puppet" (Williams 1982).

Deletions of the proximal region on the long arm of chromosome 15 (bands 15q11–q13) are found in most individuals with two distinct genetic disorders, AS and Prader-Willi syndrome (PWS) (Donlon 1988). Although the deleted genomic regions, which have been defined cytogenetically and with the use of cloned DNA probes, are similar in the two syndromes, the deletion is maternal in origin for AS and paternal in origin for PWS (Knoll 1989; Williams 1990). AS and PWS have become classic examples of genomic imprinting in humans (Ohta 1999; Chan 1993; Glenn 1997; Hulten 1991), given that completely different phenotypes are generated by the absence of maternal (AS) or paternal (PWS) contributions to the q11–q13 region of chromosome 15, which can also result from uniparental disomy instead of deletion (Engel 1980 and 1993).

Most cases of AS are sporadic (Clayton-Smith 1992) and the genetic mechanism underlying familial AS has remained enigmatic. Recent evidence implicates autosomal dominant inheritance of a defect in a gene (or genes) at 15q11–q13 that is subject to genomic imprinting (Meijers-Heijboer 1992). The *UBE3A* gene, which encodes a ubiquitin-protein ligase involved in protein degradation and processing, is mutated in many but not all patients with AS and is considered a major candidate gene for this condition. Other potential candidate genes located in the designated genomic region include a cluster of three $GABA_A$ receptor subunit genes (Saitoh 1992; Wagstaff 1991). Indeed, *GABRB3*, which encodes the β_3 subunit of the $GABA_A$ receptor, is deleted in most individuals with AS (Wagstaff 1991). The corresponding gene is located on chromosome 7 in the mouse, where it is tightly linked to two other genes that

in humans have also been mapped to the 15q11–q13 region (Wagstaff 1991). *GABRB3* is thus a candidate gene for the disturbances in central neurotransmission in AS (Saitoh 1993). The absence of this gene in mice results in craniofacial abnormalities and neurological impairment characterized by seizures (Homanics 1997; DeLorey 1998). The similarities in phenotype between homozygous *Gabrb3* knockout mice and humans with AS have suggested that these mice might prove useful as an animal model of the human disease (DeLorey 1998).

The genes for the α_5 (*GABRA5*) (Glatt 1997) and γ_3 (*GABRG3*) (Greger 1995) subunits of the $GABA_A$ receptor have also been localized to the AS and PWS region of chromosome 15q11–q13 in a cluster with *GABRB3*. The possible role of $GABA_A$ receptor–mediated inhibitory neurotransmission in the pathogenesis of AS was examined by positron emission tomography in patients with a maternal deletion of 15q11–q13 that encompasses *GABRB3* (Holopainen 2001). The binding of flumazenil in the frontal, parietal, hippocampal, and cerebellar regions of the brain was significantly reduced in these patients compared with that in control subjects, suggesting that the genomic deletion results in down-regulation of $GABA_A$ receptors, possibly explaining in part the neurological deficits of AS patients. The plasma concentration of GABA in patients with AS or PWS was also found to be two to three times that in matched control subjects and appeared unrelated to whether the chromosome 15 anomaly was due to deletion or disomy (Ebert 1997). These findings possibly represent a compensatory increase in presynaptic GABA release in response to hyposensitivity of a subset of $GABA_A$ receptors; such a response might result in increased postsynaptic activation of other normal GABA receptors, leading to complex changes in GABAergic function throughout the brain. More than 90% of AS patients exhibit seizures typical of generalized epilepsy. Indeed, AS has been considered a model of symptomatic generalized epilepsy associated with deletion of *GABRB3* (Sugimoto 1992). The electroencephalographic abnormality of AS patients are typical and can resemble in some aspects that of individuals with generalized epilepsy (Rubin 1997; Viani 1995; Boyd 1988). Various types of seizures occur, most prominently myoclonus and atypical absence seizures. The variability in the severity of seizures among AS patients, however, suggests a potential molecular diversity in the genetic mechanism, possibly indicating the involvement of more than one gene.

1.6.2. Epilepsy

It has long been suggested that disruption of GABAergic neurotransmission underlies epilepsy (Olsen 1999). Many genes that confer a predisposition to epilepsy have been identified in humans, and we here review certain types of epilepsy that have been associated with mutations in $GABA_A$ receptor genes.

1.6.2a. Juvenile myoclonic epilepsy

Juvenile myoclonic epilepsy (JME) is a common epileptic syndrome that accounts for up to 26% of all individuals with idiopathic generalized epilepsy (IGE) (Janz 1997). The clinical features of JME differ substantially from those

of generalized epilepsy with febrile seizures, another IGE syndrome (Cossette 2002). Individuals with JME have afebrile seizures only that are characterized by an onset in adolescence (rather than in childhood) and by myoclonic jerks (usually absent in generalized epilepsy with febrile seizures). A family history of "epilepsy" is sometimes noted.

JME has been shown to be caused by mutation of *GABRA1* on chromosome 5q34–q35 (Cossette 2002) as well as by mutations in other genes not directly related to GABAergic neurotransmission (Escayg 2000; Haug 2003). An alanine-322 to aspartate mutation in *GABRA1*, which encodes the α_1 subunit of the $GABA_A$ receptor, was identified in 14 members of a French-Canadian family with JME (Cossette 2002). All affected family members manifested a similar phenotype, with no history of febrile seizures. The condition was inherited in an autosomal dominant manner, with affected individuals spanning more than four generations. $GABA_A$ receptors that contain the mutant α_1 subunit showed a smaller amplitude of GABA-activated currents in vitro than did the corresponding wild-type receptors, indicating that seizures might result from a loss of function of this inhibitory ligand-gated channel.

1.6.2b. Generalized epilepsy with febrile seizures plus

Febrile seizures affect about 3% of children under 6 years of age and constitute the most common seizure disorder. A small proportion of children with febrile seizures later develop ongoing epilepsy with afebrile seizures. Segregation analysis suggests that most cases of febrile seizures have complex inheritance, but rare families appear to show an autosomal dominant pattern. A clinical subset of epilepsy, termed "generalized epilepsy with febrile seizures plus" (GEFS+), has been described, with affected individuals manifesting a highly variable phenotype characterized by febrile seizures, generalized seizures (often precipitated by fever at age 6 years or older), and partial seizures (Scheffer 1997; Singh 1999).

A family in which members of three successive generations have a phenotype consistent with GEFS+ has been studied (Baulac 2001). Whereas some family members have febrile seizures, some have afebrile epileptic seizures and others have both. The affected individuals harbor a lysine-289 to methionine mutation in *GABRG2*, which affects a highly conserved residue located in the extracellular loop between TM2 and TM3 of the γ_2 subunit of the $GABA_A$ receptor. Analysis of $GABA_A$ receptors containing the mutant or wild-type γ_2 subunit in *Xenopus laevis* oocytes showed that the mutation results in a decrease in the amplitude of GABA-activated currents.

A mutation of *GABRG2* that converts the codon for glutamine-351 to a termination codon has also been described in a family with GEFS+, including one individual who developed severe myoclonic epilepsy in infancy (Harkin 2002). This mutation is located in the intracellular loop between TM3 and TM4 of the γ_2 subunit. Expression of $GABA_A$ receptors containing the mutant subunit in *Xenopus* oocytes revealed a complete loss of GABA sensitivity, and fluorescence microscopic analysis showed that receptors containing the mutant subunit labeled with green fluorescent protein were retained in the lumen of the endoplasmic reticulum.

1.6.2c. Childhood absence epilepsy and febrile convulsions

Members from four generations of a family in which childhood absence epilepsy and febrile seizures occurred alone or in combination were found to harbor an arginine-43 to glutamine mutation in *GABRG2* (Wallace 2001). Receptors containing the mutant subunit manifest a complete loss of sensitivity to diazepam in vitro, suggesting the possibility that endogenous benzodiazepine-like compounds (endozepines) exist and play a physiological role in preventing seizures. The observations that the childhood absence epilepsy and febrile seizures had different ages of onset in affected family members and that the physiology of these two conditions is distinct suggest that the mutation has age-dependent effects on different neuronal networks that underlie their expression.

Screening of 135 unrelated German patients with idiopathic absence epilepsy resulted in the identification of a family with a mutation in *GABRG2*. Two siblings had both febrile seizures and childhood absence epilepsy and their father had febrile seizures as a child (Kananura 2002). All three affected individuals harbored the mutation, a T to G substitution in the splice donor site of intron 6, which was predicted to result in exon skipping, premature termination of translation, and the production of a nonfunctional protein.

1.6.3. Insomnia

The screening of 124 individuals for single nucleotide polymorphisms of *GABRA1*, *GABRB3*, and *GABRG2* in the regions that encode the ligand binding domain (Buhr 2002) revealed a heterozygous G to A transition in exon 6 of *GABRB3* that results in replacement of arginine-192 with histidine in an individual with insomnia who also had relatives affected by the same condition. The β_3 subunit of the $GABA_A$ receptor had been implicated in sleep processes independently by the observation that mice lacking this subunit do not exhibit the hypnotic response to oleamide (Laposky 2001). Functional analysis of the mutant human β_3 subunit suggested the possibility that decreased GABAergic inhibition might contribute to the insomnia of the affected family members (Buhr 2002).

1.6.4. Huntington's disease

Huntington's disease (HD) is inherited in an autosomal dominant manner, affects about 1 in 10,000 individuals, and is characterized by progressive and selective neural cell death associated with choreic movements as well as cognitive and psychiatric symptoms and dementia. The onset of symptoms typically occurs in the third or fourth decade of life, although the disease may develop at any age. The molecular basis of HD is the expansion of a CAG trinucleotide repeat in the first exon of the huntingtin gene on chromosome 4p16.3. This mutation results in corresponding expansion of a tract of glutamine residues in the huntingtin protein, from 6 to 39 residues in normal individuals to 36 to 180 residues in HD patients. The normal function of huntingtin and the mechanism by which expansion of the polyglutamine tract causes HD remain unclear. Huntingtin appears to be associated with synaptic vesicles or microtubules in neurons and is implicated in vesicular transport or in the binding of vesicles to the cytoskeleton. It is not known whether overactivity of the

normal function of huntingtin or the gain of a new function is responsible for the toxicity of the mutant protein. It is possible that the formation of intraneuronal aggregates of mutant huntingtin affects gene transcription, protein-protein interactions, protein transport inside the nucleus and cytoplasm, or vesicular transport.

Gross examination has revealed that the brains of about 80% of HD patients show atrophy of the frontal lobes, whereas a bilateral, symmetric atrophy of the striatum is observed in 95% of HD brains. The mean brain weight in HD patients is about 30% lower than that in normal individuals. The striatal degeneration shows an ordered and topographically specific distribution, with the tail of the caudate nucleus being affected more than the head. A gradual atrophy and neuronal loss in other brain regions become apparent as the disease progresses. Projection neurons of the striatum and cerebral cortex are much more susceptible to the disease than are interneurons. The amount of GABA is also decreased in the neostriatum (Bonilla 2000).

Mutation of $GABA_A$ receptor genes has not been detected in association with HD. However, the clinical symptoms of HD are primarily related to the progressive loss of medium spiny GABAergic neurons of the striatum and impairment of GABAergic transmission in several brain regions. Although chorea is associated with dysfunction of the basal ganglia, the underlying molecular mechanism of its development is poorly understood. Analysis of postmortem tissue from the temporal and frontal cortex of HD patients has revealed decreases in the concentrations of GABA and glutamate, suggesting that the corresponding neuronal deficits contribute to the behavioral symptoms of the disease (Pearson 1994). The selective loss of medium spiny neurons in the striatum is also accompanied by a decreased level of associated neurotransmitters, including GABA (Furtado 1995). Neurochemical correlates of chorea in HD were studied with postmortem striatal and pallidal tissue from HD patients with mild or severe chorea. Whereas the amount of GABA was reduced in these brain regions of all HD patients, those with mild chorea had significantly less GABA in the medial pallidum than did those with severe chorea. These observations suggest that chorea in HD may be related to the balance of residual GABAergic innervation between specific regions of the basal ganglia, consistent with results obtained with primate models of dyskinesias (Pearson 1990).

Single photon emission computerized tomography (SPECT) analysis has demonstrated a selective decrease in the number of benzodiazepine receptors in the striatum of HD patients (Pinborg 2001). This finding is in agreement with the previous demonstration by PET with flumazenil (Holopainen 2001) showing that benzodiazepine receptor density was significantly decreased in the caudate nucleus of drug-free patients with early HD (Holthoff 1993). Similar changes in benzodiazepine receptor density in the caudate nucleus were also apparent by postmortem autoradiographic analysis.

A model for the development of chorea based on experimental studies in primates proposes that a loss of striatal GABAergic inhibitory projections to the globus pallidus externa leads to increased activity of inhibitory GABAergic neurons in the latter brain region that project to the subthalamic nucleus. The

loss of GABAergic input to the globus pallidus externa is thought to precede a loss of GABAergic input to the globus pallidus interna, which is associated with the development of rigidity and bradykinesia (Storey 1993).

2. $GABA_B$ Receptors

2.1. Structure and Function

As previously stated, $GABA_B$ receptors are G-protein-coupled metabotropic receptors, and are responsible for the late and slow component of the synaptic inhibitory transmission (Bowery 2002; Couve 2000; Marshall 1999). These receptors represent for GABA, as in the case for other neurotransmitters, an alternative mechanism of inhibitory signal transduction in addition to that mediated by ionotropic $GABA_A$ receptors. $GABA_B$ receptors were first described about two decades ago on the bases of the pharmacological responses to GABA and to antagonists such as bicuculline (Bowery 1980). By investigating the inhibitory effects of GABA on the evoked presynaptic release of the neurotransmitter, it was observed that this action was mediated by a GABA receptor that was not sensitive to the blockade by bicuculline and was not dependent on Cl^- ions, factors that were characteristics of the classic GABA receptors. Such studies demonstrated that baclofen (β-4-chloro-phenyl-GABA), a drug used clinically to treat muscle spasm, mimicked, in a stereoselective manner, the effects of GABA in this model. The term $GABA_B$ was then proposed in order to distinguish this receptor from the bicuculline-sensitive one, which was named $GABA_A$ receptor. Subsequent research allowed to determine that $GABA_B$ receptors are localized, subcellularly, both at presynaptic, on GABAergic (autoreceptors) and non-GABAergic (heteroreceptors) terminal, and postsynaptic sites.

$GABA_B$ receptors are coupled to G-protein belonging generally to $G_{i\alpha}/G_{o\alpha}$ G-protein family which are sensitive to pertussis toxin (PTX), although in some cases, some presynaptic effects of baclofen result PTX-insensitive. $GABA_B$ receptor-associated effector mechanism include the adenylate cyclase system and voltage-dependent Ca^{2+} and K^+ channels (Bowery 2002). $GABA_B$ receptor agonists such as baclofen cause an inhibition of both basal and forskolin-evoked adenylate cyclase activity, producing a decrease in intracellular levels of cAMP. This effect can be blocked by selective $GABA_B$ antagonists such as CGP 54626 (Bowery 2002). Following their activation, $GABA_B$ receptors induce a decrease in Ca^{2+} conductance and an increase in K^+ conductance in neuronal membranes. The effect on Ca^{2+} conductance appears to be mainly associated to an action on P/Q and N type of voltage-dependent Ca^{2+} channels; thus, a reduction of Ca^{2+} influx in the presynaptic terminal through these channels represents the principal mechanism of $GABA_B$ receptor-mediated inhibition of neurotransmitter release. Modulation of K^+ conductance consequent to $GABA_B$ receptor activation is associated mostly with the postsynaptic site; an increase in K^+ permeability and the efflux of K^+ ions from the cell result in an hyperpolarization of the cell membrane with an increased threshold of neuronal excitability.

The molecular structure of the $GABA_B$ receptor was described in detail only following the molecular cloning, in 1997, of two separate genes encoding for two closely related receptor proteins, termed $GABA_BR1$ and $GABA_BR2$ (Kaupmann 1997 and 1998; Jones 1998). These are glycoproteins of about 950 aminoacids which show about 35% identity and 54% similarity. Each of these proteins is present, in rat and human brain, as multiple splice variants. As predicted from previous neurochemical and pharmacological studies, $GABA_BR1$ and $GABA_BR2$ belong to the superfamily of metabotropic receptors, presenting in their structure, in addition to the 7α-helic transmembrane domain, an extended extracellular amino-terminal region, and an intracellular carboxy-tail. These structural features and the high molecular weight make them more similar to class C metabotropic receptors which include also glutamate receptors. In fact, $GABA_B$ receptors share with metabotropic glutamate receptors about 20% identity and 45% similarity. However, $GABA_B$ receptors possess some very peculiar characteristics that make them unique among all the other metabotropic receptors. In fact, expression studies with recombinant $GABA_B$ receptors allowed to establish that the optimal functional arrangement of this receptor is the heterodimer $GABA_BR1$ – $GABA_BR2$ (Jones 1998). These studies have, in fact, showed that the expression of only $GABA_BR1$ or $GABA_BR2$ produce receptors that are not functionally coupled with G-proteins and the predicted effector systems. However, recombinant $GABA_BR1$ receptors retain a certain capability to form a recognition site for the agonist, although the affinity of this receptor is markedly lower than that of native $GABA_B$ receptors. Other studies revealed that the recombinant $GABA_BR1$ is poorly translocated into the cell membrane, remaining confined in the endoplasmic reticulum. However, the fully functional form of the receptor is obtained with the co-expression of both $GABA_BR1$ and $GABA_BR2$. It has been suggested that $GABA_BR2$ might have the role of facilitating the translocation of $GABA_BR1$ to the cell membrane by masking a particular domain (Arg-X-Arg-Arg) present in the carboxy-terminal of $GABA_BR1$, important as a signal for retention in the endoplasmic reticulum.

The two proteins, by interacting through their carboxy-terminal domains, form a coiled-coil structure and they are transported as heterodimer on the cell membrane. The $GABA_BR2$ subunit of the $GABA_B$ receptor is also an important component capable of coupling with the G-protein, whereas the $GABA_BR1$ subunit is essential for the activation of the receptor by the agonist. Thus, the agonist, by recognizing its binding site localized into the amino-terminal domain of this subunit, produces a conformational change of the protein that allows the $GABA_BR2$ subunit to activate the G-protein and therefore the coupled effector system.

2.2. Pharmacological modulation

The molecular structure of the specific agonist baclofen has been modified in order to obtain more potent $GABA_B$ receptor agonists. In particular the 3 amino-propyl-phosphonic acid (3-APPA) and its methylated analog (3-APMPA) posses a very high affinity for the $GABA_B$ receptor which is 3 to 7 times greater

than the active isomer of baclofen. Other agonists of the $GABA_B$ receptor are the methyl-phosphonic derivatives as CGO44532 or its enantiomer (R)-(+)-CGP44533 which show nanomolar affinities. Among the most important pharmacological effects of the $GABA_B$ receptor agonists the muscle relaxant is that with the larger clinical use. This pharmacological action seems to be due to its capability to induce the release of neurotransmitter on the motoneurons in the ventral horn of the spinal cord. On the other hand some other Authors propose that this anti-spastic effect of baclofen may be due to a post-synaptic action rather then pre-synaptic action on motoneurons. Another pharmacological property of baclofen is its anti-pain activity, for this reason it can be prescribed in the treatment of headache, muscular pain and trigeminal pain. This anti-pain action seems to be mediated at both cerebral and spinal level. The role of $GABA_B$ receptors in the control of pain is supported by observations made in $GABA_B$R1 knockout mice, which show a pronounced hyperalgesia to mechanic and thermal stimuli. Other clinical uses of baclofen and other $GABA_B$ receptor agonists include the treatment of urological problems by inducing bladder relaxation, and gastric problems by inducing the mobility of the gastrointestinal tract.

Among the antagonists of the $GABA_B$ receptor there are some baclofen derivatives such as faclofen, saclofen and 2-hydroxy saclofen. Even though they posses a modest affinity for the $GABA_B$ receptors, they have played a pivotal role in the study of the physiological and pharmacological properties of these receptors. Today new compounds are available such as CGP52423, CGP54626A and CGP55845a which show affinities 10,000 times higher compared to the old derivatives. Animal studies suggest a potential therapeutic use of these compounds as anti-epileptic agents in the absence. In addition they are able to improve the cognitive performance in several animal tests. Accordingly the $GABA_B$R1 knockout mice show an impairment in learning and memory compared to the wild-type mice. The $GABA_B$ receptor antagonists have been proven to be active against neurotoxicity induced by glutamate and NMDA. In support of these data there is the observation that $GABA_B$ receptor antagonists increased the hippocampal abundance of NGF and BDNF. This effect has been observed also in the rat cerebral cortex and spinal cord suggesting that these antagonists could be useful against the degenerative processes.

2.3. Drug addiction

A number of different clinical and experimental studies indicate that $GABA_B$ receptors may be neurochemical targets for drugs useful in the treatment of drug addiction (Couve 2000). Gamma-hydroxybutyrate (GHB), a derivative of GABA (Jones 1998), has been shown to be effective in decreasing alcohol intake (Agabio 1998; Colombo 1998; Biggio 1992; Gallimberti 1992; Poldrugo 1999) and in attenuating alcohol withdrawal symptoms (Fadda 1989; Gallimberti 2000). The inhibitory effect of GHB can be selectively blocked by $GABA_B$ receptor antagonists (Xie 1992). In line with this idea, the $GABA_B$ receptor agonist baclofen was shown to reduce alcohol consumption in rats and

alcoholics (Addolorato 2000; Colombo 2000) and the severity of alcohol withdrawal syndrome (Colombo 2000; File 1993).

Baclofen has also been proposed to be effective in reducing the reinforcing properties of alcohol as well as other addictive drugs, by inhibiting the drug-stimulated release of dopamine from the mesocorticolimbic system (Shoaib 1998). Baclofen reduced cocaine and opiate self administration (Shoaib 1998; Brebner 1999; Roberts 1997; Xi 1999). In a clinical trial, baclofen reduced craving for cocaine and induced an overall decrease in drug use indicating that $GABA_B$ receptor agonists appear to be potential useful therapeutic agents.

3. REFERENCES

Addolorato, G., et al., 2000, Ability of baclofen in reducing alcohol craving and intake: II–Preliminary clinical evidence. *Alcohol Clin Exp Res*. **24**(1): p. 67-71.

Agabio, R., et al., 1998, Gamma-hydroxybutyric acid reducing effect on ethanol intake: evidence in favour of a substitution mechanism. *Alcohol Alcohol*. **33**(5): p. 465-74.

Angelman, H., 1965, "Puppet children": a report of three cases. Dev Med Child Neurol. 7: p. 681-688.

Awapara, J., 1950, Occurrence of free gamma-aminobutyric acid in brain and its formation from L-glutamic acid. *Tex Rep Biol Med*. **8**(4): p. 443-7.

Barnard, E.A., et al., 1998, International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev*. **50**(2): p. 291-313.

Baulac, S., et al., 2001, First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat Genet*. **28**(1): p. 46-8.

Becker, H.C. and Anton, R.F., 1989, The benzodiazepine receptor inverse agonist RO15-4513 exacerbates, but does not precipitate, ethanol withdrawal in mice. *Pharmacol Biochem Behav*. **32**(1): p. 163-7.

Becker, H.C. and Jarvis, M.F., 1996, Chronic ethanol exposure selectively increases diazepam-insensitive [3H]RO15-4513 binding in mouse cerebellum. *Eur J Pharmacol*. **296**(1): p. 43-6.

Belhage, B., et al., 1998, Effects of gamma-aminobutyric acid (GABA) on synaptogenesis and synaptic function. *Perspect Dev Neurobiol*. **5**(2-3): p. 235-46.

Ben-Ari, Y., et al., 1994, Gamma-Aminobutyric acid (GABA): a fast excitatory transmitter which may regulate the development of hippocampal neurones in early postnatal life. *Prog Brain Res*. **102**: p. 261-73.

Biggio, G., et al., 1992, Suppression of voluntary alcohol intake in rats and alcoholics by gamma-hydroxybutyric acid: a non-GABAergic mechanism. *Adv Biochem Psychopharmacol*. **47**: p. 281-8.

Bonilla, E., 2000, [Huntington disease. A review]. Invest Clin. **41**(2): p. 117-41.

Bower, B.D. and Jeavons, P.M., 1967, The "happy puppet" syndrome. Arch Dis Child. **42**(223): p. 298-302.

Bowery, N.G., et al., 1980, (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*. **283**(5742): p. 92-4.

Bowery, N.G., et al., 2002, International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev*. **54**(2): p. 247-64.

Boyd, S.G., Harden, A. and Patton, M.A., 1988, The EEG in early diagnosis of the Angelman (happy puppet) syndrome. *Eur J Pediatr*. **147**(5): p. 508-13.

Brebner, K., et al., 1999, The GABA(B) agonist CGP 44532 decreases cocaine self-administration in rats: demonstration using a progressive ratio and a discrete trials procedure. *Neuropharmacology*. **38**(11): p. 1797-804.

Brussaard, A.B. and Herbison, A.E., 2000, Long-term plasticity of postsynaptic GABAA-receptor function in the adult brain: insights from the oxytocin neurone. *Trends Neurosci*. **23**(5): p. 190-5.

Brussaard, A.B., et al., 1997, Plasticity in fast synaptic inhibition of adult oxytocin neurons caused by switch in GABA(A) receptor subunit expression. *Neuron*. **19**(5): p. 1103-14.

Buck, K.J., et al., 1991, Chronic ethanol treatment alters brain levels of gamma-aminobutyric acidA receptor subunit mRNAs: relationship to genetic differences in ethanol withdrawal seizure severity. *J Neurochem*. **57**(4): p. 1452-5.

Buhr, A., et al., 2002, Functional characterization of the new human GABA(A) receptor mutation beta3(R192H). *Hum Genet*. **111**(2): p. 154-60.

Cagetti, E., et al., 2003, Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABAA receptors. *Mol Pharmacol*. **63**(1): p. 53-64.

Chan, C.T., et al., 1993, Molecular mechanisms in Angelman syndrome: a survey of 93 patients. *J Med Genet.* **30**(11): p. 895-902.

Charlton, M.E., et al., 1997, Chronic ethanol administration regulates the expression of GABAA receptor alpha 1 and alpha 5 subunits in the ventral tegmental area and hippocampus. J Neurochem. **68**(1): p. 121-7.

Clayton-Smith, J. and Pembrey, M.E., 1992, Angelman syndrome. *J Med Genet*. **29**(6): p. 412-5.

Clayton-Smith, J., et al., 1992, Further evidence for dominant inheritance at the chromosome 15q11-13 locus in familial Angelman syndrome. *Am J Med Genet*. **44**(2): p. 256-60.

Collinson, N., et al., 2002, Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. *J Neurosci*. **22**(13): p. 5572-80.

Colombo, G., et al., 1998, Gamma-hydroxybutyric acid intake in ethanol-preferring sP and -nonpreferring sNP rats. *Physiol Behav*. **64**(2): p. 197-202.

Colombo, G., et al., 2000, Ability of baclofen in reducing alcohol intake and withdrawal severity: I–Preclinical evidence. *Alcohol Clin Exp Res*. **24**(1): p. 58-66.

Concas, A., et al., 1998, Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc Natl Acad Sci USA*. **95**(22): p. 13284-9.

Concas, A., et al., 1999, Physiological modulation of GABA(A) receptor plasticity by progesterone metabolites. *Eur J Pharmacol*. **375**(1-3): p. 225-35.

Cossette, P., et al., 2002, Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet*. **31**(2): p. 184-9.

Couve, A., Moss, S.J. and Pangalos, M.N., 2000, GABAB receptors: a new paradigm in G protein signaling. *Mol Cell Neurosci.* **16**(4): p. 296-312.

Criswell, H.E., et al., 1993, Molecular basis for regionally specific action of ethanol on gamma-aminobutyric acidA receptors: generalization to other ligand-gated ion channels. *J Pharmacol Exp Ther*. **267**(1): p. 522-37.

DeLorey, T.M., et al., 1998, Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci*. **18**(20): p. 8505-14.

Devaud, L.L., et al., 1997, Bidirectional alterations of GABA(A) receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. *J Neurochem.* **69**(1): p. 126-30.

Donlon, T.A., 1988, Similar molecular deletions on chromosome 15q11.2 are encountered in both the Prader-Willi and Angelman syndromes. *Hum Genet*. **80**(4): p. 322-8.

Ebert, M.H., et al., 1997, Elevated plasma gamma-aminobutyric acid (GABA) levels in individuals with either Prader-Willi syndrome or Angelman syndrome. *J Neuropsychiatry Clin Neurosci*. **9**(1): p. 75-80.

Eghbali, M., Gage, P.W. and Birnir, B., 2000, Pentobarbital modulates gamma-aminobutyric acid-activated single-channel conductance in rat cultured hippocampal neurons. *Mol Pharmacol*. **58**(3): p. 463-9.

Engel, E., 1980, A new genetic concept: uniparental disomy and its potential effect, isodisomy. *Am J Med Genet*. **6**(2): p. 137-43.

Engel, E., 1993, Uniparental disomy revisited: the first twelve years. *Am J Med Genet.* **46**(6): p. 670-4.

Escayg, A., et al., 2000, Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. *Am J Hum Genet*. **66**(5): p. 1531-9.

Essrich, C., et al., 1998, Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. *Nat Neurosci*. **1**(7): p. 563-71.

Fadda, F., et al., 1989, Suppression by gamma-hydroxybutyric acid of ethanol withdrawal syndrome in rats. *Alcohol Alcohol*. **24**(5): p. 447-51.

Fenelon, V.S. and Herbison, A.E., 1996, Plasticity in GABAA receptor subunit mRNA expression by hypothalamic magnocellular neurons in the adult rat. *J Neurosci*. **16**(16): p. 4872-80.

File, S.E., and Andrews, N., 1993, Benzodiazepine withdrawal: behavioural pharmacology and neurochemical changes. *Biochem Soc Symp*. **59**: p. 97-106.

Follesa, P., et al., 1998, Molecular and functional adaptation of the GABA(A) receptor complex during pregnancy and after delivery in the rat brain. *Eur. J. Neurosci*. **10**(9): p. 2905-12.

Follesa, P., et al., 2000, Allopregnanolone synthesis in cerebellar granule cells: roles in regulation of GABA(A) receptor expression and function during progesterone treatment and withdrawal. *Mol Pharmacol*. **57**(6): p. 1262-70.

Follesa, P., et al., 2001, Role of allopregnanolone in regulation of GABA(A) receptor plasticity during long-term exposure to and withdrawal from progesterone. *Brain Res Brain Res Rev*. **37**(1-3): p. 81-90.

Follesa, P., et al., 2002, Changes in GABAA receptor gamma 2 subunit gene expression induced by long-term administration of oral contraceptives in rats. *Neuropharmacology*. **42**(3): p. 325-36.

Follesa, P., et al., 2003, Gamma-hydroxybutyric acid and diazepam antagonize a rapid increase in GABA(A) receptors alpha(4) subunit mRNA abundance induced by ethanol withdrawal in cerebellar granule cells. *Mol Pharmacol*. **63**(4): p. 896-907.

Follesa, P., et al., 2004, Ethanol withdrawal-induced up-regulation of the alpha2 subunit of the GABAA receptor and its prevention by diazepam or gamma-hydroxybutyric acid. *Brain Res Mol Brain Res.* **120**(2): p. 130-7.

Furtado, S. and Suchowersky, O., 1995, Huntington's disease: recent advances in diagnosis and management. *Can J Neurol Sci*. **22**(1): p. 5-12.

Gallimberti, L., et al., 1992, Gamma-Hydroxybutyric acid in the treatment of alcohol dependence: a double-blind study. *Alcohol Clin Exp Res*. **16**(4): p. 673-6.

Gallimberti, L., et al., 2000, Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence. *Alcohol*. **20**(3): p. 257-62.

Glatt, K., Glatt, H. and Lalande, M., 1997, Structure and organization of GABRB3 and GABRA5. *Genomics*. **41**(1): p. 63-9.

Glenn, C.C., et al., 1997, Genomic imprinting: potential function and mechanisms revealed by the Prader-Willi and Angelman syndromes. *Mol Hum Reprod*. **3**(4): p. 321-32.

Greger, V., et al., 1995, The gamma-aminobutyric acid receptor gamma 3 subunit gene (GABRG3) is tightly linked to the alpha 5 subunit gene (GABRA5) on human chromosome 15q11-q13 and is transcribed in the same orientation. *Genomics*. **26**(2): p. 258-64.

Harkin, L.A., et al., 2002, Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet*. **70**(2): p. 530-6.

Haug, K., et al., 2003, Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. *Nat Genet*. **33**(4): p. 527-32.

Herbison, A.E., 2001, Physiological roles for the neurosteroid allopregnanolone in the modulation of brain function during pregnancy and parturition. *Prog Brain Res.* **133**: p. 39-47.

Holopainen, I.E., et al., 2001, Decreased binding of [11C]flumazenil in Angelman syndrome patients with GABA(A) receptor beta3 subunit deletions. *Ann Neurol.* **49**(1): p. 110-3.

Holt, R.A., Bateson, A.N. and Martin, I.L., 1996, Chronic treatment with diazepam or abecarnil differently affects the expression of GABAA receptor subunit mRNAs in the rat cortex. *Neuropharmacology*. **35**(9-10): p. 1457-63.

Holt, R.A., Martin, I.L. and Bateson, A.N., 1997, Chronic diazepam exposure decreases transcription of the rat GABA(A) receptor gamma2-subunit gene. *Brain Res Mol Brain Res*. 48(1): p. 164-6.

Holthoff, V.A., et al., 1993, Positron emission tomography measures of benzodiazepine receptors in Huntington's disease. *Ann Neurol*. **34**(1): p. 76-81.

Homanics, G.E., et al., 1997, Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc Natl Acad Sci USA*. **94**(8): p. 4143-8.

Hulten, M., et al., 1991, Genomic imprinting in an Angelman and Prader-Willi translocation family. *Lancet*. **338**(8767): p. 638-9.

Impagnatiello, F., et al., 1996, Modifications of gamma-aminobutyric acidA receptor subunit expression in rat neocortex during tolerance to diazepam. *Mol Pharmacol*. **49**(5): p. 822-31.

Janz, D. and Durner, M., 1997, Juvenile myoclonic epilepsy, in Epilepsy: A Comprehensive Textbook, E.J. Engel and T.A. Pedley, Editors, Lippincott-Raven: Philadelphia. p. 2389-2400.

Jones, K.A., et al., 1998, GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature*. **396**(6712): p. 674-9.

Kananura, C., et al., 2002, A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol*. **59**(7): p. 1137-41.

Kaupmann, K., et al., 1997, Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature*. **386**(6622): p. 239-46.

Kaupmann, K., et al., 1998, GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature*. **396**(6712): p. 683-7.

Kaupmann, K., et al., 1998, Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K+ channels. *Proc Natl Acad Sci USA.* **95**(25): p. 14991-6.

Knoll, J.H., et al., 1989, Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. *Am J Med Genet*. **32**(2): p. 285-90.

Lambert, J.J., et al., 2003, Neurosteroid modulation of GABAA receptors. *Prog Neurobiol*. **71**(1): p. 67-80.

Laposky, A.D., et al., 2001, Deletion of the GABA(A) receptor beta 3 subunit eliminates the hypnotic actions of oleamide in mice. *Neuroreport*. **12**(18): p. 4143-7.

Liu, Q.Y., et al., 2000, Persistent activation of GABA(A) receptor/Cl(-) channels by astrocyte-derived GABA in cultured embryonic rat hippocampal neurons. *J Neurophysiol.* 84(3): p. 1392-403.

Low, K., et al., 2000, Molecular and neuronal substrate for the selective attenuation of anxiety. *Science*. **290**(5489): p. 131-4.

Mahmoudi, M., et al., 1997, Chronic intermittent ethanol treatment in rats increases GABA(A) receptor alpha4-subunit expression: possible relevance to alcohol dependence. *J Neurochem*. **68**(6): p. 2485-92.

Maitre, M., 1997, The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog Neurobiol*. **51**(3): p. 337-61.

Majchrowicz, E., 1975, Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia*. **43**(3): p. 245-54.

Marshall, F.H., et al., 1999, GABAB receptors - the first 7TM heterodimers. Trends Pharmacol Sci. **20**(10): p. 396-9.

McKernan, R.M. and Whiting, P.J., 1996, Which GABAA-receptor subtypes really occur in the brain? *Trends Neurosci*. **19**(4): p. 139-43.

McKernan, R.M., et al., 2000, Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. *Nat Neurosci*. **3**(6): p. 587-92.

Mehta, A.K. and Ticku, M.K., 1988, Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves gamma-aminobutyric acidA-gated chloride channels. *J Pharmacol Exp Ther.* **246**(2): p. 558-64.

Mehta, A.K. and Ticku, M.K., 1989, Chronic ethanol treatment alters the behavioral effects of Ro 15-4513, a partially negative ligand for benzodiazepine binding sites. *Brain Res,* 1989. **489**(1): p. 93-100.

Mehta, A.K. and Ticku, M.K., 1990, Are GABAB receptors involved in the pharmacological effects of ethanol? *Eur J Pharmacol*. **182**(3): p. 473-80.

Mehta, A.K. and Ticku, M.K., 1999, An update on GABAA receptors. *Brain Res Brain Res Rev.* **29**(2-3): p. 196-217.

Meijers-Heijboer, E.J., et al., 1992, Linkage analysis with chromosome 15q11-13 markers shows genomic imprinting in familial Angelman syndrome. *J Med Genet*. **29**(12): p. 853-7.

Mhatre, M. and Ticku, M.K., 1994, Chronic ethanol treatment upregulates the GABA receptor beta subunit expression. *Brain Res Mol Brain Res*. **23**(3): p. 246-52.

Mhatre, M., Mehta, A.K. and Ticku, M.K., 1988, Chronic ethanol administration increases the binding of the benzodiazepine inverse agonist and alcohol antagonist [3H]RO15-4513 in rat brain. *Eur J Pharmacol*. **153**(1): p. 141-5.

Mhatre, M.C. and Ticku, M.K., 1992, Chronic ethanol administration alters gamma-aminobutyric acidA receptor gene expression. *Mol Pharmacol*. **42**(3): p. 415-22.

Mhatre, M.C., et al., 1993, Antibodies specific for GABAA receptor alpha subunits reveal that chronic alcohol treatment down-regulates alpha-subunit expression in rat brain regions. *J Neurochem*. **61**(5): p. 1620-5.

Montpied, P., et al., 1991, Gamma-Aminobutyric acid (GABA) induces a receptor-mediated reduction in GABAA receptor alpha subunit messenger RNAs in embryonic chick neurons in culture. *J Biol Chem*. **266**(10): p. 6011-4.

Montpied, P., et al., 1991, Prolonged ethanol inhalation decreases gamma-aminobutyric acidA receptor alpha subunit mRNAs in the rat cerebral cortex. *Mol Pharmacol.* **39**(2): p. 157-63.

Morrow, A.L., et al., 1990, Chronic ethanol and pentobarbital administration in the rat: effects on GABAA receptor function and expression in brain. *Alcohol*. **7**(3): p. 237-44.

Nusser, Z., Sieghart, W. and Somogyi, P., 1998, Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci*. **18**(5): p. 1693-703.

Ohta, T., et al., 1999, Molecular mechanism of angelman syndrome in two large families involves an imprinting mutation. *Am J Hum Genet*. **64**(2): p. 385-96.

Olsen, R.W., et al., 1999, GABA receptor function and epilepsy. *Adv Neurol.* **79**: p. 499-510.

Pearson, S.J. and Reynolds, G.P., 1994, Neocortical neurotransmitter markers in Huntington's disease. *J Neural Transm Gen Sect*. **98**(3): p. 197-207.

Pearson, S.J., Heathfield, K.W., and Reynolds, G.P., 1990, Pallidal GABA and chorea in Huntington's disease. *J Neural Transm Gen Sect.* **81**(3): p. 241-6.

Pinborg, L.H., et al., 2001, Benzodiazepine receptor quantification in Huntington's disease with [(123)I]omazenil and SPECT. *J Neurol Neurosurg Psychiatry*. **70**(5): p. 657-61.

Poldrugo, F. and Addolorato, G., 1999, The role of gamma-hydroxybutyric acid in the treatment of alcoholism: from animal to clinical studies. *Alcohol Alcohol*. **34**(1): p. 15-24.

Rivera, C., et al., 1999, The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. **397**(6716): p. 251-5.

Roberts, D.C., and Andrews, M.M., 1997, Baclofen suppression of cocaine self-administration: demonstration using a discrete trials procedure. *Psychopharmacology* (Berl). **131**(3): p. 271-7.

Roberts, E. and Frankel, S., 1950, Gamma-Aminobutyric acid in brain: its formation from glutamic acid. *J Biol Chem*, **187**(1): p. 55-63.

Roca, D.J., et al., 1990, Chronic agonist exposure induces down-regulation and allosteric uncoupling of the gamma-aminobutyric acid/benzodiazepine receptor complex. *Mol Pharmacol*. **37**(1): p. 37-43.

Roca, D.J., et al., 1990, Gamma-Aminobutyric acidA receptor regulation in culture: altered allosteric interactions following prolonged exposure to benzodiazepines, barbiturates, and methylxanthines. *Mol Pharmacol*. **37**(5): p. 710-9.

Rubin, D.I., et al., 1997, Angelman's syndrome: clinical and electroencephalographic findings. *Electroencephalogr Clin Neurophysiol*. **102**(4): p. 299-302.

Rudolph, U., et al., 1999, Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature*. **401**(6755): p. 796-800.

Saitoh, S. and Niikawa, N., 1993, [The GABAA receptor beta 3-subunit gene (GABRB3) as a candidate responsible for central nerve disturbances in Angelman syndrome (AS)]. *Nippon Rinsho*. **51**(9): p. 2409-13.

Saitoh, S., et al., 1992, Familial Angelman syndrome caused by imprinted submicroscopic deletion encompassing GABAA receptor beta 3-subunit gene. *Lancet*. **339**(8789): p. 366-7.

Sanna, E., et al., 2002, Comparison of the effects of zaleplon, zolpidem, and triazolam at various GABA(A) receptor subtypes. *Eur J Pharmacol*. **451**(2): p. 103-10.

Sanna, E., et al., 2003, Changes in GABA(A) receptor gene expression associated with selective alterations in receptor function and pharmacology after ethanol withdrawal. *J Neurosci*. **23**(37): p. 11711-24.

Scheffer, I.E., and Berkovic, S.F., 1997, Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. *Brain*. **120** (Pt 3): p. 479-90.

Schweizer, E. and Rickels, K., 1998, Benzodiazepine dependence and withdrawal: a review of the syndrome and its clinical management. *Acta Psychiatr Scand Suppl*. **393**: p. 95-101.

Semyanov, A., et al., 2004, Tonically active GABA A receptors: modulating gain and maintaining the tone. *Trends Neurosci*. **27**(5): p. 262-9.

Shoaib, M., et al., 1998, The GABAB agonist baclofen modifies cocaine self-administration in rats. *Behav Pharmacol*. **9**(3): p. 195-206.

Sieghart, W., 1995, Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. *Pharmacol Rev*. **47**(2): p. 181-234.

Singh, R., et al., 1999, Generalized epilepsy with febrile seizures plus: a common childhood-onset genetic epilepsy syndrome. *Ann Neurol*. **45**(1): p. 75-81.

Smith, S.S., et al., 1998, GABA(A) receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroid. *Nature*. **392**(6679): p. 926-30.

Smith, S.S., et al., 1998, Withdrawal from 3alpha-OH-5alpha-pregnan-20-One using a pseudopregnancy model alters the kinetics of hippocampal GABAA-gated current and increases the GABAA receptor alpha4 subunit in association with increased anxiety. *J Neurosci*. **18**(14): p. 5275-84.

Steffenburg, S., et al., 1996, Autism in Angelman syndrome: a population-based study. *Pediatr Neurol*. 14(2): p. 131-6.

Storey, E. and Beal, M.F., 1993, Neurochemical substrates of rigidity and chorea in Huntington's disease. *Brain*. **116** (Pt 5): p. 1201-22.

Sugimoto, T., et al., 1992, Angelman syndrome in three siblings: characteristic epileptic seizures and EEG abnormalities. *Epilepsia*. **33**(6): p. 1078-82.

Sur, C., et al., 1999, Preferential coassembly of alpha4 and delta subunits of the gamma-aminobutyric acidA receptor in rat thalamus. *Mol Pharmacol*. **56**(1): p. 110-5.

Ticku, M.K. and Kulkarni, S.K., 1988, Molecular interactions of ethanol with GABAergic system and potential of RO15-4513 as an ethanol antagonist. *Pharmacol Biochem Behav*. **30**(2): p. 501-10.

Tseng, Y.T., Miyaoka, T. and Ho, I.K., 1993, Region-specific changes of GABAA receptors by tolerance to and dependence upon pentobarbital. *Eur J Pharmacol*. **236**(1): p. 23-30.

Viani, F., et al., 1995, Seizure and EEG patterns in Angelman's syndrome. *J Child Neurol*. **10**(6): p. 467-71.

Wagstaff, J., Chaillet, J.R. and Lalande, M., 1991, The GABAA receptor beta 3 subunit gene: characterization of a human cDNA from chromosome 15q11q13 and mapping to a region of conserved synteny on mouse chromosome 7. *Genomics*. **11**(4): p. 1071-8.

Wagstaff, J., et al., 1991, Localization of the gene encoding the GABAA receptor beta 3 subunit to the Angelman/Prader-Willi region of human chromosome 15. *Am J Hum Genet.* **49**(2): p. 330-7.

Wallace, R.H., et al., 2001, Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. **28**(1): p. 49-52.

Whiting, P., McKernan, R.M., and Iversen, L.L., 1990, Another mechanism for creating diversity in gamma-aminobutyrate type A receptors: RNA splicing directs expression of two forms of gamma 2 phosphorylation site. *Proc Natl Acad Sci USA*, **87**(24): p. 9966-70.

Whiting, P.J., 2003, The GABAA receptor gene family: new opportunities for drug development. *Curr Opin Drug Discov Devel*. **6**(5): p. 648-57.

Wieland, H.A., Luddens, H. and. Seeburg, P.H., 1992, A single histidine in GABAA receptors is essential for benzodiazepine agonist binding. *J Biol Chem*. **267**(3): p. 1426-9.

Williams, C.A. and Frias, J.L., 1982, The Angelman ("happy puppet") syndrome. *Am J Med Genet*. 11(4): p. 453-60.

Williams, C.A., et al., 1990, Maternal origin of 15q11-13 deletions in Angelman syndrome suggests a role for genomic imprinting. *Am J Med Genet*. **35**(3): p. 350-3.

Xi, Z.X. and Stein, E.A., 1999, Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J Pharmacol Exp Ther*. **290**(3): p. 1369-74.

Xie, X. and Smart, T.G., 1992, Gamma-hydroxybutyrate hyperpolarizes hippocampal neurones by activating GABAB receptors. *Eur J Pharmacol*. **212**(2-3): p. 291-4.

Yu, R., Follesa, P. and Ticku, M.K., 1996, Down-regulation of the GABA receptor subunits mRNA levels in mammalian cultured cortical neurons following chronic neurosteroid treatment. *Brain Res Mol Brain Res*. **41**(1-2): p. 163-8.

16. CYTOCHROMES P450

Marja-Liisa Dahl and Maria Gabriella Scordo*

1. INTRODUCTION

The pharmacokinetics of antidepressant and antipsychotic drugs are subject to large interindividual variability, leading to great differences in the doses required to achieve the same serum drug concentration at steady state. Subsequently, this pharmacokinetic variability can partly explain the wide range of doses required for optimal therapeutic effect. This is especially true for the classical tricyclic antidepressants (TCA) where up to 30-fold differences in both pharmacokinetics and dose requirement have been shown (Hammer 1967).

The classical antidepressants and antipsychotics have a relatively narrow therapeutic index, concentration-dependent adverse effects occurring at levels similar to or only slightly higher than those required for therapeutic effect. The newer antipsychotics, sometimes referred to as atypical, have a broader therapeutic index with respect to extrapyramidal side effects. Similarly, newer antidepressant compounds, especially of the selective serotonin reuptake inhibitor group, lack the cardiovascular and CNS toxicity shown by TCAs. Still, concentration-dependent adverse effects do exist even for these compounds. Polypharmacy is common in psychiatric practice, and exposes the patients to the risk of pharmacokinetic interactions. Adverse dug reactions may resemble the symptoms of the disease treated, and, finally, patients not seldom comply poorly with the drug regimen. Thus, dosage optimisation is an important issue for all compounds used in psychiatry.

All antipsychotics and antidepressants, with the exception of lithium, are highly lipophilic compounds subject to extensive metabolism in the body before excretion. Many have active metabolites contributing to the pharmacological effects of the drug. Much of the pharmacokinetic variability can be traced back to interindividual differences in the metabolism of these compounds, even though other pharmacokinetic processes such as distribution and absorption may also vary. The metabolic capacity in its turn is subject to genetic, environmental

* Marja-Liisa Dahl & Maria Gabriella Scordo, Department of Medical Sciences, Clinical Pharmacology, Uppsala University, SE-75185 Uppsala, Sweden

as well as pathophysiological regulation. Enzymes belonging to the cytochrome P450 (CYP) superfamily have a central role in the oxidative metabolism of psychotropic drugs. CYPs typically show distinct but overlapping substrate specificity as well as large inter- and sometimes intraindividual differences in activity. Each CYP isoform is a specific gene product, and a number of variant alleles of each gene have been characterised. The allelic variants may be due to deletion of the entire gene, single nucleotide polymorphisms (SNPs), deletion or insertion of fragments of DNA within the gene, or multiple copies of a gene, leading to absent, deficient or enhanced enzyme activity. Some of the enzymes, notably CYP2D6, CYP2C9 and CYP2C19, are polymorphic also at phenotype level, allowing division of a population into at least two phenotypes, extensive metabolisers (EM) and poor metabolisers (PM), and, with respect to CYP2D6, ultrarapid metabolisers (UM). The activity of these enzymes is to a high extent genetically regulated while the activities of CYP1A2 and CYP3A4/5 are influenced by a number of constitutional and environmental factors.

The clinical importance of polymorphic metabolism depends on a number of factors including whether the parent compound, metabolite(s) or both are metabolised by the polymorphic enzyme; whether the parent compound, metabolite(s) or both are pharmacologically active; the relative potency of the active compounds, and the overall contribution of the polymorphic pathway to the total clearance of the drug. Furthermore, the therapeutic index (narrow-broad), possible saturation of the polymorphic pathway and the contribution of other enzymes need to be considered. The clinical consequences of polymorphic genes are expected to be extreme for subjects homozygous for defect genes (PM), or for those with duplicate or amplified functional genes (UM). In the former case, impaired enzyme activity may cause diminished first pass metabolism, increased bioavailability, decreased elimination and thus possibly an exaggerated response. If the enzyme activates the drug to an active metabolite, one might predict loss of therapeutic efficacy among PMs. On the contrary, UMs may not achieve therapeutic levels of the drug given at a standard dose and this might account for lack of therapeutic effect. Furthermore, in cases where the enzyme bioactivates the drug, increased plasma levels of the active metabolite can be expected in UMs. Therefore, the possibility to start the therapy at the right dosage, according to the patient's metabolic capacity, would be expected to improve treatment outcome by decreasing the occurrence of concentration-dependent adverse effects and therapeutic failure or delayed response.

The recent advances in the understanding of the molecular genetics of drug metabolism in general, and CYPs in particular, have created expectations of the use of pharmacogenetic testing for optimization of drug treatment. Pharmacogenetic tools available are phenotyping, i.e. prediction of a specific enzyme activity by use of a probe drug, and genotyping, analysis of functionally important mutations, or markers thereof, in the gene coding for the specific enzyme.

2. CYTOCHROME P450 ENZYMES

The CYP system is a superfamily of isoenzymes located in the smooth endoplasmic reticulum, mainly in the liver, but also in extrahepatic tissues (e.g. intestinal mucosa, lung, kidney, brain, lymphocytes, placenta, etc.) (Gonzales 1992 and Guengerich 1995). These enzymes are responsible for the oxidative metabolism of a number of drugs and other exogenous compounds, as well as many endogenous substrates, such as prostaglandins, fatty acids and steroids. The CYP enzymes are classified into families, subfamilies and isoenzymes according to a systematic nomenclature based on similarities in their amino acid sequences (Nelson 1996). In humans, 18 families and 43 subfamilies of CYP genes have so far been identified (http://drnelson.utmem.edu/P450lect.html). The major CYP enzymes involved in drug metabolism in humans belong to families 1, 2 and 3, the specific isoforms being CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

Figure 1. The proportions of individual CYPs of total liver CYP content

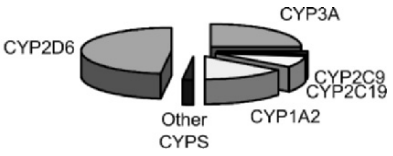


Figure 2. The proportions of psychoactive drugs metabolized by specific CYPs

2.1. CYP2D6

Although expressed at rather low levels compared with other human CYPs (2% of hepatic CYP content; Fig. 1), this isoform plays an important role in the metabolism of a variety of drugs, including most classical antidepressant and antipsychotic drugs (Fig. 2; Tab. 1). In contrast to all other CYPs involved in drug metabolism, CYP2D6 is not inducible.

The CYP2D6 polymorphism was described first in the 70's for the drugs debrisoquine and sparteine (Mahgoub 1977; Tucker 1997; Eichelbaum 1979). Debrisoquine, sparteine, dextromethorphan and desmethylimipramine have been validated as probe drugs for CYP2D6. The metabolic ratio (MR; the urinary ratio between the parent compound and its main metabolite) for these drugs is bimodally distributed. Approximately 7% of Caucasians, but only 1 to 2% of Orientals, lack CYP2D6 activity and are classified as PMs (Evans 1980; Alvan 1990; Bertilsson 1992). Among the EMs, the catalytic activity varies largely. A subgroup of subjects with extremely high enzyme activity (low MR) can be identified among the EMs, and classified as UMs (Johansson 1993; Dahl 1995).

The *CYP2D6* gene is extremely polymorphic. To date, more than 70 allelic variants have been described (http://www.imm.ki.se/CYPalleles). Four major mutated alleles, *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6*, account for 90-95% of the PM alleles in Caucasians. The most common allele associated with the PM phenotype is *CYP2D6*4*, with an allele frequency of ~ 21% in

Caucasian populations (Ingelman-Sundberg 1999). *CYP2D6*4* is almost absent in Orientals, which accounts for the low incidence of PM in these populations (Bertilsson 1992; Johansson 1994). On the other hand, the high frequency (up to 50%) of the *CYP2D6*10* allele, causing decreased but not absent CYP2D6 activity, among Orientals, and its absence among Caucasians, explains the lower CYP2D6 activity found in Oriental EMs compared to Caucasians (Johansson 1994). The frequency of the *CYP2D6*5* allele, characterised by deletion of the entire CYP2D6 gene (Gaedigk 1991), is 4-6%, very similar in different ethnic populations (Ingelman-Sundberg 1999). Other detrimental alleles frequently (2-3%) found among Caucasians are *CYP2D6*3* and *CYP2D6*6* (Gaedigk 1991; Kagimoto 1990; Broly 1991). Individuals heterozygous for the defect alleles have higher median MR than homozygous EM (Dahl 1992; Sachse 1997). Furthermore, alleles with duplication or multiduplication of a functional *CYP2D6* gene (**1* or **2*), causing increased CYP2D6 activity, have been described (Johansson 1993; Dahl 1995). The frequency of subjects carrying extra copies of a functional gene (UM) varies among different Caucasian populations, between 1-2 % in Swedes (Dahl 1995) and 7-10% in Spaniards (Agundez 1995; Bernal 1999) and southern Italians (Scordo 1999 and 2000).

2.2. CYP2C9

The human CYP2C subfamily consists of at least four isoforms, 2C8, 2C9, 2C18 and 2C19, whose genes are located together on chromosome 10. CYP2C9, the most abundant among human CYP2C isoforms, represents ~ 18% of total hepatic CYPs (Fig. 1). This enzyme metabolizes a number of important drugs, many of which have a narrow therapeutic range, but it does not seem to play a major role in the metabolism of antipsychotics or antidepressants (Miners 1998) (Fig. 2; Tab. 1).

To date, three different allelic variants (*CYP2C9*1*, *CYP2C9*2*, *CYP2C9*3*), coding for enzymes with different catalytic activity, have been well characterized (Miners 1998). *CYP2C9*2* encodes an enzyme that displays reduced affinity for P450 oxidoreductase, and *CYP2C9*3* yields an enzyme with reduced affinity for many substrates. The **3* allele confers a higher *in vitro* reduction in metabolic activity compared to the **2* allele (Lee 2002). Similarly, the **3/*3* genotype is associated with significant alterations in the pharmacokinetics of many CYP2C9 substrates *in vivo* (Lee 2002; Scordo 2002; Brandolese 2001; Yasar 2002). The frequencies of the *CYP2C9*2* and *CYP2C9*3* alleles vary between 8–12% and 3–8%, respectively, among Caucasians, and are lower in Orientals and black Africans (Miners 1998; Scordo 2001). Additional rare defect alleles have been described, but their impact on enzyme activity *in vivo* is so far unclear (http://www.imm.ki.se/CYPalleles).

2.3. CYP2C19

The polymorphic CYP2C19 accounts for ~ 3% of total hepatic CYPs (Fig. 1). The enzyme catalyses the 4-hydroxylation of the *S*-enantiomer of the anticonvulsant mephenytoin and contributes to the clearance of diazepam,

omeprazole, proguanil, citalopram, *R*-warfarin and many antidepressants (Goldstein 2001) (Fig. 2; Tab. 1). The frequency of the PM phenotype varies in populations of different racial origin, being approximately 3% in Caucasians, 15 to 25% in Orientals, and 4 to 7% in black Africans. The PM phenotype is inherited as an autosomal recessive trait.

The best-characterized defect *CYP2C19* alleles responsible for the PM phenotype are *CYP2C19*2*, the most common among Caucasians and Orientals (De Morais 1994), and *CYP2C19*3*, found at a frequency of about 12% among Oriental populations, but almost absent among Caucasians (De Morais 1994). *CYP2C19*2* accounts for 75% of the defective alleles in Orientals (De Morais 1994), and 93% in Caucasians (Chang 1995). The remaining 25% of defective alleles in Orientals are accounted for by *CYP2C19*3*. Additional rare defect alleles have been described (http://www.imm.ki.se/CYPalleles), but their impact on enzyme activity has to be further clarified.

Table 1. Major cytochrome P450 isoforms involved in drug metabolism.

Enzymes	***Substrates***	***Inhibitors***	***Inducers***	***Polymorphism***	***Probe-drug***
CYP1A2	*Antidepressants*: amitriptyline, clomipramine, imipramine, fluvoxamine, mirtazapine *Antipsychotics*: haloperidol, clozapine, olanzapine, thioridazine *Miscellaneous*: paracetamol, phenacetin, tacrine, *R*-warfarin, theophylline, caffeine	Fluvoxamine Ciprofloxacin	Smoking Rifampicin Barbiturates Phenytoin Carbamazepine	Yes	Caffeine
CYP2C9	*NSAIDs*: diclofenac, ibuprofen, naproxen, piroxicam *Antiepileptics*: phenytoin, phenobarbital, valproic acid *Miscellaneous*: *S*-warfarin, tolbutamide, losartan, torasemide, fluoxetine, sertraline	Sulfaphenazole Fluconazole Miconazole Fluoxetine Fluvoxamine	Rifampicin Barbiturates Phenytoin Carbamazepine	Yes	Losartan Warfarin Tolbutamide Phenytoin
CYP2C19	*Antidepressants*: amitriptyline, clomipramine, imipramine, citalopram, moclobemide, fluoxetine, sertraline	Omeprazole Ticlopidine Fluvoxamine	Rifampicin Barbiturates Phenytoin Carbamazepine	Yes	Omeprazole Mephenytoin

	Miscellaneous: phenytoin, diazepam, omeprazole, propranolol, proguanil, *S*-mephenytoin, *R*-warfarin				
CYP2D6	*Antidepressants*: amitriptyline, clomipramine, imipramine, desipramine, nortriptyline, trazodone, fluoxetine, paroxetine, fluvoxamine, citalopram, venlafaxine, mianserin, mirtazapine, maprotiline *Antipsychotics*: thioridazine, perphenazine, zuclopenthixol, haloperidol, risperidone, olanzapine, sertindole *Opiates*: codeine, dextromethorphan, tramadol, oxycodone *β-blockers*: alprenolol, bufuralol, metoprolol, propanolol, timolol, pindolol *Antiarrhythmics*: encainide, flecainide, propafenone *Miscellaneous*: debrisoquine, sparteine, diltiazem	Quinidine Thioridazine Perphenazine Fluoxetine Paroxetine Cimetidine Ritonavir	*None known*	Yes	Debrisoquine Dextromethorphan Spartein Metoprolol Desipramine
CYP3A4	*Antidepressants*: amitriptyline, clomipramine, imipramine, trazodone, sertraline, nefazodone, mirtazapine, citalopram, venlafaxine, reboxetine *Antipsychotics*: haloperidol, clozapine, risperidone,	Ketoconazole Itraconazole Fluconazole Erythromycin Troleandomycin Nefazodone Grapefruit juice	Rifampicin Barbiturates Phenytoin Carbamazepine	No/?	Cortisol Dapsone Erythromycin Quinine Midazolam

quetiapine, ziprasidone, sertindole *Benzodiazepines*: alprazolam, midazolam, triazolam *Ca-antagonists*: diltiazem, felodipine, nifedipine, verapamil *Immunosuppressants*: cyclosporin, tacrolimus *Miscellaneous*: cisapride, terfenadine, astemizole, carbamazepine, erythromycin, clarytromycin, tamoxifen, amiodarone, quinidine, methadone, ethinylestradiol, levonorgestrel, *statins*

Modified from ref (Scordo 2002).

2.4. CYP1A2

CYP1A2 accounts for approximately 13% of total CYPs expressed in human liver (Fig. 1). There is increasing awareness of the importance of CYP1A2 in drug metabolism (Tab. 1, Fig. 2). CYP1A2 activity shows wide, 40-fold, interindividual variability. Women have a lower average activity than men (Relling 1992). This enzyme is potently inhibited by fluvoxamine, and induced by polycyclic aromatic hydrocarbons, cigarette smoking and by rifampicin, phenytoin and, to a lesser extent, omeprazole (Table 1). Several polymorphisms have been suggested to be associated with the inducibility of CYP1A2 (Sachse 1999; Nakajima 1999; Chida 1999) (http://www.imm.ki.se/CYPalleles), but their impact on drug metabolism remains controversial.

2.5. CYP3A4

The human CYP3A subfamily is composed of 3 isoforms, 3A4, 3A5 and 3A7, encoded by genes located on chromosome 7. CYP3A4 is the most abundant in human liver (approximately 30% of total CYP content; Fig. 1) and intestine (Shimada 1994). This isoform catalyzes the biotransformation of a large number of structurally diverse drugs and endogenous compounds (Fig. 2; Tab. 1). CYP3A4 activity varies more than 20-fold among individuals, but is, in contrast to the polymorphic enzymes, unimodally distributed in the population. The wide interindividual variability may be caused, at least partially, by ethnic or cultural differences, presumably related to an interaction between race and

diet, and/or be a result of enzyme induction and inhibition (Table 1). The existence of an allelic variant, *CYP3A4*2*, associated with impaired clearance of nifedipine *in vitro*, has been demonstrated (Sata 2000), but its importance for the metabolism of other CYP3A4 substrates remains unclear.

3. PSYCHOTROPIC DRUGS AND CYTOCHROMES P450

3.1. Antidepressants

3.1.1. Tricyclic antidepressants (TCAs)

TCAs have been the corner stone of antidepressant treatment for three decades and still remain an important option for severe depression. The major problem in the clinical use of TCAs is the large interindividual variability in their metabolism, leading to pronounced variation in steady state concentrations and, consequently, in drug response. TCAs have a narrow therapeutic index and considerable toxicity associated with elevated plasma concentrations. Therapeutic plasma concentration ranges are reasonably well established, at least for amitriptyline, nortriptyline, imipramine and desipramine, allowing therapeutic drug monitoring (TDM) to be used as a tool for dose optimisation (Gram 1977; Task Force Laboratory Tests in Psychiatry 1985).

The tertiary amine TCAs amitriptyline, imipramine and clomipramine are demethylated to the pharmacologically active secondary amines nortriptyline, desipramine and desmethylclomipramine, respectively. Nortriptyline and desipramine are themselves also available as antidepressant drugs. All compouds undergo hydroxylation and further glucuronidation before excretion in urine. The hydroxy metabolites of TCAs possess pharmacological activity and may contribute to the antidepressant effects of the parent compounds (Nordin 1995).

Twin and family studies early established that genetic factors were a major determinant of the steady state plasma concentrations of TCAs (Alexanderson 1969). The discovery of the debrisoquine hydroxylation polymorphism (CYP2D6) in 1977 aroused a renewed interest in the pharmacogenetics of TCAs. Pharmacokinetic studies in healthy, drug-free EMs and PMs suggested that the hydroxylation reactions of TCAs were associated with the CYP2D6 phenotype while several enzymes catalysed the demethylation reactions. The role of CYP2D6 in the hydroxylation of nortriptyline is clearly shown in a single dose study in healthy Swedish subjects which included, in addition to PM subjects, EMs homo- or heterozygous for a functional *CYP2D6* allele, as well as individuals with 3, 4 or 13 functional *CYP2D6* genes (UMs) (Dalén 1998). The plasma concentrations of both nortriptyline and its 10-hydroxy metabolite were closely related to the number of functional *CYP2D6* genes, PM having the highest and UMs extremely low plasma levels of nortriptyline. The plasma concentrations of 10-hydroxynortriptyline showed the opposite pattern. In a similar study in Chinese subjects, the *CYP2D6*10* allele was associated with higher plasma levels of nortriptyline compared with the *CYP2D6*1* allele (Yue

1998). The 2-hydroxylation of desipramine has similarly been shown to be highly dependent on the CYP2D6 phenotype (Spina 1987; Dahl 1992 and 1993).

The situation is more complex for the tertiary TCAs. In contrast to the hydroxylation reactions catalysed by CYP2D6, the demethylation of imipramine and amitriptyline was found not to be associated with the CYP2D6 polymorphism (Mellström 1983; Brosen 1986). However, when only nonsmokers were studied, a significant correlation was found between amitriptyline demethylation clearance and urinary debrisoquine MR (Mellström 1986). This suggested that the demethylation reactions were catalysed by CYP2D6 as well as an enzyme inducible by smoking. *In vitro* and *in vivo* studies have later indicated the involvement of CYP1A2, CYP2C19 as well as CYP3A4 (Lemoine 1993; Skjelbo 1991; Chiba 1994).

Considering that the hydroxylation reactions are rate limiting for the elimination of TCAs and that their therapeutic and adverse effects are concentration-dependent, the potential clinical implications of polymorphic metabolism seem obvious. A handfull of case reports in patients with extremely high or low CYP2D6 activity have been described in the literature. A PM patient, earlier treated with normal nortriptyline doses with subsequent adverse effects, could be succesfully treated with doses as low as 20 mg/day (Bertilsson 1981). On the other hand, an UM patient with an extra functional CYP2D6 gene equired doses up to 300-500 mg/day in order to reach plasma concentrations in the recommended range (Bertilsson 1985 and 1993).

In a prospective study in depressed patients starting treatment with oral desipramine, the steady state plasma concentrations were significantly correlated with CYP2D6 activity, determined by the dextromethorphan test before treatment (Spina 1997). Two patients had a PM phenotype and showed the highest plasma concentrations and complained of severe adverse effects requiring dose reduction. No significant correlation was found between the plasma levels of desipramine or the sum of desipramine and its hydroxy metabolite, and the antidepressant effect. This study indicated that the CYP2D6 phenotype is a relatively good predictor of steady state plasma levels of desipramine and may identify patients at risk of concentration-dependent adverse effects, but not therapeutic response. Similarly, the CYP2D6 phenotype has been shown to predict the plasma levels of nortriptyline (Nordin 1985). The *CYP2D6* genotype was found to explain about 40% of the interindividual variability in the steady state plasma concentrations of nortriptyline (Dahl 1996; Kvist 2001). In a recent study (Bertilsson 2002), the steady state concentrations of nortriptyline were simulated in the different CYP2D6 genotypes using the single dose pharmacokinetic data from Dalén *et al.* (1998). At the usually recommended daily dose of 150 mg, subjects with 0 or 1 functional genes were predicted to attain levels above the recommended therapeutic intervall of 200-600 nmol/L and might therefore be at risk of adverse drug reactions, while subjects with 2 functional genes (the majority of the Caucasian population) would be in the middle of the recommended interval. Subjects with gene duplication or multiduplication (UM) were predicted to require doses of 225 mg per day or higher in order to reach recommended plasma concentrations of nortriptyline. These data are in line with the published case reports. However, it

must be remembered that these simulations assumed linear kinetics at the dose range used. Dose dependent kinetics may occur in EM at high nortriptyline doses as CYP2D6 is saturated at high substrate concentrations (Brosen 1986). Moreover, as 10-hydroxynortriptyline is pharmacologically active (Nordin 1995), it could contribute to the effects of the drug, especially in UM patients.

The influence of the Oriental *CYP2D6*10* allele on the steady state plasma levels of nortriptyline and 10-hydroxynortriptyline has been shown in Japanese patients (Morita 2000). However, as *CYP2D6*10* codes for an enzyme with decreased but not absent enzyme activity, the effect of it is less pronounced than that of alleles encoding no enzyme at all (such as *CYP2D6*3, *4, *5* and *6*).

3.1.2. Selective serotonin reuptake inhibitors (SSRIs)

Over the past decade, SSRIs have supplemented TCAs as antidepressants and anxiolytics of first choice due to their greater safety and tolerability profile (Caccia 1998). SSRIs have flat and overlapping dose-effect curves for the antidepressant and adverse effets, and no clear-cut correlation between plasma levels and clinical response has been documented (Rasmussen 2000). However, even these compounds show wide interindividual differences in pharmacokinetics, and are involved in many pharmacokinetic interactions. Therefore, the clinical implications of the genetically and environmentally induced differences in their metabolism might have been underestimated so far.

3.1.2a. Fluoxetine

Fluoxetine is primarily N-demethylated to the active metabolite norfluoxetine. Both fluoxetine and norfluoxetine are chiral compounds. The S- and R- enantiomers of fluoxetine are equipotent as serotonin reuptake inhibitors, while S-norfluoxetine is about 20 times more potent in this respect than its R-enantiomer. *In vitro* studies have implicated CYP2D6 and CYP2C9 as the primary enzymes catalysing the N-demethylation of fluoxetine, with a possible contribution of CYP3A (Margolis 2000; Ring 2001). CYP2D6 seems to be the major enzyme catalysing the demethylation of S-fluoxetine while R-fluoxetine is metabolised by CYP2D6 as well as CYP2C9 (Ring 2001). *In vivo*, the disposition of both fluoxetine and norfluoxetine co-segregated with the CYP2D6 phenotype in healthy volunteers after a single oral dose of the drug (Hamelin 1996). Another single dose study showed that CYP2D6 catalyses the metabolism of both S- and R-fluoxetine and most likely also the further metabolism of S-norfluoxetine, but not of R-fluoxetine (Fjordside 1999). In one further study (Eap 2001), the steady state levels of fluoxetine and norfluoxetine enantiomers were analysed after multiple dosing in 11 CYP2D6 EM subjects and 3 PM subjects. The plasma levels of S-fluoxetine were higher and those of S-norfluoxetine lower in PM than in EM. The role of CYP2C19 in the N-demethylation of fluoxetine has been suggested in Orientals, both *in vitro* (Liu 2001) and *in vivo* (Liu 2001). Furthermore, there is *in vitro* evidence for the involvement of CYP2C19 in the O-dealkylation of fluoxetine, the second major route of fluoxetine metabolism (Liu 2001).

Two recent studies have explored the relationship between the steady state plasma concentrations of fluoxetine and norfluoxetine, and the genotypes for *CYP2D6*, *CYP2C9* (Llerena 2003; Scordo 2003) and *CYP2C19* (Scordo 2003). Both found again evidence for the role of CYP2D6 as well as CYP2C9. In the study of Scordo *et al.* (2003), in which the enantiomers of fluoxetine and norfluoxetine were also determined, *CYP2D6* genotype was associated with the demethylation of S-fluoxetine, and *CYP2C9* with that of R-fluoxetine. No association between the *CYP2C19* genotype and the plasma levels of any of the compounds was found in this Caucasian population.

3.1.2b. Fluvoxamine

Fluvoxamine undergoes extensive metabolism, with at least 11 metabolites having been identified in urine. Significantly lower plasma concentrations of fluvoxamine have been reported in smokers as compared to non-smokers, indicating the role of CYP1A2 (Spigset 1995; Carrillo 1996). A significant correlation between CYP1A2 activity, measured using caffeine as a probe drug, and fluvoxamine clearance has also been found in a study involving both smokers and non-smokers (Carrillo 1996), but not in another with only non-smokers (Spigset 1999). After single oral doses of the drug, PM of CYP2D6 had significantly higher plasma concentrations of fluvoxamine than EM, suggesting a role for CYP2D6 as well (Carrillo 1996; Spigset 1997). Spigset *et al.* (1997) showed in an *in vivo* study that CYP2D6 seems to catalyse the formation of the major 5-demethoxylated carboxylic acid metabolite of fluvoxamine, whereas CYP1A2 catalyses other pathways. The CYP2C19 phenotype or genotype does not appear to influence the kinetics of fluvoxamine (Spigset 1997; Jan 2002).

3.1.2c. Paroxetine

PMs of CYP2D6 were found to have seven times times higher median area under the plasma concentration vs time curve (AUC) than EM after a single oral dose of paroxetine (Sindrup 1992). However, in the same subjects, the difference was only 2-fold at steady state. This could be due to saturation of CYP2D6, with resultant non-linear kinetics in EM but not in PM after repeated and higher doses. In another study by the same group, a 25-fold variation was found in the steady state plasma levels of paroxetine at a dose level of 30 mg/day (Sindrup 1992). There was a significant correlation between the sparteine MR and the steady state paroxetine levels within the EM group. In human liver microsomes, quinidine, a potent inhibitor of CYP2D6, only inhibited about two thirds of paroxetine metabolism, indicating a contribution by other CYPs as well (Bloomer 1992). Özdemir *et al.* (1999) found 2-fold higher median steady state plasma concentrations of paroxetine in heterozygous EM compared to homozygous EM, with a significant overlap in the distribution of concentrations between the two genotype groups. Unfortunately, no PM or UM were included in this study. One study has also shown the role of the *CYP2D6*10* allele on the disposition of paroxetine in Korean subjects (Yoon 2000).

3.1.2d. Sertraline

Sertraline is N-demethylated to the inactive metabolite desmethylsertraline. No significant differences were found in the pharmacokinetics of sertraline or desmethylsertraline between EM and PM of CYP2D6 (Hamelin 1996). On the other hand, single dose data from Chinese subjects indicate the major role of CYP2C19 (Wang 2001). *In vitro* studies indicate the contribution of multiple CYPs including CYP3A4, CYP2C19 and CYP2C9 (Kobayashi 1999; Xu 1999).

3.1.2e. Citalopram and escitalopram

With regard to citalopram metabolism, both CYP2D6 and CYP2C19 seem to be involved, CYP2C19 catalysing the demethylation of citalopram to its active metabolite desmethylcitalopram and CYP2D6 the further metabolism of desmethylcitalopram to didesmethyl-citalopram (Sindrup 1993). *In vitro* studies have indicated the involvement of not only CYP2C19 but also CYP3A4 in the demethylation of citalopram (Kobayashi 1997; Rochat 1997). Escitalopram, the pharmacologically active enantiomer of citalopram, has recently been introduced as an antidepressant itself. There are *in vitro* data indicating that CYP2D6, CYP2C19 and CYP3A4 all catalyse the demethylation of escitalopram (Von Moltke 2001).

3.1.3. Other antidepressants

3.1.3a. Venlafaxine

Venlafaxine undergoes CYP2D6 dependent metabolism to the active major metabolite O-desmethylvenlafaxine, while its N-demethylation is catalysed by CYP3A4, and possibly CYP2C19 and CYP2C9 (Otton 1996; Fogelman 1999). PM of CYP2D6 had a more than 4-fold lower oral clearance of venlafaxine compared to EM, mainly due to decreased capacity to form the O-demethylated metabolite (Lessard 1999). In a study on 33 depressed patients treated with 225 mg venlafaxine per day, a significant relationship between the *CYP2D6* genotype and the O-desmethylvenlafaxine/venlafaxine ratio was found (Veefkind 2000). A significant relationship between the *CYP2D6* genotype (i.e. the presence of *CYP2D6*10* allele) and the plasma kinetics of venlafaxine and its O-desmethyl metabolite has also been shown in Japanese subjects (Fukuda 2000). This study also suggested the involvement of CYP2C19 in the metabolism of venlafaxine. Since venlafaxine and O-desmethylvenlafaxine have similar pharmacological properties, the clinical implications of polymorphic metabolism are uncertain.

Venlafaxine is a chiral compound and, according to *in vitro* data, S(-)-venlafaxine inhibits both noradrenaline and serotonin presynaptic reuptake, while R(+)-venlafaxine primarily inhibits serotonin reuptake (Holliday 1995). According to a recent *in vivo* study, CYP2D6 seems to catalyse the O-demethylation of both enantiomers, but with a marked stereoselectivity towards the R-enantiomer (Eap 2003). Whether this has clinical relevance remains to be established.

3.1.3b. Maprotiline

The metabolism of the tetracyclic antidepressant maprotiline appears to be CYP2D6 dependent as Cmax and AUC were significantly higher in PM than in EM of CYP2D6 after a single oral dose of the drug (Firkusny 1994). The major role of CYP2D6, with additional contribution of CYP1A2, is supported by *in vitro* data (Brachtendorf 2002).

3.1.3c. Mianserin

Mianserin and its major metabolite desmethylmianserin appear to be metabolised by CYP2D6 as significantly higher plasma concentrations of both compounds were found in PM than in EM of CYP2D6 after a single oral dose (Dahl 1994). The CYP2D6-dependent disposition showed marked stereoselectivity for the major and more potent S(+)-enantiomer of mianserin. A study in Japanese patients suggested that the *CYP2D6* genotype (i.e. presence of *CYP2D6*10* allele) plays a major role in controlling the steady state plasma levels of S(+)-mianserin (Mihara 1997). On the other hand, no significant differences were found in the mean concentration-to-dose ratios for S- or R-mianserin or desmethylmianserin between Caucasian homo- or hetero-zygous EMs and one PM subject (Eap 1998). *In vitro* studies have indicated the involvement of CYP2D6 in the hydroxylation and CYP1A2 in the N-demethylation of both mianserin enantiomers (Koyama 1996).

3.1.3d. Mirtazapine

Mirtazapine is structurally related to mianserin. *In vitro* studies in human liver microsomes and cells expressing different CYPs have suggested that racemic mirtazapine is predominantly metabolised by CYP2D6, CYP3A4 as well as by CYP1A2 (Dahl 1997; Störmer 2000). According to the urinary excretion profile in humans, 25% of mirtazapine is demethylated by CYP3A to desmethylmirtazapine, 40% is hydroxylated by CYP2D6 and/or CYP1A2 to 8-hydroxymirtazapine, 10% is N-oxidised by CYP3A4, and 25% glucuronidated (Preskorn 1993).

In a single dose study, no significant differences in the pharmacokinetic parameters of either mirtazapine or desmethylmirtazapine were found between PM and EM of CYP2D6 (Dahl 1997). Analysis of the enantiomers, however, revealed CYP2D6-related differences in the plasma levels of the minor, but more active S(+)-enantiomer of mirtazapine (Delbressine 1997). The role of CYP2D6 in the metabolism of S(+)-mirtazapine has been confirmed *in vitro* (Dodd 2001).

3.1.3e. Moclobemide

Moclobemide has been shown to be a substrate of CYP2C19, based on a panel study in PM and EM of CYP2C19 (Gram 1995). No significant differences were found in the pharmacokinetics of moclobemide after multiple dosing between 4 EMs and 2 PMs of CYP2D6, indicating no major role of CYP2D6 (Härtter 1996). *In vitro*, the N-oxidation of moclobemide has been shown to be catalysed predominantly by flavin-containing mono-oxygenase activity, with a potentially small contribution of cytochromes P450 (Hoskins 2001).

3.1.3f. Reboxetine.

Based on *in vitro* studies, the metabolism of reboxetine seems to be principally catalysed by CYP3A4 (Wienkers 1999). No studies have evaluated the contribution of different CYP enzymes on the pharmacokinetics of reboxetine *in vivo*.

3.1.3g. Nefazodone

Nefazodone has a complex metabolism, and both *in vitro* and *in vivo* data indicate that CYP3A4 is the major enzyme involved. In a panel study in PM and EM of CYP2D6, no differences in the pharmacokinetics of nefazodone or its hydroxy metabolite were found between the two phenotypes (Barbhaiya 1996). However, the plasma concentrations of the mCCP metabolite were higher in PM, suggesting that the further metabolism of mCCP is catalysed by CYP2D6. This has also been confirmed *in vitro* (Rotzinger 1998).

3.1.3h. Trazodone

Trazodone undergoes oxidative metabolism primarily to mCCP. CYP2D6 has been suggested to be involved in the metabolism of both trazodone and mCCP (Yasui 1995). However, in depressed Japanese patients, the CYP2D6 genotype was not found to predict the steady state plasma levels of either trazodone or mCCP (Mihara 1997). Other enzymes including CYP1A2 and CYP3A4 are probably involved, as indicated by lower plasma concentrations in smokers compared to non-smokers and during coadministration of carbamazepine, an inducer of CYP3A (Ishida 1995; Otani 1996).

3.2. Antipsychotic drugs

The antipsychotics currently available can be divided into older or "typical" compounds, such as phenothiazines, butyrophenones, thioxanthenes, and newer or "atypical" agents, such as clozapine, olanzapine, quetiapine, risperidone and ziprasidone. While classical compounds frequently cause extrapyramidal side effects and hyperprolactinemia, the newer agents have a lower propensity to induce such adverse effects. They have a higher efficacy in the treatment of negative symptoms of schizophrenia, compared to the classical drugs. Many classical compounds including haloperidol, perphenazine, zuclopenthixol and thioridazine are metabolised to a significant extent by the polymorphic CYP2D6, while many of the newer compounds are substrates of CYP1A2 and CYP3A4.

3.2.1. Classical antipsychotics

3.2.1a. Chlorpromazine

Although the major metabolic pathways of chlorpromazine and its metabolites have been identified (Hubbard 1993; Fang 1999), the enzymes catalysing these reactions have not been completely elucidated. An *in vitro* study indicated that 7-hydroxylation of chlorpromazine, the major pathway in humans,

is catalyzed mainly by CYP2D6 and partially by CYP1A2 (Yoshii 2000). No data are available on the role of CYP2D6 in the metabolism of chlorpromazine *in vivo*. The finding that cigarette smoking reduces chlorpromazine plasma concentrations (Desai 2001) suggests a role for CYP1A2 *in vivo*.

3.2.1b. Thioridazine

Thioridazine is metabolised to two active metabolites, thioridazine 2-sulfoxide (mesoridazine) and thioridazine 2-sulfone (sulforidazine), as well as to thioridazine 5-sulfoxide, which is cardiotoxic (Dahl 1982; Hale 1996). Although the enzymes catalysing the metabolism of thioridazine have not been fully identified, it has been suggested that CYP2D6 is involved in the formation of mesoridazine and, possibly, thioridazine 5-sulfoxide (Von Bahr 1991; Eap 1996). PM of CYP2D6 reached higher plasma levels of thioridazine and thioridazine 5-sulfoxide than EMs after a single oral dose of the drug, suggesting that PMs might be at greater risk of cardiovascular side effects of thioridazine (Von Bahr 1991). Low doses of fluvoxamine (25 mg twice a day) have been reported to increase the mean concentrations of thioridazine, mesoridazine and sulforidazine by more than 2-fold in schizophrenic patients (Carrillo 1999). Therefore, it has been hypothesised that CYP1A2 and/or CYP2C19 may also contribute to the metabolism of thioridazine and its metabolites. The contribution of *CYP2D6* genotype and smoking behaviour on steady state plasma levels of thioridazine and its metabolites has recently been confirmed in a clinical study in psychiatric patients (Berecz 2003).

3.2.1c. Perphenazine

Perphenazine is mainly metabolised in humans by N-dealkylation, sulfoxidation and 7-hydroxylation, while N-oxidation and direct glucuronidation are minor pathways (Joergensen 1986). Although the CYP isoforms involved have not been completely characterised, indirect evidence points towards a major role of CYP2D6. In a study in healthy volunteers, after a single oral dose, the plasma concentrations of perphenazine were 3-4 times higher in PMs than in EMs of debrisoquine (Dahl-Puustinen 1989). Similarly, in a study investigating the relationship between *CYP2D6* genotype and steady-state plasma levels of perphenazine in patients on long-term therapy, Linnet and Wiborg (Linnet 1996) found a 2-fold higher median concentration per dose unit (C/D) in PM than in EM patients without interacting co-medication. There was, however, an almost 30-fold variation in C/D among the EMs and a large overlap between EM and PM genotypes (Linnet 1996). In another study, a 3-fold difference in the oral clearance of the drug between PM and homozygous EM genotypes was seen in patients on long-term therapy (Jerling 1996). The important role of CYP2D6 is also indirectly supported by an interaction study showing that paroxetine, a potent CYP2D6 inhibitor, increased plasma perphenazine concentrations from 2- to 13-fold, together with the occurrence of CNS side-effects, including oversedation, extrapyramidal symptoms and impairment of psychomotor performance and memory (Özdemir 1997). The results of an *in vitro* study suggest that CYP isoforms 1A2, 2C19, 2D6 and 3A4 are involved in perphenazine dealkylation (Olesen 2000). No data are available to

assess the contribution of enzymes other than CYP2D6 to the elimination of perphenazine *in vivo*.

3.2.1d. Zuclopenthixol

Zuclopenthixol is a thioxanthene derivative structurally related to perphenazine. In a panel study in volunteers, after single oral doses, the plasma elimination half-life of zuclopenthixol was significantly longer in PM than in EM of debrisoquine, and the apparent oral clearance in PMs was in average one-third of that in EMs (Dahl 1991). Similarly, in studies performed in patients, an average 2-fold difference in the oral clearance of zuclopenthixol between PM and homozygous EM patients (Jerling 1996) and a 60% higher median C/D of zuclopenthixol in PM compared with EM patients not receiving interacting drugs (Linnet 1996) were found. Similar differences between EM and PM of CYP2D6 were also found after intramuscular administration of zuclopenthixol decanoate (Jaanson 2002).

3.2.1e. Haloperidol

The butyrophenone haloperidol undergoes complex metabolism. Its major metabolic pathways include reduction of the ketone group to form reduced haloperidol, N-dealkylation and aromatic hydroxylation (Fang 1999). The involvement of CYP2D6 in the metabolism of haloperidol and reduced haloperidol has been demonstrated both *in vitro* (Pan 1999) and *in vivo*. Llerena *et al.* (1992) first demonstrated that, after single oral doses of haloperidol, PMs of debrisoquine eliminated haloperidol significantly slower than EMs, and also had higher plasma levels of reduced haloperidol. In a further study in schizophrenic patients receiving haloperidol decanoate, the only subject with a PM genotype for CYP2D6 had the highest plasma concentration of haloperidol and the highest dopamine D2 receptor occupancy, as determined by positron emission tomography (Nyberg 1995). The results of another clinical study, showing the capability of fluoxetine to interact with haloperidol (Avenoso 1997), provide further, indirect evidence of the involvement of CYP2D6 in haloperidol metabolism.

Several studies in Japanese patients (Suzuki 1997; Mihara 1999; Someya 1999) have shown a relationship between increased steady state concentrations of haloperidol and reduced haloperidol and the presence of *CYP2D6*5* and/or **10* alleles, while in another report no genotype-related differences were found (Shimoda 2000). Among Caucasians, a 4-fold higher C/D of reduced haloperidol was found in PMs than in EMs, while there was no difference in the C/D of haloperidol between the genotype groups (Pan 1999). In a recent study (Roh 2001), a relationship between the *CYP2D6* genotype and the C/Ds of haloperidol and reduced haloperidol was found in Korean patients receiving less than 20 mg of haloperidol daily, but not in those with higher doses, suggesting that the importance of *CYP2D6* genotype may depend on the dose range used. A plausible explanation is that the high-affinity low-capacity CYP2D6 plays an important role at low concentrations/doses of haloperidol, while the low-affinity high-capacity CYP3A4 becomes dominant at higher doses. As suggested by *in vitro* studies (Pan 1999; Fang 1997), and by the *in vivo* evidence of elevated

plasma concentrations of haloperidol and reduced haloperidol during itraconazole administration (Yasui 1999), CYP3A4 also appears to be involved in haloperidol metabolism. On the other hand, the findings that fluvoxamine markedly increases (Daniel 1994) and smoking decreases haloperidol serum concentrations (Pan 1999; Shimoda 1999), suggest the involvement of CYP1A2 as well. A recent study, addressing the influence of *CYP1A2* gene polymorphisms on steady-state plasma haloperidol levels in Japanese schizophrenic patients, however, did not show any relationship between CYP1A2 activity and haloperidol levels (Mihara 2000). The results of a large study in 172 psychiatric inpatients (Brockmoller 2002) indicate that haloperidol clearance correlates significantly with the number of active *CYP2D6* genes.

3.2.2. Atypical antipsychotics

3.2.2a. Clozapine

Pharmacokinetic studies and case reports have clearly documented that fluvoxamine, a potent inhibitor of CYP1A2, may increase plasma clozapine concentration up to 5–10-fold, possibly resulting in toxic effects (Hiemke 1994; Kuo 1998; Wetzel 1998). It has been speculated that the inhibitory effects of fluvoxamine on CYP2C19 and CYP3A4, in addition to CYP1A2, might contribute to this interaction. A correlation has been demonstrated between clozapine clearance and a caffeine index reflecting CYP1A2 activity (Bertilsson 1994). Several reports indicate that plasma concentrations of clozapine and norclozapine are lower in smokers compared with nonsmokers (Haring 1990; Hasegawa 1993), confirming the major role of the smoking-inducible CYP1A2. Environmentally induced or constitutively high CYP1A2 expression might theoretically explain treatment resistance in some patients. Özdemir *et al.* (2001) suggested that ultrarapid CYP1A2 activity sustained by a genetic polymorphism might affect therapeutic response to conventional doses of clozapine, leading to subtherapeutic concentrations and treatment resistance. The results of a recent study (Van Der Weide 2003), however, suggest that the *CYP1A2*1F* polymorphism has no significant clinical impact on clozapine clearance and daily dose.

Fluoxetine has been reported to increase plasma clozapine concentrations up to 2-fold, presumably through inhibition of CYP2D6 and CYP3A4 (Centorrino 1994 and 1996; Spina 1998). The evidence on the role of CYP2D6 in the metabolism of clozapine *in vitro* is contradictory, since some studies have indicated a minor involvement of CYP2D6 (Fischer 1992; Linnet 1997), while others found no evidence for this (Pirmohamed 1995; Eiermann 1997). No differences in the disposition of clozapine were found *in vivo* between EM and PM of debrisoquine after a single oral dose of the drug (Dahl 1994). Paroxetine, a potent and probably relatively selective inhibitor of CYP2D6, has also been associated with a significant elevation in the plasma levels of clozapine in some (Centorrino 1996; Spina 2000), but not all studies (Wetzel 1998). *In vitro* studies have confirmed that CYP1A2 but also CYP3A4 catalyse the metabolism of clozapine (Eiermann 1997). Two case reports have indicated that co-administration of erythromycin, a macrolide antibiotic with a potent inhibitory effect on

CYP3A4, may increase plasma concentrations of clozapine, leading to signs of clozapine toxicity, such as seizures, somnolence, slurred speech, desorientation and incontinence (Funderburg 1994; Cohen 1996). On the other hand, in a formal pharmacokinetic investigation in healthy volunteers, erythromycin was found not to modify the pharmacokinetics of clozapine, suggesting that CYP3A4 is probably of minor importance (Hagg 1999). In agreement with this, two other strong inhibitors of CYP3A4, itraconazole and nefazodone, were found not to affect plasma concentrations of clozapine and norclozapine in schizophrenic patients (Raaska 1998; Taylor 1999). While the results in an *in vitro* study suggested an involvement of CYP2C19 in clozapine metabolism (Linnet 1997), data from healthy volunteers did not show any differences in clozapine disposition between EMs and PMs of mephenytoin (Dahl 1994).

3.2.2b. Olanzapine

Direct glucurono-conjugation appears to be a major metabolic pathway of olanzapine in humans, olanzapine 10-*N*-glucuronide being its main circulating metabolite (Kassahun 1997). The oxidative metabolism of olanzapine has been characterized *in vitro* (Kassahun 1997; Ring 1996; Caccia 2000). It has been shown that CYP1A2 catalyzes the formation of *N*-desmethylolanzapine and 7-hydroxyolanzapine, while CYP2D6 catalyzes the formation of 2-hydroxyolanzapine. Potent inhibitors of CYP1A2, such as fluvoxamine and ciprofloxacin, have been reported to cause significant changes in olanzapine pharmacokinetics (Mäenpää 1997; Markowitz 1999; De Jong 2001). On the other hand, inducers of CYP1A2, such as cigarette smoking and carbamazepine, may accelerate the metabolism of olanzapine. Consistent with this, several pharmacokinetic studies and analyses, summarized by Callaghan *et al.* (1999) have shown that olanzapine clearance is about 40% higher in smokers than in non-smokers. Interaction studies in healthy volunteers and in psychiatric patients have indicated that carbamazepine may cause an approximately 30–40% decrease in plasma concentrations of olanzapine (Lucas 1998; Olesen 1999), by induction of CYP1A2 and, possibly, UDP glucuronosyltransferase.

3.2.2c. Quetiapine

Quetiapine has complex metabolism, with at least 20 metabolites (Devane 2001). *In vitro* studies showed that CYP3A4 is the main isoenzyme involved in quetiapine sulfoxidation and *N*- and *O*-dealkylation, while its 7-hydroxylation is thought to be partly mediated by CYP2D6 in addition to CYP3A4 (Caccia 2000). *In vivo* studies in healthy volunteers, summarised by Dev and Raniwalla (2000), have demonstrated that the pharmacokinetics of quetiapine are significantly affected by co-administration of inhibitors or inducers of CYP3A4, confirming the major role of this isoform.

3.2.2d. Risperidone

Risperidone undergoes 9- and 7-hydroxylation, N-dealkylation and glucuronidation (Mannens 1993). 9-hydroxyrisperidone is the major metabolite in plasma and approximately equipotent to risperidone in terms of receptor affinity (Van Beijsterveldt 1994). *In vitro* studies in human liver microsomes

indicated that the 9-hydroxylation is catalysed mainly by CYP2D6 but also by CYP3A4 (Fang 1999). The major role of CYP2D6 was confirmed in a single dose study in healthy volunteers showing that the formation of 9-hydroxyrisperidone was strongly related to the CYP2D6 phenotype (Huang 1993). In a study in schizophrenic patients on risperidone monotherapy, the steady state C/Ds of risperidone and the risperidone/9-hydroxyrisperidone ratio were strongly associated with the *CYP2D6* genotype, although there was an extremely high (up to 30-fold) interindividual variability among EM subjects (Scordo 1999). Furthermore, no genotype-related differences were found in the C/D of 9-hydroxyrisperidone or the sum of risperidone and 9-hydroxyrisperidone (Scordo 1999). Similar data have been reported by Olesen *et al.* (1998). Since risperidone and its 9-hydroxymetabolite are considered to be equipotent, the CYP2D6 polymorphism, and the co-administration of agents that affect CYP enzyme activity, modifying the risperidone/9-hydroxyrisperidone ratio, but not the active fraction, would be expected to be of limited clinical importance for the outcome of treatment. There are, however, anecdotal reports suggesting that PMs might be more prone to side effects (Bork 1999) and UMs to therapeutic failure (Güsey 2000) when compared to EMs. Besides, since PM subjects lack CYP2D6 activity, they depend almost completely on CYP3A4 to metabolise the drug. Therefore, they might be more susceptible to drug-drug interactions when CYP3A4 inhibitors or inducers are co-administered (Spina 2001). Recent evidence indicates that certain inhibitors of CYP2D6, i.e. paroxetine and fluoxetine (Spina 2001 and 2002), or inducers of CYP3A4, i.e. carbamazepine (Spina 2000), may cause a significant increase or decrease in total plasma risperidone concentrations (risperidone plus 9-hydroxyrisperidone). Even though no clear-cut correlation has been found between plasma concentrations of risperidone and 9-hydroxyrisperidone and antipsychotic response, plasma levels of the active moiety may correlate with the occurrence of extrapyramidal side effects (Spina 2001).

9-OH-risperidone has a chiral carbon atom and thus two enantiomers, (+)- and (-)-9-hydroxyrisperidone. Recently, it has been shown *in vitro* that CYP2D6 catalyses the formation of the (+)-enantiomer while CYP3A4 catalyses that of the (-)-form (Yasui-Furukori 2001). (+)-9-hydroxyrisperidone is the major enantiomer in EMs, subjects homozygous for *CYP2D6*1* having significantly higher (+)/(-)-enantiomeric ratios of 9-hydroxyrisperidone than those heterozygous for *CYP2D6*4* or **5* (Yasui-Furukori 2001). No data are available on the pharmacological activity of the two enantiomers. If the pharmacological properties of the enantiomers differ, one might expect genotype-related differences in the clinical effects of risperidone despite similar plasma concentrations of the active moiety.

3.2.2e. Ziprasidone

Ziprasidone is metabolised in human liver microsomes to ziprasidone sulphoxide, S-sulphone, oxidole acetic acid and benzisothiazole piperazine (BITP), sulphoxide and sulphone being the major circulating metabolites (Prakash 2000). *In vitro* experiments indicate that CYP3A4 is the major enzyme involved (Prakash 2000). Accordingly, studies in healthy volunteers have indicated that the pharmacokinetics of ziprasidone is modified by inhibitors and inducers of CYP3A4 (Miceli 2000 and 2000).

Table 2. Major cytochrome P450 (CYP) enzymes involved in the metabolism of antidepressant drugs.

Drug	CYP isoenzymes				
	1A2	2C9	2C19	2D6	3A4
Amitriptyline	+	-	+	+	+
Imipramine	+	-	+	+	+
Desipramine	±	-	±	+	±
Nortriptyline	±	-	±	+	±
Fluoxetine	-	+	±	+	±
Fluvoxamine	+	-	-	+	-
Paroxetine	-	-	-	+	-
Sertraline	-	+	+	-	+
Citalopram	-	-	+	+	+
Venlafaxine	-	+	+	+	+
Maprotiline	+	-	-	+	-
Mianserin	-	-	-	+	-
Mirtazapine	+	-	-	+	+
Moclobemide	-	-	+	-	-
Reboxetine	-	-	-	-	+
Nefazodone	-	-	-	±	+
Trazodone	+	-	-	+	+

+ Data support role ± Data are contradictory
- Data do not support role or are lacking

Table 3. Major cytochrome P450 (CYP) enzymes involved in the metabolism of antipsychotic drugs.

Drug	CYP isoenzymes				
	1A2	2C9	2C19	2D6	3A4
Chlorpromazine	+	-	-	+	-
Thioridazine	+	-	±	+	-
Perphenazine	+	-	+	+	+
Zuclopenthixol	-	-	-	+	-
Haloperidol	+	-	-	+	+
Clozapine	+	-	±	±	+
Olanzapine	+	-	+	+	-
Quetiapine	-	-	-	+	+
Risperidone	-	-	-	+	+
Ziprasidone	-	-	-	-	+

+ Data support role ± Data are contradictory
- Data do not support role or are lacking

4. PHENOTYPE/GENOTYPE AND CLINICAL EFFECTS OF PSYCHOTROPIC DRUGS

Tables 2 and 3 summarise the present-day knowledge on the involvement of various CYPs in the metabolism of different antidepressant and antipsycohotic drugs. As discussed above, the quantitative importance of various CYPs, and the polymorphic CYP2D6 and CYP2C19 in particular, for the total clearance varies largely between the different psychoactive drugs, and is probably also dependent on the dose level used. Thus, each compound needs to be studied individually at therapeutic dose levels in order to fully understand the clinical implications of polymorphic metabolism. While the pharmacokinetic consequences of polymorphic metabolism are relatively well characterised for most psychoactive drugs, the documentation on its importance with respect to therapeutic response and dosing remains scanty.

4.1. Therapeutic effects

Only 5 studies on antidepressants have been found which included efficacy data related to CYP2D6 (amitriptyline, desipramine, mianserin) or CYP2C19 (amitriptyline, imipramine) pheno/genotype (Spina 1997; Mihara 1997; Baumann 1986; Koyama 1996; Morinobu 1997). With the exception of the mianserin study (Mihara 1997), no significant differences were found between the pheno/genotype groups. The number of PMs is low in all studies, resulting in low statistical power. Mihara *et al.*(1997), however, reported a higher proportion of responders, together with higher plasma concentrations of S-mianserin, among 9 Japanese patients heterozygous for *CYP2D6*10* than among 5 patients homozygous for the *CYP2D6*1* allele. The dosage of mianserin was 30 mg per day and treatment duration three weeks.

Only few studies have evaluated the possible relationship between CYP2D6 pheno- or genotype and therapeutic response to antipsychotic agents. In a study in 18 schizophrenic patients treated with haloperidol (10 mg/day) for two weeks, no correlation between CYP2D6 phenotype and improvement of psychotic symptoms was found, although a significant relationship between CYP2D6 activity and steady-state plasma concentrations of both haloperidol and reduced haloperidol was observed (Lane 1997). Similarly, Pollock *et al.* (1995) showed no significant differences in improvement of agitation and psychotic symptoms associated with dementia between 5 PM and 40 EM patients treated with perphenazine, at a dosage of 0.1 mg/kg, for 17 days.

In a retrospective study involving 235 patients with treatment refractory schizophrenia, Aitchison *et al.* (1999) addressed the correlation between *CYP2D6* UM status and lack of response to typical antipsychotics. The findings that only 0.9% of treatment-refractory patients were genotypically UM, as compared with 4.1% of non-refractory patients in the control group, clearly indicated that the UM status is not a major cause of treatment resistance. These results are consistent with the findings that the UM status is unlikely to be a protective condition against the occurrence of extrapyramidal side effects (Scordo 2000). On the other hand, in a recent report involving 749 schizophrenic

inpatients of Caucasian origin, Sachse *et al*. (2000) observed that the number of different drugs administered and the number of re-hospitalizations were higher in UMs carrying three or more functional *CYP2D6* genes, compared to patients of other *CYP2D6* genotypes, suggesting a possible clinical importance of the UM genotype for the clinical outcome.

4.2. Side effects

With respect to antidepressant drugs, only a few studies have reported explicit data on adverse drug reactions in relation to CYP2D6 (amitriptyline, despiramine, mianserin, venlafaxine) or CYP2C19 (imipramine) pheno/ genotype (Spina 1997; Lessard 1999; Dahl 1994; Baumann 1986; Morinobu 1997). Again, most studies are small with only a few PM subjects and fail to find significant differences between the groups. In the study of Spina et al. (1997), adverse effects requiring dose decrease were reported in both two PMs but in only one of 29 CYP2D6 EMs treated with desipramine. A relationship between cardiovascular side effects of venlafaxine and CYP2D6 phenotype has been suggested based on four patients with PM phenotype (either genetic or due to inhibition of CYP2D6 by concomitant drugs) (Lessard 1999). The side effects included palpitations, shortness of breath and proarrhythmias.

Spigset *et al.* (1997) retrospectively studied cases of seizures or myoclonus during treatment with antidepressant drugs, reported to the Swedish Adverse Drug Reaction Advisory Committee. A total of 27 cases of seizures and 7 of myoclonus were identified. Eleven of the patients could be genotyped for *CYP2D6* and *CYP2C19*, and none of them was a PM of either polymorphic enzyme. On the other hand, 47 % of the patients were concomitantly treated with drugs that are potential inhibitors of CYP2D6. Thus, the concomitant treatment could have converted the patients to phenotypically PMs, despite EM genotype, leading to an increased risk of concentration-dependent adverse drug reactions.

In contrast, many studies have examined the relationship between CYP2D6 pheno- or genotype and adverse effects of antipsychotic drugs (Scordo 2002; Dahl 2002). Some studies found evidence for oversedation to be more likely to develop in PM than in EM during treatment with classical antipsychotics metabolised by CYP2D6. In a retrospective study in schizophrenic patients, Spina *et al.* (1992) found that 8 out of 24 patients (33%), who had developed severe adverse effects within the first days of treatment with phenotiazines or haloperidol, were phenotypically PM of debrisoquine, while in a control group of 29 patients without side effects, the PM frequency was 7%. Therefore, an association between the PM phenotype and acute antipsychotic-induced side effects such as oversedation, postural hypotension and autonomic effects, was suggested. However, in a further investigation, no differences in the prevalence of PMs for debrisoquine were observed between 26 patients with neuroleptic-induced dystonic reactions and a control group of 53 patients with no history of dystonia (11.5% vs 9.4%) (Spina 1992). Thus, the occurrence of acute dystonic reactions did not appear to be related to polymorphic oxidation (Spina 1992). Meyer *et al.* (1990) described abnormally high plasma levels of thioridazine,

with subsequent appearance of oversedation, in a PM patient taking a standard dose of the drug. Pollock *et al*. (1995) found that among elderly demented patients treated with perphenazine, 5 PMs had significantly more severe side effects during the first 10 days of treatment compared with 40 EM patients. The difference, however, diminished by day 17. It was suggested that the smaller difference between EM and PM subjects after 17 days of treatment would be due to saturation kinetics of perphenazine in EM, leading to diminished contribution of CYP2D6 to the overall clearance of perphenazine at steady state. Unfortunately, the plasma levels of perphenazine were not measured in this study.

Several further studies have investigated the possible association between *CYP2D6* genotype and movement disorders induced by antipsychotic drugs. No clear relationship was observed between CYP2D6 activity or genotype and the occurrence of acute dystonia, akathisia and neuroleptic malignant syndrome (Scordo 2002; Dahl 2002). However, these adverse effects are probably not concentration-dependent and would therefore not be expected to be genotype-related.

The results of studies evaluating the relationship between CYP2D6 activity and parkinsonism and/or tardive dyskinesia have been inconsistent. In a pilot study in 16 schizophrenic Caucasian patients with antipsychotic-induced tardive dyskinesia (Arthur 1995), a relationship was suggested between the degree of impaired CYP2D6 activity and the severity of EPS, although there was no overrepresentation of PM among the investigated patients. The results of studies carried out in larger numbers of patients (Armstrong 1997; Andreassen 1997) indicated that, although *CYP2D6* genotype is unlikely to be a determinant of susceptibility to acute dystonic reactions, it may contribute to the occurrence and severity of other antipsychotic-induced movement disorders, including tardive dyskinesia. Consistent with these findings, in a recent retrospective investigation (Scordo 2000), all four PM subjects found among 119 schizophrenic patients had EPS (parkinsonism or tardive dyskinesia) while treated with different classical antipsychotics. In a study in 100 Japanese schizophrenics treated with several antipsychotics, Ohmori *et al.* (1998) found a significant association between the detrimental *CYP2D6*10* allele and the total AIMS score and a moderate association with tardive dyskinesia.

Many of the studies on the relationship between CYP2D6 and antipsychotic related adverse effects have methodological problems that may influence the results obtained. In many studies the patients were taking various antipsychotics including those not metabolized by CYP2D6, or on polytherapy with drugs that are potent inhibitors of CYP2D6. Pharmacokinetic drug interactions due to concomitant use of two or more antipsychotics (or other drugs inhibiting CYP2D6) may lead to elevated plasma concentrations and an increased risk of concentration-dependent side effects irrespective of the genotype. Most studies are also fairly small and may thus fail to detect a true association. The studies published so far, with the exception of that by Pollock *et al*. (1995), have been retrospective and used rough criteria or case record data for evaluation of adverse effects. Also, the plasma levels of the antipsychotics have not been measured in any of the studies. Thus, prospective studies using careful effect

and side effect evaluation are required to establish whether the risk of adverse effects of antipsychotics is related to the *CYP2D6* genotype.

In a 1-year follow-up study of 100 consecutive psychiatric in-patients genotyped for *CYP2D6* on admission, Chou *et al.* (2000) found a trend towards an increasing number of adverse effects in patients treated with drugs primarily metabolized by CYP2D6 (tricyclic antidepressants, thioridazine, perphenazine, haloperidol and risperidone), when one moves from UM to PM genotypes. The costs of treating patients with extremes of CYP2D6 activity (i.e. PM and UM) were on average 4000-6000 dollars greater per year than the costs of treating patients with homozygous or heterozygous EM genotypes. The total duration of hospital stay also tended to be longer for patients in the PM group. The authors, however, calculated that 1500 to 2000 patients should be evaluated over at least one year to determine whether the genetic variation of CYP2D6 significantly alters the duration and costs of hospital stay.

Recently, Tamminga *et al.* (2003) retrospectively analysed the utilization of psychotropic drugs in 241 hospitalized psychiatric patients genotyped for CYP2D6. Drugs metabolised by CYP2D6 were less frequently prescribed in PMs than in EMs and the average duration of prescriptions was shorter in PMs. A similar but not significant trend was found between EM and UM patients. No differences were found between the genotype groups with respect to the doses prescribed. Interestingly, drugs against parkinsonian symptoms were given twice as frequently in PMs as in EMs, indicating that PMs would have been more prone to adverse drug reactions. It is to be noted that the patients´ genotypes were unknown to the physicians during the study period.

Basile and coll. (2000) suggested that the genetic polymorphism in the first intron of the *CYP1A2* gene, influencing the inducibility of CYP1A2 by smoking, might represent a genetic risk factor for the development of tardive dyskinesia in schizophrenic patients. However, in another study, no support for this hypothesis was found (Schulze 2001).

4.3. Genotype-based dosage recommendations

The first preliminary average dose suggestions based on *CYP2D6* or *CYP2C19* genotypes have been published for 14 different antidepressant drugs (Kirchheiner 2001). The recommendations are based on a thorough review of studies with pharmacokinetic data on various antidepressants among PM, EM and UM subjects. Most of the data are from Caucasian populations. For TCAs, dosage reductions to about 50% of the manufacturer's usual recommendation were suggested in CYP2D6 PMs, while the difference was smaller for SSRIs. For the few drugs with (sparse) data from UM, the preliminary recommended doses were 200-300% of the usual dose. This is the first attempt to apply the pharmacogenetic knowledge to practical dose recommendations. Prospective clinical studies with large patient populations and proper outcome measurement are required to evaluate these recommendations.

5. CONCLUSION

Although the role of polymorphic CYPs, especially CYP2D6, in the metabolism of many antipsychotics and antidepressants has been assessed both *in vitro* and *in vivo*, no clear correlation has been demonstrated between polymorphic CYP activity and clinical response to these compounds. Theoretically, when treated with standard doses of CYP2D6 substrates, PMs should be more prone to develop concentration-dependent adverse effects, while UMs would be at higher risk of therapeutic failure. Accordingly, some studies suggest that PMs of CYP2D6 are more prone to oversedation and possibly parkinsonism during treatment with classical antipsychotics. Other studies have, however, been negative or inconclusive and the newer antipsychotics have not been studied in this respect. On the other hand, many studies showing a lack of relationship between CYP2D6 pheno/genotype and drug effects have methodological limitations that reduce their validity. The clinical outcome data are even scantier for antidepressant drugs.

Whether pheno- or genotyping can be used prospectively to predict an optimal dose range and to improve the therapeutic outcome and minimise side effects remains to be evaluated. The recently published preliminary genotype-based dose recommendations for antidepressants pave the way for such urgently needed prospective studies. There is increasing evidence of the role of other CYPs, especially CYP1A2 and CYP3A4, as causes of interindividual variability in the kinetics and, presumably, clinical response to psychotropic drugs. Whether geno- or haplotype analysis of these or other drug metabolising enzyme genes will further increase our knowledge on the genetic basis of variability in drug metabolic capacity remains to be shown. With respect to CYP2D6 and CYP2C19, genotyping can presently be recommended as a complement to plasma concentration determination when aberrant metabolic capacity (poor or ultrarapid) is suspected. On the other hand, as newer antipsychotic and antidepressant drugs show a broader therapeutic index with a less severe adverse effect profile and appear to be less dependent on polymorphic metabolic pathways, they might be a safer choice in patients with extremely high or low CYP2D6 activity.

6. REFERENCES

Agundez, J.A., Ledesma, M.C., Ladero, J.M. et al., 1995, Prevalence of CYP2D6 gene duplication and its repercussion on the oxidative phenotype in a white population, *Clin. Pharmacol. Ther.* **57**, 265-269.

Aitchison, K.J., Munro, J., Wright, P. et al., 1999, Failure to respond to treatment with typical antipsychotics is not associated with CYP2D6 ultrarapid hydroxylation, *Br. J. Clin. Pharmacol.* **48**, 388-394.

Alexanderson, B., Evans, D.A., Sjöqvist, F., 1969, Steady-state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy, *Br. Med. J.* **4**, 764-768.

Alvan, G., Bechtel, P., Iselius, L. et al., 1990, Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations, *Eur. J. Clin. Pharmacol*. **39**, 533-537.

Andreassen, O.A., Macewan, T., Gulbrandsen, A.K., Mccreadie, R.G., Steen, V.M., 1997, Non-functional CYP2D6 alleles and risk for neuroleptic-induced movement disorders in schizophrenic patients, *Psychopharmacology*. **131**, 174-179.

Armstrong, M., Daly, A.K., Blennerhassett, R., Ferrier, N., Idle, J., 1997, Antipsychotic drug-induced movement disorders in schizophrenics in relation to CYP2D6 genotype, *Br. J. Psychiatry*. **170**, 23-26.

Arthur, H., Dahl, M.L., Siwers, B., Sjöqvist, F., 1995, Polymorphic drug metabolism in schizophrenic patients with tardive dyskinesia, *J. Clin. Psychopharmacol*. **15**, 211-216.

Avenoso, A., Spina, E., Campo, G. et al., 1997, Interaction between fluoxetine and haloperidol: pharmacokinetic and clinical implications, *Pharmacol. Res.* **35**, 335-339.

Barbhaiya, R.H., Buch, A.B., Greene, D.S., 1996, Single and multiple dose pharmacokinetics of nefazodone in subjects classified as extensive and poor metabolizers of dextromethorphan, *Br. J. Clin. Pharmacol.* **42**, 573-581.

Basile, V.S., Özdemir, V., Masellis, M. et al., 2000, A functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene: association with tardive dyskinesia in schizophrenia, *Mol. Psychiatry* **5**, 410-417.

Baumann, P., Jonzier Perey, M., Koeb, L., Küpfer, A., Tinguely, D., Schopf, J., 1986, Amitriptyline pharmacokinetics and clinical response: II. Metabolic polymorphism assessed by hydroxylation of debrisoquine and mephenytoin, *Int. Clin. Psychopharmacol.* **1**, 102-112.

Berecz, R., De la Rubia, Dorado, A.P., Fernandez-Salguero, P., Dahl, M.L., Llerena, A., 2003, Thioridazine steady-state plasma concentrations are influenced by tobacco smoking and CYP2D6, but not by the CYP2C9 genotype, *Eur. J. Clin. Pharmacol*. **59**(1), 45-50.

Bernal, M.L., Sinues, B., Johansson, I. et al., 1999, Ten percent of North Spanish individuals carry duplicated or triplicated CYP2D6 genes associated with ultrarapid metabolism of debrisoquine, *Pharmacogenetics.* **9**, 657-660.

Bertilsson, L., Åberg-Wistedt, A., Gustafsson, L.L., Nordin, C., 1985, Extremely rapid hydroxylation of debrisoquine: a case report with implication for treatment with nortriptyline and other tricyclic antidepressants, *Ther. Drug Monit.* **7**, 478-480.

Bertilsson, L., Carrillo, J.A., Dahl, M.L. et al., 1994, Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test, *Br. J. Clin. Pharmacol*. **38**, 471-473.

Bertilsson, L., Dahl, M.L., Sjöqvist, F., Åberg-Wistedt, A., Humble, M., Johansson, I., Lundqvist, E., Ingelman-Sundberg, M., 1993, Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine [letter], *Lancet.* **341**, 63.

Bertilsson, L., Dahl, M-L., Dalén, P., Al-Shurbaji, A., 2002, Molecular genetics of CYP2D6: Clinical relevance with focus on psychotropic drugs, *Br. J. Clin. Pharmacol.* **53**, 111-122.

Bertilsson, L., Lou, Y., Q., Du, Y.L. et al., 1992, Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquine and S-mephenytoin, *Clin. Pharmacol. Ther*. **51**, 388-397.

Bertilsson, L., Mellström, B., Sjöqvist, F., Martenson, B., Åsberg, M., 1981, Slow hydroxylation of nortriptyline and concomitant poor debrisoquine hydroxylation: clinical implications [letter], *Lancet.* **1**, 560-561.

Bloomer, J.C., Woods, F.R., Haddock, R.E., Lennard, M.S., Tucker, G.T., 1992, The role of cytochrome P4502D6 in the metabolism of paroxetine by human liver microsomes, *Br. J. Clin. Pharmacol.* **33**, 521-523.

Bork, J.A., Rogers, T., Wedlund, P.J. et al., 1999, A pilot study on risperidone metabolism: the role of cytochromes P450 2D6 and 3A, *J. Clin. Psychiatry.* **60**, 469-476.

Brachtendorf, L., Jetter, A., Beckurts, K.T., Hölscher, A.H., Fuhr, U., 2002, Cytochrome P-450 enzymes contributing to demethylation of maprotiline in man, *Pharmacology & Toxicology.* **90**, 144-149.

Brandolese, R., Scordo, M.G., Spina, E., Gusella, M., Padrini, R. 2001, Severe phenytoin intoxication in a homozygous CYP2C9*3 metabolizer, *Clin. Pharmacol. Ther*. **70**, 391-394.

Brockmoller, J., Kirchheiner, J., Schmider, J., Walter, S., Sachse, C., Muller-Oerlinghausen, B., Roots, I., 2002, The impact of the CYP2D6 polymorphism on haloperidol pharmacokinetics and on the outcome of haloperidol treatment, *Clin. Pharmacol. Ther*. **72**, 438-452.

Broly, F., Gaedigk, A., Heim, M., Eichelbaum, M., Morike, K., Meyer, U.A., 1991, Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of CYP2D6 in a European population, *DNA Cell. Biol*. **10**, 545-558.

Brøsen, K., Gram, L.F., Klysner, R., Bech, P., 1986, Steady-state levels of imipramine and its metabolites: significance of dose-dependent kinetics, *Eur. J. Clin. Pharmacol.* **30**, 43-49.

Brøsen, K., Klysner, R., Gram, L.F., Otton, S.V., Bech, P., Bertilsson, L., 1986, Steady-state concentrations of imipramine and its metabolites in relation to the sparteine/debrisoquine polymorphism, *Eur. J. Clin. Pharmacol.* **30**, 679-684.

Caccia, S., 1998, Metabolism of the newer antidepressants. An overview of the pharmacological and pharmacokinetic implications, *Clin. Pharmacokinet.* **34**, 281-302.

Caccia, S., 2000, Biotransformation of post-clozapine antipsychotics: pharmacological implications, *Clin. Pharmacokinet*. **38**, 393-414.

Callaghan, J.T., Bergstrom, R.F., Ptak, L.R., Beasley, C.M., 1999, Olanzapine: pharmacokinetic and pharmacodynamic profile, *Clin. Pharmacokinet*. **37**, 177-193.

Carrillo, J.A., Dahl, M.L., Svensson, J.O., Alm, C., Rodriguez, I., Bertilsson, L., 1996, Disposition of fluvoxamine in humans is determined by the polymorphic CYP2D6 and also by the CYP1A2 activity, *Clin. Pharmacol. Ther.* **60**, 183-190.

Carrillo, J.A., Ramos, S.I., Herraiz, A.G. et al., 1999, Pharmacokinetic interaction of fluvoxamine and thioridazine in schizophrenic patients, *J. Clin. Psychopharmacol*. **19**, 494-499.

Centorrino, F., Baldessarini, R.J., Frankenburg, F. et al., 1996, Serum levels of clozapine and norclozapine in patients treated with selective serotonin re-uptake inhibitors, *Am. J. Psychiatry.* **153**, 820–822.

Centorrino, F., Baldessarini, R.J., Kando, J., Frankenburg, F.R., Volpicelli, S.A., Flood, J.G., 1994, Serum concentrations of clozapine and its metabolites: effects of cotreatment with fluoxetine or valproate, *Am. J. Psychiatry.* **151**, 123–125.

Chang, M., Dahl, M.L., Tybring, G., Gotharson, E., Bertilsson, L., 1995, Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype, *Pharmacogenetics.* **5**, 358-363.

Chiba, K., Saitoh, A., Koyama, E., Tani, M., Hayashi, M., Ishizaki, T., 1994, The role of S-mephenytoin 4'-hydroxylase in imipramine metabolism by human liver microsomes: a two-enzyme kinetic analysis of N-demethylation and 2-hydroxylation, *Br. J. Clin. Pharmacol.* **37**, 237-242.

Chida, M., Yokoi, T., Fukui, T., Kinoshita, M., Yokota, J., Kamataki, T., 1999, Detection of three genetic polymorphisms in the 5'-flanking region and intron 1 of human CYP1A2 in the Japanese population, *Jpn. J. Cancer Res*. **90**, 899-902.

Chou, W.H., Yan, F.X., De Leon, J. et al., 2000, Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness, *J. Clin. Psychopharmacol*. **20**, 246-251.

Cohen, L.G., Chesley, S., Eugenio, L., Flood, J.G., Fisch, J., Goff, D.C., 1996, Erythromycin-induced clozapine toxic reaction, *Arch. Int. Med*. **156**, 675–677.

Dahl, M.L., Bertilsson, L., Nordin, C., 1996, Steady-state plasma levels of nortriptyline and its 10-hydroxy metabolite: relationship to the CYP2D6 genotype, *Psychopharmacology (Berl).* **123**, 315-319.

Dahl, M.L., Ekqvist B., Widen, J., Bertilsson, L., 1991, Disposition of the neuroleptic zuclopenthixol cosegregates with the polymorphic hydroxylation of debrisoquine in humans, *Acta Psychiatr. Scand.* **84**, 99-102.

Dahl, M.L., Iselius, L., Alm, C., Svensson, J.O., Lee, D., Johansson, I., Ingelman-Sundberg, M., Sjoqvist F., 1993, Polymorphic 2-hydroxylation of desipramine. A population and family study, *Eur. J. Clin. Pharmacol*. **44**, 445-450.

Dahl, M.L., Johansson, I., Bertilsson, L. et al, 1995, Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis, *J. Pharmacol. Exp. Ther.* **274**, 516-520.

Dahl, M.L., Johansson, I., Palmertz, M.P., Ingelman-Sundberg, M., Sjöqvist, F., 1992, Analysis of the CYP2D6 gene in relation to debrisoquin and desipramine hydroxylation in a Swedish population, *Clin. Pharmacol. Ther.* **51**, 12-17.

Dahl, M.L., Llerena, A., Bondesson, U. et al., 1994, Disposition of clozapine in man: lack of association with debrisoquine and S-mephenytoin hydroxylation polymorphisms, *Br. J. Clin. Pharmacol.* **37**, 71-74.

Dahl, M.L., Tybring, G., Elwin, C.E., Alm, C., Andreasson, K., Gyllenpalm, M., Bertilsson, L., 1994, Stereoselective disposition of mianserin is related to debrisoquin hydroxylation polymorphism. *Clin. Pharmacol. Ther.* **56**, 176-183.

Dahl, M.L., Voortman, G., Alm, C., Elwin, C.E., Delbressine, L., Vos, R., Bogaards, J.J.P., Bertilsson, L., 1997, In vitro and in vivo studies on the disposition of mirtazapine in humans, *Clin. Drug Invest.* **13**, 37-46.

Dahl, M-L., 2002, Cytochrome P450 phenotyping/genotyping in patients receiving antipsychotics. Useful aid to prescribing? *Clin. Pharmacokinet.* **41**(7), 453-470.

Dahl, S.G., 1982, Active metabolites of neuroleptic drugs: possible contribution to therapeutic and toxic effects, *Ther. Drug Monit.* **4**, 33-40.

Dahl-Puustinen, M.L., Liden, A. Alm, C., Nordin, C., Bertilsson, L., 1989, Disposition of perphenazine is related to polymorphic debrisoquin hydroxylation in human beings, *Clin. Pharmacol. Ther.* **46**, 78-81.

Dalén, P., Dahl, M.L., Ruiz, M.L., Nordin, J., Bertilsson, L., 1998, 10-Hydroxylation of nortriptyline in white persons with 0, 1, 2, 3, and 13 functional CYP2D6 genes, *Clin. Pharmacol. Ther.* **63**, 444-452.

Daniel, D.G., Randolph, C., Jaskiw, G. et al., 1994, Coadministration of fluvoxamine increases serum concentrations of haloperidol, *J. Clin. Psychopharmacol*. **14**, 340-343.

De Jong, J., Hoogenboom, B., Van Troostwijck, L.D., De Hann, L., 2001, Interaction of olanzapine with fluvoxamine, *Psychopharmacology.* **156**, 219–220.

De Morais, S.M., Wilkinson, G.R., Blaisdell, J. et al., 1994, Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese, *Mol. Pharmacol* **46**, 594-598.

De Morais, S.M., Wilkinson, G.R., Blaisdell, J. et al., 1994, The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans, *J. Biol. Chem.* **269**, 15419-15422.

Delbressine, L.P., Vos, R.M., 1997, The clinical relevance of preclinical data: Mirtazapine, a model compound, *J. Clin. Psychopharmacol.* **17**, 29S-33S.

Desai, H.D., Seabolt, J., Jann, M.W., 2001, Smoking in patients receiving psychotropic medications: a pharmacokinetic perspective, *CNS Drugs*. **15**, 469-494.

Dev, V., Raniwalla, J., 2000, Quetiapine: a review of its safety in the management of schizophrenia, *Drug Saf*. **23**, 295-307.

Devane, C.L., Nemeroff, C.B., 2001, Clinical pharmacokinetics of quetiapine: an atypical antipsychotic, *Clin. Pharmacokinet*. **40**, 509-522.

Dodd, S., Boulton, D.W., Burrows, G.D., DeVane, C.L., Norman, T.R., 2001, In vitro metabolism of mirtazapine enantiomers by human cytochrome P450 enzymes, *Hum. Psychopharmacol. Clin. Exp.* **16**, 541-544.

Eap, C.B., Bondolfi, G., Zullino, D., Savary-Cosendai, L., Powell-Golay, K., Kosel, M., Baumann, P., 2001, Concentrations of the enantiomers of fluoxetine and norfluoxetine after multiple doses of fluoxetine in cytochrome P4502D6 poor and extensive metabolizers, *J. Clin. Psychopharmacol.* **21**, 330-334.

Eap, C.B., Lessard, E., Baumann, P., Brawand-Amey, M., Yessine, M-A., O'Hara, G., Turgeon, J., 2003, Role of CYP2D6 in the stereoselective disposition of venlafaxine in humans, *Pharmacogenetics.* **13,** 39-47.

Eap, C.B., Lima, C. A., Macciardi, F., Woggon, B., Powell, K., Baumann, P., 1998, Steady state concentrations of the enantiomers of mianserin and desmethylmianserin in poor and in homozygous and heterozygous extensive metabolizers of debrisoquine, *Ther. Drug. Monit.* **20**(1), 7-13.

Eap, C.B., Guentert, T.W., Schaublin M., Loidl et al., 1996, Plasma levels of the enantiomers of thioridazine, thioridazine 2-sulfoxide, thioridazine 2-sulfone, and thioridazine 5-sulfoxide in poor and extensive metabolizers of dextromethorphan and mephenytoin, *Clin. Pharmacol. Ther.* **59**, 322-331.

Eichelbaum, M., Spannbrucker, N., Steincke, B. et al., 1979, Defective N-oxidation of sparteine in man: a new pharmacogenetic defect, *Eur. J. Clin. Pharmacol.* **16**, 183-187.

Eiermann, B., Engel, G., Johansson, I. et al., 1997, The involvement of CYP1A2 and CYP3A4 in the metabolism of clozapine, *Br. J. Clin. Pharmacol*. **44**, 439-446.

Evans, D.A., Mahgoub, A., Sloan, T.P. et al., 1980, A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population, *J. Med. Genet.* **17**, 102-105.

Fang, J., Baker, G.B., Silverstone, P.H. et al., 1997, Involvement of CYP3A4 and CYP2D6 in the metabolism of haloperidol, *Cell. Mol. Neurobiology.* **17**, 227-233.

Fang, J., Bourin, M., Baker, G.B., 1999, Metabolism of risperidone to 9-hydroxyrisperidone by human cytochromes P450 2D6 and 3A4, *Naunyn-Schmiedeberg's Arch. Pharmacol*. **359**, 147- 151.

Fang, J., Gorrod, J.W., 1999, Metabolism, pharmacogenetics and metabolic drug-drug interactions of antipsychotic drugs, *Cell. Mol. Neurobiol*. **19**, 491-510.

Firkusny, L., Gleiter, C.H., 1994, Maprotiline metabolism appears to co-segregate with the genetically- determined CYP2D6 polymorphic hydroxylation of debrisoquine. *Br. J. Clin. Pharmacol.* **37**, 383-388.

Fischer, V., Vogels, B., Maurer, G. et al., 1992, The antipsychotic clozapine is metabolized by the polymorphic human microsomal and recombinant cytochrome P450 2D6, *J. Pharmacol. Exp. Ther.* **260**, 1355-1360.

Fjordside, L., Jeppesen, U., Eap, C.B., Powell, K., Baumann, P., Brøsen, K., 1999, The stereoselective metabolism of fluoxetine in poor and extensive metabolizers of sparteine, *Pharmacogenetics.* **9**, 55-60.

Fogelman, S.M., Schmider, J., Venkatakrishnan, K., Von Moltke, L.L., Harmatz, J.S., Shader, R.I., Greenblatt, D. J., 1999, O- and N-demethylation of venlafaxine in vitro by human liver microsomes and by microsomes from cDNA-transfected cells: Effect of metabolic inhibitors and SSRI antidepressants, *Neuropsychopharmacol.* **20**, 480-490.

Fukuda, T., Nishida, Y., Zhou, Q., Yamamoto, I., Kondo, S., Azuma, J., 2000, The impact of the CYP2D6 and CYP2C19 genotypes on venlafaxine pharmacokinetics in a Japanese population, *Eur. J. Clin. Pharmacol.* **56**, 175-180.

Funderburg, L.G., Vertrees, J.E., True, J.E., Miller, A.L., 1994, Seizure following addition of erythromycin to clozapine therapy, *Am. J. Psychiatry.* **151**, 1840–1841.

Gaedigk, A., Blum, M., Gaedigk, R., Eichelbaum, M., Meyer, U.A., 1991, Deletion of the entire cytochrome P450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism, *Am. J. Hum. Genet*. **48**, 943-950.

Goldstein, J.A., 2001, Clinical relevance of genetic polymorphisms in the human CYP2C subfamily, *Br. J. Clin. Pharmacol*. **52**, 349-355.

Gonzales, F.J., 1992, Human cytochrome P450: problems and prospects, *Trends Pharmacol. Sci*. **13**, 346-352.

Gram, L.F., 1977, Plasma level monitoring of tricyclic antidepressant therapy, *Clin. Pharmacokinet.* **2**, 237-251.

Gram, L.F., Guentert, T.W., Grange, S., Vistisen, K., Brøsen, K., 1995, Moclobemide, a substrate of CYP2C19 and an inhibitor of CYP2C19, CYP2D6, and CYP1A2: a panel study. *Clin. Pharmacol. Ther.* **57**, 670-677.

Guengerich, F.P., 1995, Human cytochrome P450 enzymes. In: Cytochrome P450: structure, mechanism, and biochemistry, Ortiz De La Montellano PR (Ed.), Plenum Press, New York, 473-535.

Güsey, C., Aamo, T., Spigset, O., 2000, Risperidone metabolism and the impact of being a cytochrome P450 2D6 ultrarapid metabolizer, *J. Clin. Psychiatry.* **61**, 600-601.

Hagg, S., Spigset, O., Mjorndal, T., Granberg, K., Persbo-Lundqvist, G., Dahlqvist, R., 1999, Absence of interaction between erythromycin and a single dose of clozapine, *Eur. J. Clin. Pharmacol.* **55**, 221-226.

Hale, P., Poklis, A., 1996, Thioridazine cardiotoxicity, *J. Toxicol. Clin. Toxicol*. **34**, 127-130.

Hamelin, B.A., Turgeon, J., Vallee, F., Belanger, P.M., Paquet, F., LeBel, M., 1996, The disposition of fluoxetine but not sertraline is altered in poor metabolizers of debrisoquin. *Clin. Pharmacol. Ther.* **60**, 512-521.

Hammer, W., Sjöqvist, F., 1967, Plasma levels of monomethylated tricyclic antidepressants during treatment with imipramine-like compounds, *Life Sci.* **6**, 1895-1903.

Haring, C., Fleischhacker, W., Schett, P., Humpel, C., 1990, Influence of plasma related variables on clozapine levels, *Am. J. Psychiatry*.**147**, 1471-1475.

Härtter, S., Dingemanse, J., Baijer, D., Ziegler, G., Hiemke, C., 1996, The role of cytochrome P450 2D6 in the metabolism of moclobemide, *Eur. Neuropsychopharmacol.* **6**(3), 225-230.

Hasegawa, M., Guiterrez, R., Way, L., Meltzer, H.Y., 1993, Relationship between clinical efficacy and clozapine concentration in plasma in schizophrenia: effect of smoking, *J. Clin. Psychopharmacol.* **13**, 383-390.

Hiemke, C., Weigmann, H., Härtter, S. et al., 1994, Elevated levels of clozapine in serum after addition of fluvoxamine*, J. Clin. Psychopharmacol.* **14**, 279-281.

Holliday, S.M., Benfield, P., 1995, Venlafaxine – a review of its pharmacology and therapeutic potential in depression, *Drugs.* **49**, 280-294.

Hoskins, J., Shenfield, G., Murray, M., Gross, A., 2001, Characterization of moclobemide N-oxidation in human liver microsomes, *Xenobiotica.* **31**(7), 387-397.

Huang, M.L., Van Peer, A., Woestenborghs, R. et al., 1993, Pharmacokinetics of the novel antipsychotic agent risperidone and the prolactin response in healthy subjects, *Clin. Pharmacol. Ther.* **54**, 257-268.

Hubbard, J.W., Midha, K.K., Hawe, E.M. et al., 1993, Metabolism of phenothiazine and butyrophenone antipsychotic drugs: a review of some recent research findings and clinical implications, *Br. J. Psychiatry.***163**, 19-24.

Ingelman-Sundberg, M., Oscarsson, M., Mc Lellan, R.A., 1999, Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment, *Trends Pharmacol. Sci.* **20**, 342-349 (1999).

Ishida, M., Otani, K., Kaneko S., Ohkubo, T., Osanai, T., Yasui N., Mihara, K., Higuchi H., Sugawara, K., 1995, Effects of various factors on steady state plasma concentrations of trazodone and its active metabolite m-chlorophenylpiperazine. *Int. Clin. Psychopharmacol.* **10**, 143-146.

Jaanson, P., Marandi, T., Kiivet, R. A., Vasar, V., Vään, S., Svensson, J. O., Dahl, M. L., 2002, Maintenance therapy with zuclopenthixol decanoate: associations between plasma concentrations, neurological side effects and CYP2D6 genotype, *Psychopharmacol.* **162**, 67-73.

Jan, M.W., ZumBrunnen, T.L., Kazmi, Y.R., VanDenBerg, C.M., Desai,. H.D., Weidler, D.J., Flockhart, D.A., 2002, Pharmacokinetics of fluvoxamine in relation to CYP2C19 phenotype and genotype, *Drug. Metabol. Drug. Interact.* **19**(1), 1-11.

Jerling, M., Dahl, M.L., Åberg-Wistedt, A. et al., 1996, The CYP2D6 genotype predicts the oral clearance of the neuroleptic agents perphenazine and zuclopenthixol, *Clin. Pharmacol. Ther.* **59**, 423-428.

Joergensen, A., 1986, Metabolism and pharmacokinetics of antipsychotic drugs, *Prog. Drug Metabolism.* **9**, 111-174.

Johansson, I., Lundqvist, E., Bertilsson, L. et al., 1993, Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine, *Proc. Natl. Acad. Sci. USA.* **90**, 11825-11829.

Johansson, I., Oscarson, M., Yue, Q.Y. et al., 1994, Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation, *Mol. Pharmacol.* **46**, 452-459.

Kagimoto, M., Heim, M., Kagimoto, K., Zeugin, T., Meyer, U.A., 1990, Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes, *J. Biol. Chem.* **265**, 17209-17214.

Kassahun, K., Mattiuz, E., Nyhart, E. et al., 1997, Disposition and biotransformation of the antipsychotic agent olanzapine in humans, *Drug Metab. Dispos*. **25**, 81-93.

Kirchheiner, J., Brøsen, K., Dahl, M-L., Gram L. F., Kasper, S., Roots, I., Sjöqvist, F., Spina, E., Brockmöller, J., 2001, CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages, *Acta. Psychiatr. Scand.* **104**, 173-192.,

Kobayashi, K., Chiba, K., Yagi, T., Shimada, N., Taniguchi, T., Horie, T., Tani, M., Yamamoto, T., Ishizaki, T., Kuroiwa, Y., 1997, Identification of cytochrome P450 isoforms involved in citalopram N-demethylation by human liver microsomes, *J. Pharmacol. Exp. Ther.* **280**, 927-933.

Kobayashi, K., Ishizuka, T., Shimada, N., Yoshimura, Y., Kamijima, K., Chiba, K., 1999, Sertraline N-demethylation is catalyzed by multiple isoforms of human cytochrome P-450 in vitro, *Drug Metab. Dispos*. **27**, 763-766.

Koyama, E., Chiba, K., Tani, M., Ishizaki, T., 1996, Identification of human cytochrome P450 isoforms involved in the stereoselective metabolism of mianserin enantiomers, *J. Pharmacol. Exp. Ther.* **278**, 21-30.

Koyama, E., Tanaka, T., Chiba, K., Kawakatsu, S., Morinobu, S., Totsuka, S., Ishizaki, T., 1996, Steady-state plasma concentrations of imipramine and desipramine in relation to S-mephenytoin 4'-hydroxylation status in Japanese depressive patients, *J. Clin. Psychopharmacol.* **16**, 286-293.

Kuo, F.J., Lane, H.Y., Chang, W.H., 1998, Extrapyramidal symptoms after addition of fluvoxamine to clozapine*, J. Clin. Psychopharmacol*. **53**, 483-484.

Kvist, E.E., Al-Shurbaji, A., Dahl, M-L., Nordin, C., Alván, G., Ståhle, L., 2001, Quantitative pharmacogenetics of nortriptyline. A novel approach, *Clin. Pharmacokinet.* **40**(11), 869-877.

Lane, H.Y., Hu, O.Y.P., Jann, M.W. et al., 1997, Dextromethorphan phenotyping and haloperidol disposition in schizophrenic patients, *Psychiatry Res.* **69**, 105-111.

Lee, C.R., Goldstein, J.A., Pieper, J.A., 2002, Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in vitro and human data, *Pharmacogenetics.* **12**, 251-263.

Lemoine, A., Gautier, J.C., Azoulay, D., Kiffel, L., Belloc, C., Guengerich, F.P., Maurel, P., Beaune, P., Leroux, J.P., 1993, Major pathway of imipramine metabolism is catalyzed by cytochromes P-450 1A2 and P-450 3A4 in human liver, *Mol. Pharmacol.* **43**, 827-832.

Lessard, E., Yessine, M.A., Hamelin, B.A., O'Hara, G., LeBlanc, J., Turgeon, J., 1999, Influence of CYP2D6 activity on the disposition and cardiovascular toxicity of the antidepressant agent venlafaxine in humans, *Pharmacogenetics.* **9**, 435-443.

Linnet, K., Olesen O.V., 1997, Metabolism of clozapine by cDNA expressed human cytochrome P450 enzymes, *Drug Metab. Dispos.* **25**, 1379-1382.

Linnet, K., Wiborg, O., 1996, Steady-state serum concentrations of the neuroleptic perphenazine in relation to CYP2D6 genetic polymorphism, *Clin. Pharmacol. Ther.* **60**, 41-47.

Linnet, K., Wiborg, O., 1996, Influence of CYP2D6 genetic polymorphism on ratios of steady-state serum concentrations to dose of the neuroleptic zuclopenthixol. *Ther. Drug. Monit.* **18**, 629-634.

Liu, Z-Q., Cheng, Z-N., Huang, S-L., Chen, X-P., Ou-Yang, D-S., Jiang, C-H., Zhou, H-H., 2001, Effect of the CYP2C19 oxidation polymorphism on fluoxetine metabolism in Chinese healthy subjects, *J. Clin. Pharmacol.* **52**, 96-99.

Liu, Z-Q., Shu, Y., Huang, S-L., Wang, L-S., He, N., Zhou, H-H., 2001, Effects of CYP2C19 genotype and CYP2C9 on fluoxetine N-demethylation in human liver microsomes, *Acta. Pharmacol. Sin.* **22**(1), 85-90.

Liu, Z-Q., Zhu, B., Tan, Y-F., Tan, Z-R., Wang, L-S., Huang, S-L., Shu, Y., Zhou, H-H., 2001, O-Dealkylation of fluoxetine in relation to CYP2C19 gene dose and involvement of CYP3A4 in human liver microsomes**,** *J. Pharmacol. Exp. Ther.* **299**, 105-111.

Llerena, A., Alm, C., Dahl, M.L. et al., 1992, Haloperidol disposition is dependent on debrisoquine hydroxylation phenotype*, Ther. Drug Monit*. **14**, 92-97.

Llerena, A., Dahl, M.L., Ekqvist, B. et al., 1992, Haloperidol disposition is dependent on the debrisoquine hydroxylation phenotype: increased plasma levels of the reduced metabolite in poor metabolizers, *Ther. Drug Monit.* **14**, 261-264.

Llerena, A., Dorado, P., Berecz, R., González, A.P., Cáceres, M.C., 2003, Influence of CYP2D6 and CYP2C9 genotypes on fluoxetine plasma concentrations in psychiatric patients: *European Collaboration:Towards Drug Development and Rational Drug Therapy,* eds. F. C. Tulunay, M. Orme, Proceedings of the Sixth Congress of the European Association for Clinical Pharmacology and Therapeutics, Istanbul, June 24-28.

Lucas, R.A., Gilfillan, D.J., Bergstrom, R.F., 1998, A pharmacokinetic interaction between carbamazepine and olanzapine: observations on possible mechanisms, *Eur. J. Clin. Pharmacol.* **54**, 639-643.

Mäenpää, J., Wrightson, S., Bergstrom, R. et al., 1997, Pharmacokinetic (Pk) and pharmacodynamic (Pd) interactions between fluvoxamine and olanzapine, *Clin. Pharmacol. Ther.* **61**, 225.

Mahgoub, A., Idle, J.R., Dring, L.G., et al., 1977, Polymorphic hydroxylation of debrisoquine in man, *Lancet* **2**, 584-586.

Mannens, G., Huang, M.L., Meuldermans, W. et al., 1993, Absorption, metabolism, and excretion of risperidone in humans*, Drug Metab. Dispos.* **21**, 1134-1141.

Margolis, J.M., O'Donnell, J.P., Mankowski, D.C., Ekins, S., Obach, R.S., 2000, (R)-, (S)-, and racemic fluoxetine N-demethylation by human cytochrome P450 enzymes, *Drug. Metab. Dispos.* **28**, 1187-1191.

Markowitz, J.S., Devane, C.L., 1999, Suspected ciprofloxacin inhibition of olanzapine resulting in increased plasma concentrations, *J. Clin. Psychopharmacol.* **19**, 289-291.

Mellström, B., Bertilsson, L., Lou, Y.C., Säwe, J., Sjöqvist, F., 1983, Amitriptyline metabolism: relationship to polymorphic debrisoquine hydroxylation, *Clin. Pharmacol. Ther.* **34**, 516-520.

Mellström, B., Säwe, J., Bertilsson, L., Sjöqvist, F., 1986, Amitriptyline metabolism: association with debrisoquin hydroxylation in nonsmokers, *Clin. Pharmacol. Ther.* **39**, 369-371.

Meyer, J.W., Woggon, B., Baumann, P. et al., 1990, Clinical implications of slow sulphoxidation of thioridazine in a poor metabolizer of the debrisoquine type, *Eur. J. Clin. Pharmacol*. **39**, 613-614.

Miceli, J.J., Anziano, R.J., Robarge, L., Hansen, R.A., Laurent, A., 2000, The effect of carbamazepine on the steady-state pharmacokinetics of ziprasidone in healthy volunteers, *Br. J. Clin. Pharmacol.* **49** (Suppl. 1), 65s-70s.

Miceli, J.J., Smith, M., Robarge, L., Morse, T., Laurent, A., 2000, The effects of ketoconazole on ziprasidone pharmacokinetics: a placebo-controlled crossover study in healthy volunteers, *Br. J. Clin. Pharmacol.* **49** (Suppl. 1), 71s-76s.

Mihara, K., Otani, K., Suzuki A., Yasui, N., Nakano, H., Meng, X., Ohkubo T., Nagasaki T., Kaneko, S., Tsuchida, S., Sugawara, K.,. Gonzalez, F.J., 1997, Relationship between the CYP2D6 genotype and the steady-state plasma concentrations of trazodone and its active metabolite m-chlorophenylpiperazine, *Psychopharmacology (Berl)* **133**, 95-98.

Mihara, K., Otani, K., Tybring, G., Dahl, M.L., Bertilsson, L., Kaneko, S., 1997, The CYP2D6 genotype and plasma concentrations of mianserin enantiomers in relation to therapeutic response to mianserin in depressed Japanese patients, *J. Clin. Psychopharmacol.* **17**, 467-471.

Mihara, K., Suzuki, A., Kondo, T. et al., 1999, Effects of the CYP2D6*10 allele on the steady-state plasma concentrations of haloperidol and reduced haloperidol in Japanese patients with schizophrenia, *Clin. Pharmacol. Ther.* **65**, 291-294.

Mihara, K., Suzuki, A., Kondo, T. et al., 2000, Effect of a genetic polymorphism of CYP1A2 inducibility on the steady state plasma concentrations of haloperidol and reduced haloperidol in Japanese patients with schizophrenia, *Ther. Drug Monit*. **22**, 245-249.

Miners, J.O., Birkett, D.J., 1998, Cytochrome P4502C9: an enzyme of major importance in human drug metabolism, *Br. J. Clin. Pharmacol*. **45**, 525-538.

Morinobu, S., Tanaka, T., Kawakatsu, S., Totsuka, S., Koyama, E., Chiba, K., Ishizaki, T., Kubota, T., 1997, Effects of genetic defects in the CYP2C19 gene on the N-demethylation of imipramine, and clinical outcome of imipramine therapy, *Psychiatry. Clin. Neurosci.* **51**, 253-257.

Morita, S., Shimoda, K., Someya, T., Yoshimura, Y., Kamijima, K., Kato, N., 2000, Steady-state plasma levels of nortriptyline and its hydroxylated metabolites in Japanese patients: Impact of CYP2D6 genotype on the hydroxylation of nortriptyline, *J. Clin. Psychopharmacol.* **20**, 141-149.

Nakajima, M., Yokoi, T., Mizutani, M. et al., 1999, Genetic polymorphism in the 5'-Flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans, *J. Biochem.* **125**, 803-808.

Nelson, D.R., Koymans, L., Kamataki, T. et al., 1996, P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature, *Pharmacogenetics* **6**, 1-42.

Nordin, C., Bertilsson, L., 1995, Active hydroxymetabolites of antidepressants. Emphasis on E-10-hydroxy-nortriptyline, *Clin. Pharmacokinet.* **28**, 26-40.

Nordin, C., Siwers, B., Benitez, J., Bertilsson, L., 1985, Plasma concentrations of nortriptyline and its 10-hydroxy metabolite in depressed patients–relationship to the debrisoquine hydroxylation metabolic ratio, *Br. J. Clin. Pharmacol.* **19**, 832-835.

Nyberg, S., Farde, L., Halldin, C. et al., 1995, D2 Dopamine receptor occupancy during low-dose treatment with haloperidol decanoate, *Am. J. Psychiatry* **152**, 173-178.

Ohmori, O., Suzuki, T., Kojima, H. et al., 1998, Tardive dyskinesia and debrisoquine 4-hydroxylase (CYP2D6) genotype in Japanese schizophrenics, *Schizophr. Res.* **32**, 107-113.

Olesen, O.V., Licht, R.W., Thomsen, E. et al., 1998, Serum concentrations and side effects in psychiatric patients during risperidone therapy, *Ther. Drug Monit*. **20**, 380-384.

Olesen, O.V., Linnet, K., 1999, Olanzapine serum concentrations in psychiatric patients given standard doses: the influence of comedication, *Ther. Drug Monit.* **21**, 87-90.

Olesen, O.V., Linnet, K., 2000, Identification of the human cytochrome P450 isoforms mediating *in vitro* N-dealkylation of perphenazine, *Br. J. Clin. Pharmacol*. **50**, 563-571.

Otani, K., Ishida M., Kaneko, S., Mihara, K., Ohkubo, T., Osanai, T., Sugawara, K., 1996, Effects of carbamazepine coadministration on plasma concentrations of trazodone and its active metabolite, m-chlorophenylpiperazine, *Ther. Drug Monit*.**18**, 164-167.

Otton, S.V., Ball, S.E., Cheung, S.W., Inaba, T., Rudolph, R.L., Sellers, E.M., 1996,Venlafaxine oxidation in vitro is catalysed by CYP2D6. *Br. J. Clin. Pharmacol.* **41**, 149-156.

Özdemir, V., Kalow, W., Okey, A.B. et al., 2001, Treatment-resistance to clozapine in association with ultrarapid CYP1A2 activity and the C→ A polymorphism in intron 1 of the CYP1A2 gene: effect of grapefruit juice and low-dose fluvoxamine, *J. Clin. Psychopharmacol.* **21**, 603-607.

Özdemir, V., Naranjo, C.A., Herrmann, N., Reed, K., Sellers, E.M., Kalow, W., 1997, Paroxetine potentiates the central nervous system side effects of perphenazine: contribution of cytochrome P4502D6 inhibition in vivo, *Clin. Pharmacol. Ther.* **62**, 334-347.

Özdemir, V., Tyndale, R. F., Reed, K., Herrmann, N., Sellers, E. M., Kalow, W., Naranjo, C. A., 1999, Paroxetine steady-state plasma concentration in relation to CYP2D6 genotype in extensive metabolizers, *J. Clin. Psychopharmacol.* **19**, 472-475.

Pan, L., Belpaire, F.M., 1999, *In vitro* study on the involvement of CYP1A2, CYP2D6 and CYP3A4 in the metabolism of haloperidol and reduced haloperidol, *Eur. J. Clin. Pharmacol*. **55**, 599-604.

Pan, L., Vander Stichele, R., Rosseel, M.T. et al., 1999, Effects of smoking, CYP2D6 genotype, and concomitant drug intake on the steady state plasma concentrations of haloperidol and reduced haloperidol in schizophrenic inpatients, *Ther. Drug Monit.* **21**, 489-497.

Pirmohamed, M., Williams, D., Madden, S. et al., 1995, Metabolism and bioactivation of clozapine by human liver *in vitro*, *J. Pharmacol. Exp. Ther*. **272**, 984-990.

Pollock, B.G., Mulsant, B.H., Sweet, R.A. et al., 1995, Prospective cytochrome P450 phenotyping for neuroleptic treatment in dementia, *Psychopharmacol. Bulletin* **31**, 327-331.

Prakash, C., Kamel, A., Cui, D. et al., 2000, Identification of the major human liver cytochrome P450 isoform(s) responsible for the formation of the primary metabolites of ziprasidone and prediction of possible drug interactions, *Br. J. Clin. Pharmacol.* **49** (Suppl I), 35s-42s.

Preskorn, S. H., 1993, Pharmacokinetics of antidepressants: Why and how they are relevant to treatment, *J. Clin. Psychiatry.* **54**(9), 14-34.

Raaska, K., Neuvonen, P.J., 1998, Serum concentrations of clozapine and N-desmethylclozapine are unaffected by the potent CYP3A4 inhibitor itraconazole, *Eur. J. Clin. Pharmacol*. **54**, 167–170.

Rasmussen, B.B., Brøsen, K., 2000, Is therapeutic drug monitoring a case for optimizing clinical outcome and avoiding interactions of the selective serotonin reuptake inhibitors?, *Ther. Drug. Monit.* **22**(2), 143-154.

Relling, M.V., Lin, J.S., Ayers, G.D., Evans, W.E., 1992, Racial and gender differences in N-acetyltransferase, xanthine oxidase and CYP1A2 activities, *Clin. Pharmacol. Ther*. **52**, 643-658.

Ring, B.J., Eckstein, J.A., Gillespie, J.S., Binkley, S.N., Vandenbranden M., Wrighton, S.A., 2001, Identification of the human cytochromes P450 responsible for in vitro formation of R- and S-norfluoxetine, *J. Pharmacol. Exp. Ther.* **297**, 1044-1050.

Ring, B.J., Catlow, J., Lindsay, T.J. et al., 1996, Identification of the human cytochrome P450 responsible for the *in vitro* formation of the major oxidative metabolites of the antipsychotic agent olanzapine, *J. Pharmacol. Exp. Ther.* **276**, 658-666.

Rochat, B., Amey, M., Gillet, M., Meyer, U.A., Baumann, P., 1997, Identification of three cytochrome P450 isozymes involved in N-demethylation of citalopram enantiomers in human liver microsomes, *Pharmacogenetics* **7**, 1-10.

Roh, H.K., Chung, J.Y., Oh, D.Y. et al., 2001, Plasma concentrations of haloperidol and its reduced metabolite are related to the CYP2D6 genotype at low, but not high doses of haloperidol in Korean schizophrenic patients, *Br. J. Clin. Pharmacol.* **52**, 265-271.

Rotzinger, S., Fang, J., Coutts, RT., Baker, G.B., 1998, Human CYP2D6 and metabolism of m-chlorophenylpiperazine, *Biol. Psychiatry* **44**, 1185-1191.

Sachse, C., Brockmöller, J., Bauer, S., Roots, I., 1997, Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences, *Am. J. Hum. Genet.* **60**, 284-295.

Sachse, C., Brockmöller, J., Bauer, S. et al., 1999, Functional significance of a C→ A polymorphism in Intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine, *Br. J. Clin. Pharmacol.* **47**, 445-449.

Sachse, C., Schmider, J., Kanitz, P. et al., 2000, Frequency of CYP2D6 genotypes in schizophrenic patients and relation to antipsychotic therapy. Proceedings of the VII World Conference

on Clinical Pharmacology and Therapeutics & 4th Congress of the European Association for Clinical Pharmacology and Therapeutics. Florence, Italy.

Sata, F., Sapone, A., Elizondo, G. et al., 2000, CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity, *Clin. Pharmacol. Ther.* **67**, 48-56.

Schulze, T.G., Schumacher, J., Muller, D.J. et al., 2001, Lack of association between a functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene and tardive dyskinesia in schizophrenia, *Am. J. Med. Genet.* **105**, 498-501.

Scordo, M.G., Spina, E. Dahl, M-L., Gatti, G., Perucca, E., 2003, Influence of CYP2C9, 2C19 and 2D6 genetic polymorphisms on plasma levels of fluoxetine and norfluoxetine enantiomers: *European Collaboration:Towards Drug Development and Rational Drug Therapy,* eds. F.C. Tulunay, M. Orme, Proceedings of the Sixth Congress of the European Association for Clinical Pharmacology and Therapeutics, Istanbul, June 24-28.

Scordo, M.G., Aklillu, E., Yasar, U., Dahl, M.L., Spina, E. Ingelman-Sundberg, M., 2001, Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population, *Br. J. Clin. Pharmacol.* **52**, 447-450.

Scordo, M.G., Pengo, V. Spina, E. Dahl, M.L. Gusella, M. Padrini, R., 2002, Influence of *CYP2C9* and *CYP2C19* genetic polymorphisms on warfarin maintenance dose and metabolic clearance, *Clin. Pharmacol. Ther.* **72**, 702-710.

Scordo, M.G., Spina, E., Facciolà, G. et al., 1999, Cytochrome P450 2D6 genotype and steady state plasma levels of risperidone and 9-hydroxyrisperidone, *Psychopharmacology* **147**, 300-305.

Scordo, M.G., Spina, E., Romeo, P. et al., 2000, CYP2D6 genotype and antipsychotic-induced extrapyramidal side-effecs in schizophrenic patients, *Eur. J. Clin. Pharmacol*. **56**, 679-683.

Scordo, M.G., Spina, E., 2002, Cytochrome P450 polymorphisms and response to antipsychotic therapy, *Pharmacogenomics* **3**, 201-218.

Shimada, T., Yamazaki, H., Mimura, M. et al., 1994, Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians, *J.Pharmacol. Exp. Ther.* **270**, 414-423.

Shimoda, K., Morita, S., Someya, T. et al., 2000, CYP2D6*10 alleles are not the determinant of the plasma haloperidol concentrations in Asian patients, *Ther. Drug Monit*. **122**, 392-396.

Shimoda, K., Someya, T., Morita, S. et al., 1999, Lower plasma levels of haloperidol in smoking than in nonsmoking schizophrenic patients, *Ther. Drug Monit.* **21**, 293-296.

Sindrup, S.H., Brøsen, K., Gram, L.F., 1992, Pharmacokinetics of the selective serotonin reuptake inhibitor paroxetine: nonlinearity and relation to the sparteine oxidation polymorphism, *Clin. Pharmacol. Ther.* **51**, 288-295.

Sindrup, S.H., Brøsen, K., Gram, L.F., Hallas, J., Skjelbo, E., Allen, A., Allen, G.D., Cooper, S.M., Mellows, G., Tasker, T.C.G., Zussman, B.D., 1992, The relationship between paroxetine and the sparteine oxidation polymorphism, *Clin. Pharmacol. Ther.* **51**, 278-287.

Sindrup, S.H., Brøsen, K., Hansen, M.G., Aaes-Jorgensen, T., Overo, K.F., Gram, L.F., 1993, Pharmacokinetics of citalopram in relation to the sparteine and the mephenytoin oxidation polymorphisms, *Ther. Drug. Monit.* **15**, 11-17.

Skjelbo, E., Brøsen, K., Hallas, J., Gram, L.F., 1991, The mephenytoin oxidation polymorphism is partially responsible for the N-demethylation of imipramine, *Clin. Pharmacol. Ther.* **49**, 18-23.

Someya, T., Suzuki, Y., Shimoda, K. et al., 1999, The effect of cytochrome P450 2D6 genotypes on haloperidol metabolism: a preliminary study in a psychiatric population, *Psychiatry and Clinical Neurosciences* **53**, 593-597.

Spigset, O., Axelsson, S., Norström, Å., Hägg, S., Dahlqvist, R., 2001, The major fluvoxamine metabolite in urine is formed by CYP2D6, *Eur. J. Clin. Pharmacol.* **57**, 653-658.

Spigset, O., Carleborg, L., Hedenmalm, K., Dahlqvist, R., 1995, Effect of cigarette smoking on fluvoxamine pharmacokinetics in humans, *Clin. Pharmacol. Ther.* **58**, 399-403.

Spigset, O., Granberg, K., Hagg, S., Norstrom, A., Dahlqvist, R., 1997, Relationship between fluvoxamine pharmacokinetics and CYP2D6/CYP2C19 phenotype polymorphisms, *Eur. J. Clin. Pharmacol.* **52**, 129-133.

Spigset, O., Hägg, S., Söderström, E., Dahlqvist, R., 1999, Lack of correlation between fluvoxamine clearance and CYP1A2 activity as measured by systemic caffeine clearance, *Eur. J. Clin. Pharmacol.* **54**, 943-946.

Spigset, O., Hedenmalm, K., Dahl, M-L., Wiholm, B-E., Dahlqvist, R., 1997, Seizures and myoclonus, associated with antidepressant treatment: assessment of potential risk factors, including CYP2D6 and CYP2C19 polymorphisms, and treatment with CYP2D6 inhibitors, *Acta. Psychiatr. Scand.* **96**, 379-384.

Spina, E., Ancione, M., Di Rosa, A.E. et al., 1992, Polymorphic debrisoquine oxidation and acute neuroleptic-induced adverse effects, *Eur. J. Clin. Pharmacol*. **42**, 347-348.

Spina, E., Avenoso, A., Facciolà, G. et al., 2000, Plasma concentrations of risperidone and 9-hydroxyrisperidone: effect of comedication with carbamazepine or valproate, *Ther. Drug Monit.* **22**, 481-485.

Spina, E., Avenoso, A., Facciolà, G. et al., 2001, Relationship between plasma risperidone and 9-hydroxyrisperidone concentrations and clinical response in patients with schizophrenia, *Psychopharmacology* **153**, 238-243.

Spina, E., Avenoso, A., Facciolà, G. et al.,1998, Effect of fluoxetine on the plasma concentrations of clozapine and its major metabolites in patients with schizophrenia, *Int. Clin. Psychopharmacol.* **13**, 141–145.

Spina, E., Avenoso, A., Facciolà, G., Scordo, M.G., Ancione, M., Madia, A., 2001, Plasma concentrations of risperidone and 9-hydroxyrisperidone during combined treatment with paroxetine, *Ther. Drug Monit.* **23**, 223-227.

Spina, E., Avenoso, A., Salemi, M. et al., 2000, Plasma concentrations of clozapine and its major metabolites during combined treatment with paroxetine or sertraline, *Pharmacopsychiatry* **33**, 213–217.

Spina, E., Avenoso, A., Scordo, M.G. et al., 2002, Inhibition of risperidone metabolism by fluoxetine in patients with schizophrenia: a clinically relevant pharmacokinetic drug interaction, *J. Clin. Psychopharmacol.* **22**, 419-423.

Spina, E., Gitto, C., Avenoso, A., Campo, G.M., Caputi, A.P., Perucca, E., 1997, Relationship between plasma desipramine levels, CYP2D6 phenotype and clinical response to desipramine: a prospective study, *Eur. J. Clin. Pharmacol.* **51**, 395-398.

Spina, E., Scordo, M.G., Avenoso, A., Perucca, E., 2001, Adverse drug interaction between risperidone and carbamazepine in a patient with chronic schizophrenia and deficient CYP2D6 activity, *J. Clin. Psychopharmacol*. **21**, 108-109.

Spina, E., Steiner, E., Ericsson, O., Sjöqvist, F., 1987, Hydroxylation of desmethylimipramine: dependence on the debrisoquin hydroxylation phenotype, *Clin Pharmacol Ther* **41**, 314-319.

Spina, E., Sturiale, V., Valvo, S. et al., 1992, Debrisoquine oxidation phenotype and neuroleptic-induced dystonic reactions, *Acta Psychiatr. Scand*. **86**, 364-366.

Störmer, E., Von Moltke, L.L., Shader, R.I., Greenblatt, D.J., 2000, Metabolism of the antidepressant mirtazapine in vitro: contribution of cytochromes P-450 1A2, 2D6, and 3A4, *Drug. Metab. Dispos.* **28**, 1168-1175.

Suzuki, A., Otani, K., Mihara, K. et al., 1997, Effects of the CYP2D6 genotype on the steady-state plasma concentrations of haloperidol and reduced haloperidol in Japanese schizophrenic patients, *Pharmacogenetics* **7**, 415-418.

Tamminga, W.J., Wemer, J., Oosterhuis, B., De Boer, Vranckx, A.S., Drenth, B.F.H., De Zeeuw, De Leij, R.A.L.F.M.H.,. Jonkman, J.H.G., 2003, Polymorphic drug metabolism (CYP2D6) and utilisation of psychotropic drugs in hospitalised psychiatric patients: a retrospective study, *Eur. J. Clin. Pharmacol.* **59**, 57-64.

Task Force Laboratory Tests in Psychiatry, 1985, Tricyclic antidepressants –blood level measurements and clinical outcome: an APA Task Force report. Task Force on the Use of Laboratory Tests in Psychiatry, *Am. J. Psychiatry.* **142**, 155-162.

Taylor, D., Bodani, M., Hubbeling, A., Murray, R., 1999, The effect of nefazodone on clozapine plasma concentrations, *Int. Clin. Psychopharmacol.* **14**, 185-187.

Tucker, G.T., Silas, J.H., Iyun, A.O. et al., 1977, Polymorphic hydroxylation of debrisoquine, *Lancet* **2**, 718.

Van Beijsterveldt, L.E.C., Geerts, R.J.F., Leysen, J.E. et al., 1994, The regional brain distribution of risperidone and its active metabolite 9-hydroxyrisperidone in the rat, *Psychopharmacology* **114**, 53-62.

Van Der Weide, J., Steijns, L.S., Van Weelden, M.J., 2003, The effect of smoking and cytochrome P450 CYP1A2 genetic polymorphism on clozapine clearance and dose requirement, *Pharmacogenetics* **13**, 169-172.

Veefkind, A.H., Haffmans, P.M.J., Hoencamp, E., 2000, Venlafaxine serum levels and CYP2D6 genotype, *Ther. Drug. Monit.* **22**, 202-208.

Von Bahr, C., Movin, G., Nordin, C. et al., 1991, Plasma levels of thioridazine and metabolites are influenced by the debrisoquine hydroxylation phenotype, *Clin. Pharmacol. Ther.* **49**, 234-240.

Von Moltke, L.L., Greenblatt, D.J., Giancarlo, G.M., Granda, B.W., Harmatz, J.S., Shader, R.I., 2001, Escitalopram (S-citalopram) and its metabolites in vitro: Cytochromes mediating biotransformation, inhibitory effects, and comparison to R-citalopram, *Drug Metab. Dispos*. **29,** 1102-1109.

Wang, J-H., Liu, Z-Q., Wang, W., Chen, X-P., Shu, Y., He, N., Zhou, H-H., 2001, Pharmacokinetics of sertraline in relation to genetic polymorphism of CYP2C19, *Clin. Pharmacol. Ther.* **70**, 42-47.

Wetzel, H., Anghelescu, I., Szegedi, A. et al., 1998, Pharmacokinetic interaction of clozapine with selective serotonin re-uptake inhibitors: differential effects of fluvoxamine and paroxetine in a prospective study, *J. Clin. Psychopharmacol.* **18**, 2-9.

Wienkers, L.C., Allievi, C., Hauer, M.J., Wynalda, M.A., 1999, Cytochrome P-450-mediated metabolism of the individual enantiomers of the antidepressant agent reboxetine in human liver microsomes, *Drug. Metab. Dispos.* **27**, 1334-1340.

Xu, Z-H., Wang, W., Zhao, X-J., Huang, S-L., Zhu, B., He, N., Shu, Y., Liu, Z-Q., Zhou, H-H., 1999, Evidence for involvement of polymorphic CYP2C19 and 2C9 in the N-demethylation of sertraline in human liver microsomes, *J. Clin. Pharmacol.* **48**, 416-423.

Yasar, U., Forslund-Bergengren, C., Tybring, G. et al., 2002, Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype, *Clin. Pharmacol. Ther.* **71**, 89-98.

Yasui, N., Kondo, T., Otani K. et al., 1999, Effects of itraconazole on the steady-state plasma concentrations of haloperidol and its reduced metabolite in schizophrenic patients: *in vivo* evidence of the involvement of CYP3A4 for haloperidol metabolism, *J. Clin. Psychopharmacol.* **19**, 149-154.

Yasui, N., Otani, K., Kaneko, S., Ohkubo, T., Osanai, T., Ishida, M., Mihara, K., Kondo, T., Sugawara, K., Fukushima, Y., 1995, Inhibition of trazodone metabolism by thioridazine in humans, *Ther. Drug. Monit.* **17**, 333-335.

Yasui-Furukori, N., Hidestrand, M., Spina E. et al., 2001, Different enantioselective 9-hydroxylation of risperidone by the two human CYP2D6 and CYP3A4 enzymes, *Drug Metab. Dispos.* **29**, 1263-1268.

Yoon, Y. R., Cha, I.J., Shon, J.H., Kim, K.A., Cha, Y.N., Jang, I.J., Park, C.W., Shin, S.G., Flockhart, D.A., Shin, J.G., 2000, Relationship of paroxetine disposition to metoprolol metabolic ratio and CYP2D6*10 genotype of Korean Subjects, *Clin. Pharmacol. Ther.* **67**(5), 567-576.

Yoshii, K., Kobayashi K., Tsumuji, M. et al., 2000, Identification of human cytochrome P450 isoforms involved in the 7-hydroxylation of chlorpromazine by human liver microsomes, *Life Sciences* **67**, 175-184.

Yue, Q.Y., Zhong, Z.H., Tybring, G., Dalen, P., Dahl, M.-L., Bertilsson, L., Sjöqvist, F., 1998, Pharmacokinetics of nortriptyline and its 10-hydroxy metabolite in Chinese subjects of different CYP2D6 genotypes, *Clin. Pharmacol. Ther.* **64**, 384-390.

17. SEXUAL DYSFUNCTION

Neurobiological, pharmacological and genetic consideration

Brian Mustanski and John Bancroft*

1. INTRODUCTION

Sexual side-effects are common with various types of psychotropic medications, especially antidepressants and anti-psychotic agents. Such side-effects not only affect quality of life, they are also relevant to drug compliance, of particular importance in the long-term treatment of psychotic illnesses like schizophrenia. There is however, considerable individual variability in the pattern and severity of such side-effects, raising the question of whether genetic factors may be relevant. Pharmacogenetic research on sexual dysfunctions could be productive in answering several important questions: How much positive predictive power could genetic information provide in forecasting sexual side-effects? What particular neurotransmitter receptors or transporters are related to such sexual side-effects? How can this line of research be used to help answer basic questions about the neurobiology of sexual dysfunction? Unfortunately, none of these questions can be answered as of yet. Most pharmacogenetic investigations have focused on predicting primary outcomes; recent studies have started to focus on side-effects, but so far sexual side-effects have either not been considered or not distinguished from other types of side-effects. This chapter will focus on possible candidates for pharmacogenetic investigations of sexual side-effects, especially in relation to antidepressant and anti-psychotic drugs.

* Brian Mustanski, Department of Psychology, Indiana University, Bloomington, Indiana, 47405. John Bancroft, Kinsey Institute for the Study of Sex, Gender, and Reproduction, Indiana University, Bloomington, Indiana, 47405.

2. SEXUAL NEUROBIOLOGY

We will briefly summarize current understanding of the neurobiology of sex as a first step to understanding different types of drug side-effects. The overall complexity of the neurobiology of sexual response can be simplified by using a 'dual control' model, which postulates parallel activities of a sexual excitatory and a sexual inhibitory system in the brain, which are relatively independent of each other (Bancroft, 1999). This model uses a 'conceptual nervous system' approach based on function rather than structure or anatomic localization of function (Gray, 1987). The excitatory system depends to a considerable extent on dopamine (DA) and the central effects of norepinephrine (NE), and on the activating presence of testosterone (T). The inhibitory system depends to a considerable extent on serotonin (5-HT), and the peripheral effects of NE. Neuropeptides are also clearly involved, with oxytocin playing a role in the excitatory system, and beta-endorphin in the inhibitory system. The available evidence is predominantly from male animal studies. However, inhibition of sexual response and behavior in animals has been much less studied than excitation.

Unfortunately, when we grapple with the complexities of drug effects, the use of simplified models only takes us so far. A particular challenge is the relative non-specificity of effect of the principal neurotransmitters; DA, for example, may have specific effects on sexual responsiveness in certain parts of the brain, whereas in other parts of the brain its effects are either unrelated to sex, or related to a variety of 'motivated' behaviors including sex. To add to the problem, most psychotropic drugs with sexual side-effects act on more than one neurotransmitter system.

It is also necessary to allow for considerable interaction between the three monaminergic systems, as well as with neuropeptide and steroidal mechanisms. This complexity is well illustrated in a recently studied animal model of sexual inhibition, the 'sexual satiation' model. This is an experimentally induced prolonged form of post-ejaculatory refractoriness in the male rat, which has been shown to involve 5HT, NE, opioids and DA (Rodrigues-Manzo & Fernando-Guasti, 1994, 1995; Mas et al., 1995). Also to be considered is the apparent capacity for monoamines to affect both pre-synaptic and post-synaptic receptors, depending on concentration, resulting in curvilinear dose-response relations (lower concentrations having predominantly pre-synaptic effects, and higher doses, post-synaptic).

The focus of this chapter will be on the three principal monoamine neurotransmitters, DA, NE and 5-HT as being most directly involved in sexual side-effects. However, we will also consider nitrergic transmission, as recent evidence points to its relevance.

The DA system is highly organized topographically into five sub-systems (Role & Kelly, 1991); 1. meso-striatal (projecting from the substantia nigra and ventral tegmentum to the striatum); 2. meso-limbic; 3. meso-cortical (from the ventral tegmentum to limbic and cortical areas), and two smaller, more localized sub-systems, 4. incerto-hypothalamic (or A14) periventricular, which projects to the medial pre-optic area (MPOA) and 5. tubero-infundibular (from the arcuate

nucleus of the hypothalamus to the pituitary stalk). Two of these sub-systems influence sexuality in a non-specific way, and one has specific sexual effects (Hull et al., 1998) Thus, sub-system 1 (meso-striatal) is involved in the organization of motor behavior, and 'readiness to respond', which includes copulatory as well as other non-sexual integrated motor patterns; sub-system 2 (meso-limbic) promotes 'appetite' for a variety of appetitive behaviors, including sexual; sub-system 4 (A14 periventricular) is the dopaminergic input to the MPOA which has a specifically sexual function in orchestrating genital responses and stereotyped sexual motor patterns such as mounting or thrusting. DA can be seen to have an excitatory role, though its effects on the MPOA may involve disinhibition of inhibitory tone. The relevance of the tubero-infundibular sub-system to sexuality is unclear, beyond the fact that it is involved in the pituitary control, by the gonadotrophic hormones, of production of gonadal steroids, and the pituitary secretion of the neuropeptides, oxytocin, vasopressin and prolactin.

The NE system, which in terms of numbers of neurons, is the smallest of the three cerebral monoamine systems, originates in two brain stem nuclei, the locus coeruleus (l.c.) and lateral tegmental nucleus (l.t.n.) from which there are ascending and descending projections. It is probably only the l.c. system that is relevant sexually, but again its effects are not specific to sexual response or arousal. The l.c. is activated by novel sensory input, with a role in attending to sudden contrasting or aversive situations. Its ascending projections, to fairly diverse areas, including dorsal thalamus, hypothalamus, hippocampus and frontal cortex, have both excitatory and inhibitory effects. Its descending projections, via the spinal cord, affect genital response in an inhibitory way, as well as having other non-sexual peripheral inhibitory effects on autonomic responses. NE thus presents us with the most complex and least well understood aspect of sexual response. The NE system centrally is probably fundamental to central arousal, including negative arousal states such as fear, as well as sexual arousal. This is also manifested by increased levels of NE and epinephrine (E) in circulation. The presence of testosterone (T) receptors on the l.c. suggests that T may be playing a specific role in central arousal relevant to sexual response. On the other hand, there are inhibitory sexual effects of NE in the periphery, most obvious in the male, with NE playing a crucial role in tonic inhibition of the erectile tissues (Rehman & Melman, 2001). This therefore presents us with a 'central excitatory' and 'peripheral inhibitory' role for NE. This is only partially understandable in terms of receptor types. It is the alpha receptors that are most relevant; alpha-1 activity is predominantly post-synaptic, and in the periphery is mainly manifested as inhibition of response. Alpha-2 activity is predominantly via pre-synaptic auto-receptors, and hence reduces NE transmission. However, there are post-synaptic alpha-2 receptors, the effects of which are not well understood. To add to the complexity of the NE system, a curvilinear dose-response relationship has been observed, where medium range doses may have sexually enhancing effects and high doses inhibitory effects, (e.g. Sala et al., 1990; and see Bancroft, 1995 for relevant human data).

The 5-HT system is the largest of the three in terms of numbers of neurons, the majority of which originate within the raphe nuclei of the brainstem. The

more caudal of these nuclei provide the descending 5-HT projections to the spinal cord. The upper raphe nuclei are widely projected, via the median forebrain bundle, mainly to the frontal cortex, striatum, septum and hippocampus. In general, there is evidence that drugs which increase 5-HT transmission inhibit, and those which disrupt 5-HT transmission enhance male sexual behavior (e.g. Bitran & Hull, 1987). However, these effects are not specific to sex, and can affect a variety of other behaviors (e.g. eating, aggression, general motor activity). To a considerable extent, the 5-HT inhibitory system is diffuse rather than discretely localized. However, the inhibitory effects on sexual behavior probably involve the lateral hypothalamic area (Lorrain et al., 1997). Also, the nucleus paragigantocellularis in the brain stem is involved in inhibition of erectile response in the male, an effect probably dependent on 5-HT transmission (McKenna, 2000). In general, most evidence relates to the 5-HT2 family, which mediates the inhibitory effects, and the 5-HT1A receptor, which is a pre-synaptic auto-receptor and therefore reduces 5-HT transmission when activated (Larsson & Ahlenius, 1999).

In the past 20 years or so, the importance of nitric oxide (NO) as a key factor in control of vascular response has become apparent. NO synthase (NOS) produces NO from l-arginine, which activates guanylate cyclase to produce cGMP, which in turn is responsible for smooth muscle relaxation. The action of cGMP is limited by a variety of phosphodiesterase enzymes (I to V). Around the time that it was becoming clear that penile erection depended on relaxation of the muscle in erectile tissues, it was serendipitously discovered that a phosphodiesterase V inhibitor, sildenafil, facilitated penile erection, leading to the revolutionary use of the drug in the treatment of erectile dysfunction. Initially it was believed that this nitrergic (NO induced) effect was restricted to the endothelium, and also that sildenafil did not cross the blood-brain barrier, hence restricting its action to peripheral effects. In recent years, evidence has emerged requiring a revision of this view. First, it is now clear that NO is released by neuronal as well as endothelial cells. Secondly, there is increasing evidence of NO mediated effects in the brain, some of which are relevant to sexual behavior. Thirdly, it is becoming increasingly apparent that sildenafil crosses the blood-brain barrier. And finally, recent studies have shown sildenafil to be an effective treament for premature ejaculation (Abdel-Hamid, 2004). This leaves us with the questions of how sildenafil improves rapid ejaculation, and whether such effects are centrally or peripherally mediated.

Abdel-Hamid (2004), in his review of this literature, focused on the peripheral smooth muscle relaxing effects of nitrergic transmission in the prostate, vas deferens and seminal vesicles. The sildenafil effect on ejaculation, at first sight paradoxical, becomes less so when we consider that non-striated muscle relaxation is involved in erectile response, and smooth muscle contraction in seminal emission. We still have inadequate understanding of the physiology of ejaculation, although seminal emission is only part of the process, and orgasm, a central phenomenon even less well understood, is clearly involved. Our limited understanding of orgasm partly stems from the fact that it is difficult to study in animals, and the focus has been on the behavioral

sequence of mounting, intromission, thrusting and ejaculation. We will return to this issue when we consider sexual side-effects.

3. SEXUAL PHARMACOLOGY

A variety of pharmacological agents used to treat psychiatric problems may have sexual side-effects, including benzodiazepines, lithium and related compounds. However, this chapter will focus on two classes of drugs, antidepressants and psychotropic or neurotropic drugs used to treat psychotic illnesses such as schizophrenia. Sexual side-effects are commonly reported for both classes of drug, and are important not only in their own right, but in their strong tendency to lead to discontinuation of treatment. There are, however, two major problems in understanding and explaining such side-effects. First, as already mentioned, most drugs, in both classes, are relatively non-specific in their pharmacological actions, making it difficult to interpret the side-effects in terms of neurotransmitter mediation. Second, with both affective illnesses and schizophrenia, there are sexual symptoms that result from the illness itself, making interpretation of treatment effects more complex.

3.1. Antidepressants

Loss of sexual interest is a common symptom of depressive illness; Beck (1967) found this complaint in 61% of men and women with major depressive disorder. Araujo et al. (1998) reported an association between erectile problems and depression in older men. Nocturnal penile tumescence, which can be regarded as an indicator of central 'excitatory tone', is reduced in depressive states (Thase et al., 1987). Sexual arousability is probably reduced in most depressed women also; mood appears to be a major factor in determining sexual well being in women (Bancroft, Loftus, & Long, 2003). The effects of mood on orgasmic function is less clear. In so far as negative mood states may impair sexual arousal, then orgasm is likely to be delayed. There is no clear evidence, however, of a specific effect of negative mood in inhibiting orgasm. Premature ejaculation is not obviously affected by depression, one way or the other. Anxiety, on the other hand, may aggravate premature ejaculation.

Although the effects of negative mood states on sexual interest and responsiveness are most typically negative, it has become clear that this is not always the case. Mathew & Weinman (1987) and Angst (1998) found that a proportion of men, and a somewhat smaller proportion of women, experience an increase in sexual interest and responsiveness when depressed or anxious. This paradoxical relationship between mood and sexuality has been the subject of recent research at the Kinsey Institute (Bancroft et al., 2003 a & b), and further complicates the situation. Whereas a drug-induced improvement in mood may result in an improvement in sexual interest in many depressed patients, there will be some in whom a depression or anxiety related increase in sexual interest will be reduced by effective treatment.

There is now quite an extensive literature on sexual side-effects of anti-depressants (e.g. Rosen, Lane, & Menza, 1999). In a recent comprehensive review of this literature, Montgomery, Baldwin and Riley (2002) concluded that the majority of the reported evidence was inconclusive because of methodological shortcomings, and in many cases an inadequate distinction between different types of adverse sexual effects. Sexual side-effects are commonly reported with most types of tricyclic antidepressants and 5-HT re-uptake inhibitors (SRI's). They are less often reported with pharmacologically atypical antidepressants such as bupropion, meclobemide, reboxetine, mirtazapine, and nefazadone, but as Montgomery et al. (2002) point out, such drugs have been much less widely used, and sexual side-effects were not so well recognized with more conventional anti-depressants until they had been in use for many years. Bupropion, which pharmacologically is metabolized into a NE and DA re-uptake inhibitor, has been associated with less sexual side-effects in direct comparison with sertraline (Croft et al., 1999) and various SSRI's (Modell et al., 1997). This is consistent with bupropion having a dopaminergic and central norepinephric effect. Nefadazone, which is related to trazadone, without the histaminergic sedative effect, but with a clear 5HT2 antagonist effect, has been shown to have less sexual side-effects than SRI's (Gregorian et al., 2002).

Against this complex and somewhat confused picture, one side-effect stands out; delayed ejaculation or orgasm is the most commonly reported sexual side-effect in men and in women. Across studies, 30-60% of patients on SRI's report orgasmic or ejaculatory difficulty. In earlier studies, particularly those involving tricyclic antidepressants, this may not have been adequately investigated. In a number of studies this problem only came out with direct questioning, and was not otherwise mentioned (e.g. Monteiro et al., 1987). This effect has been more evident with SRI's than with tricyclic antidepressants, and is sufficiently predictable that it is now being exploited as a pharmacological treatment of premature ejaculation. In all of the randomized controlled comparisons of atypical antidepressants, like bupropion and nefazadone, with SRI's, the side-effect which most consistently shows a significant difference is orgasmic dysfunction in women and delayed ejaculation in men, being more frequent with SRI's. Although a number of studies have directly compared one SRI with another, they have generally found no significant difference.

Although this relatively predictable effect of SRI's on orgasm and ejaculation is consistent with a serotonergic inhibitory effect, what is puzzling is why inhibition of orgasm/ejaculation is so much more predictable than inhibition of erection or other components of genital response. This reflects our relative lack of understanding of inhibitory systems as they affect sexuality, as well as uncertainty about specific receptor effects of different drugs. The recently demonstrated role of the n. paragigantocellularis in inhibiting erectile response, probably dependent on serotonergic mediation, leaves one wondering where 5-HT is acting to more specifically inhibit orgasm/ejaculation. Given the rise in 5-HT in the lateral hypothalamus following ejaculation in rats (Lorrain et al., 1997), this may be one brain area where serotonergic inhibition of ejaculation takes place. Other brain areas, such as the postero-medial bed nucleus of the stria terminalis, and the medial parvicellular subparafascicular

nucleus of the thalamus, have been shown to be active around the time of ejaculation (Veening & Coolen, 1998).

Various attempts to reduce assumed sexual side-effects of antidepressants have been made by adding a further compound to block the putative pharmacological mechanisms causing the sexual side-effect. In women, buspirone (5HT1A agonist), and amantadine (thought to increase DA availability) have been compared to placebo, with no significant benefits resulting (Michelson et al., 2000); mirtazapine (5HT2 and alpha-2 antagonist), yohimbine (alpha-2 antagonist), and olanzapine, (mixed dopaminergic and serotonergic action), were compared to placebo, again without any significant effect (Michelson et al., 2002); and ephedrine was compared to placebo without significant effect (Meston, 2004**)**. In a study of both men and women, the addition of bupropion was no better than placebo in improving SRI-related sexual side-effects (Masand et al., 2001).

The one apparently successful reduction of antidepressant induced sexual side-effects was obtained with sildenafil in men (Nurnberg et al., 2003). Improvement in erectile function with sildenafil, when attributable to antidepressant use, is to be expected; because of its assumed peripheral effect, sildenafil enhances the excitatory arm of the erectile system, whatever factor may have reduced excitation previously. But it also improves delayed ejaculation, and as discussed above, has been used effectively in the treatment of premature ejaculation. Although Abdel-Hamid (2004) emphasizes the effect of sildenafil on the smooth muscle contraction in seminal emission, we also have to consider the possibility of central nitrergic effects, plus the recent finding that SRI's have been demonstrated to reduce the production of NO by NOS (Finkel et al., 1996).

At this stage, the roles of DA and NE in mediating the sexual side-effects of antidepressants are sufficiently unclear that we should not expect pharmacogenetic evidence to have much explanatory value, or predictive value when selecting the best antidepressant for a particular patient. The serotonergic inhibition of orgasm/ ejaculation, on the other hand, and the role of nitrergic mechanisms in both erection and ejaculation, may well be illuminated by pharmacogenetic studies, increasing our understanding of the basic physiology, as well as providing clinical guidance for drug selection.

3.2. Antipsychotics

Sexual side-effects of anti-psychotics in the treatment of schizophrenia are of particular interest because of the central role that DA is believed to play in that illness and its pharmacological treatment. However, the problem of assessing such sexual effects is even more difficult than is the case with antidepressants, because of the greater difficulty in assessing the effects of the schizophrenic illness on sexuality before treatment. Although this illness can occur in people with previously normal personalities, 'pre-schizophrenic personality' is common, and likely to be associated with impaired, or in some way abnormal sexual development.

The common factor in most anti-psychotics is DA antagonism, particularly at the D2 receptor, and it is DA antagonism in the meso-limbic system which is believed to be most relevant to reduction of psychotic symptoms. However, the meso-limbic system is involved in a range of 'motivated' behaviors, including sexual behavior. Given that the florid Type 1 symptoms of schizophrenia (e.g. hallucinations, delusions and aggressive behavior) are believed to result from either increased DA activity or increased DA receptors in the meso-limbic system, one might expect that an increase in sexually motivated behavior is a feature of the acute illness, at least before 'negative' Type 2 symptoms become established. This, however, is difficult to assess. Sexual thoughts and behaviors are common in schizophrenia, and there may be a relative increase in sexual activity. However, this is typically autoerotic or 'compulsive' without any real 'object-relational' quality (Lilleleht & Leiblum, 1993). Given the distortion of the ability to relate to others that occurs in this illness, this is not surprising. It is nevertheless difficult to assess whether there is a decline or an increase in the level of sexual interest. Early studies suggested that loss of sexual interest was less likely in schizophrenia than in other types of psychiatric illness, although female schizophrenics were more likely to report a decline in sexual interest than males (Gittleson & Levine, 1966; Gittleson & Dawson-Butterworth, 1967).

The prevalence of sexual dysfunction in patients treated with anti-psychotic medication, is reported to be around 60% in men and from 30-93% in women (Smith et al., 2002), and these problems are generally assumed to be side-effects of the medication. Smith et al. (2002), recognizing the limited extent to which schizophrenic patients, treated or not, were able to engage in sexual relationships, developed a method of assessing sexual function that did not depend on such relationships. They found that the level of sexual interest did not differ from normal controls, whereas erectile dysfunction and ejaculatory dysfunction in men, and orgasmic dysfunction in women, were substantially more prevalent than in their controls. In the men, ejaculatory problems were much more common than erectile problems. They interpreted the normal levels of sexual interest as possibly resulting from the normalizing effects of the treatment, although they did not speculate on whether this was due to an increase or a reduction of sexual interest to normal levels. They found some relationship between prolactin levels and problems with sexual arousal (erection in men, and vaginal response in women), and sexual interest in women. From this they concluded that the principal sexual side-effects of the medication resulted from the drug-induced hyperprolactinaemia. A more recent study (Knegtering et al., 2004) compared the effects of two atypical antipsychotics, one, risperidone which produces hyperprolactinaemia, the other, quietapine, which does not raise prolactin. They found fewer sexual side-effects with quietapine. It is widely assumed in the literature (e.g. Kruger et al., 2002; Cutler, 2003; Halbreich et al., 2003) that prolactin has a negative effect on sexuality. However, we would argue that, except for the extremely high levels of prolactin that result from prolactin secreting tumors, the plasma level of prolactin is better seen as a marker of the dopaminergic-serotonergic balance in the tubero-infundibular system, and quite possibly in other dopaminergic systems as well. DA is the most important regulator of prolactin; as dopaminergic activity in the

tubero-infundibular system goes down, prolactin goes up. 5-HT, in contrast, has a stimulatory effect on prolactin release. Whereas prolactin within the brain may have a feedback role in the control of DA release, it is much less likely that the plasma prolactin secreted into the circulation by the anterior pituitary, which has little chance of crossing the blood-brain barrier, will have such feedback effects within the brain. Hence the degree of hyperprolactinaemia associated with anti-psychotic medication can be seen as a measure of the degree of suppression of dopaminergic activity, and of a shift in the DA/5HT balance towards a more serotonergic state.

Anti-psychotic drugs, while having in common a DA antagonist effect, tend to have 5-HT effects also. This is particularly the case with clozapin, one of the first 'atypical' anti-psychotics; here the relative absence of extrapyramidal side-effects, which are important in the management of schizophrenia, is believed to result from the serotonergic effects of the drug within the meso-striatal system. This confronts us with another source of complexity; the pharmacological profile of a particular drug does not necessarily enable us to predict its effects in all the dopaminergic systems in the brain.

Given the likely role of increased DA activity in the meso-limbic system in schizophrenia, and the relevance of this same DA system to sexual motivation, it would not be surprising if the effect of antipsychotic medication was to 'normalize' rather than suppress sexual desire. The role of DA in the incerto-hypothalamic periventricular system, which projects to the MPOA, and the lack of evidence of any alteration of DA in this system in schizophrenia, makes the drug-induced sexual side-effects of erectile problems in men and arousal problems in women, easier to understand. Drugs such as these cannot be targeted on any one of the specific DA systems. It remains more difficult to explain the most prevalent side-effect, orgasmic or ejaculatory suppression, as a result of DA antagonism, unless this is a result of a shift in the DA/5HT balance to a more serotonergic state.

While the overall complexity of sexuality among schizophrenics makes understanding and management of drug side-effects difficult, the probable importance of DA, and the relative frequency of sexual side-effects, warrants exploration of possible genetic markers. Once again this might not only benefit clinical management and selection of drug type, it may also illuminate our understanding of the sexual role of DA.

4. SEXUAL PHARMACOGENETICS

Relevant pharmacogenetic research can be usefully divided into two domains: pharmacodynamic and pharmacokinetic. Pharmacodynamic investigations focus on the mechanism of drug action, such as the receptors activated and the effects on neurotransmitter reuptake, while pharmacokinetic investigations focus on the process of drug metabolism and what effects individual differences in metabolism may have on the occurrence of side-effects. Given the current state of knowledge, the most likely pharmacodynamic candidates include the genes encoding the 5-HT and DA receptors and transporters and the genes encoding the various isoforms of the nitric oxide

synthase (NOS) enzyme. At this stage, the most promising pharmacokinetic candidates are the genes encoding the Cytochrome P450 (CYP) isoenzymes, which are involved in drug metabolism. In all of these cases we should not assume that the genes relevant to treatment response are the same genes involved in sexual side-effects.

4.1. Pharmacodynamics

4.1.1. Serotonin

As discussed earlier, several 5-HT receptors have been implicated as being related to ejaculation, the sexual response most clearly affected by SSRI medications. The best evidence seems to point to activation of pre-synaptic 5-HT1A receptors lowering and activation of the 5-HT2 family of receptors increasing the ejaculatory latency in male rats (see Larsson & Ahlenius, 1999 for a review) so these will be the only 5-HT receptors discussed in detail.

The 5-HT1A receptor has been hypothesized to be an important moderator of clinical response to SSRI drugs, and some evidence supports this assertion (e.g. Blier, Bergeron, & de Montigny, 1997). A number of polymorphisms have been identified within the 5-HT1A gene, including several that result in amino acid variation within the gene product; unfortunately, they exist in such low frequencies that they are unlikely to have much power to explain variability in SSRI induced sexual dysfunction. A relatively common polymorphism has been identified in the promoter region of this gene (Wu & Comings, 1999), and if it is found to have functional effects on gene expression it may make a suitable candidate for pharmacogenetic investigation.

The 5-HT2A receptor is a strong candidate for pharmacogenetic investigation given that selective 5-HT2A receptor agonists induce a dose-dependent decrease in ejaculation frequency and increase in ejaculation latency (Gorzalka, Mendelson, & Watson, 1990) and because there is some evidence, although it is inconsistent, that they moderate the clinical response to clozapine (Arranz et al., 1998). Murphy and colleagues (2003) published the only study focusing on using the 5-HT2A genotype to predict SSRI induced side-effects that included sexual dysfunction. Paroxetine was administered to 124 elderly patients with major depression, who were also genotyped for the 102T-C single nucleotide polymorphism (SNP). In this study, there were significantly more discontinuations due to side-effects for patients with the C/C genotype (46.3%) than for those with the T/C and T/T genotypes (16%). Unfortunately, in this study, sexual side-effects were grouped along with dizziness, headaches, and sweating. It is also important to note that the SNP used in this study does not result in an amino acid substitution, thus its effect must be due to it being in linkage disequilibrium with a nearby variant that does alter receptor functioning; the authors note that the SNP was in complete linkage disequilibrium with the -1438 promoter polymorphism, suggesting that it lies in close proximity and may be responsible for the observed effect. Given these results, it seems this gene is a good candidate for further pharmacogenetic research focusing more specifically on sexual dysfunction.

Evidence from animals administered a 5-HT2C agonist suggest that stimulation of this receptor results in inhibition of ejaculation (Larsson & Ahlenius, 1999). This fact, coupled with existence of a functional polymorphism that has been linked to clozapine clinical response (Veenstra-VanderWeele, Anderson, & Cook, 2000), make this receptor a good candidate for pharmacogenetic investigation of SRI- induced sexual dysfunction. Unfortunately, to date, no such study has been undertaken.

Several pharmacogenetic studies have focused on the role of the 5-HT transporter (5-HTT) gene in predicting treatment response to SRI medications. A number of functional polymorphisms have been identified in the coding and promoter regions of the 5-HTT gene (Veenstra-VanderWeele et al., 2000). In regards to neurobiology, psychopathology, and behavior, the most widely studied polymorphism in the 5-HTT gene is a variable number of tandem repeat (VNTR) sequences located in the promoter region (Heils et al., 1996). Two common alleles exist for this polymorphism, a 14 copy allele referred to as "short" or (s) and a 16 copy allele referred to as "long" or (l). These alleles have been found to significantly affect gene expression, with the (l) allele demonstrating approximately twice the gene expression, and consequently greater 5-HT reuptake, relative to the (s) allele (Lesch et al., 1996). Several pharmacogenetic studies have documented a relationship between the (l) allele and a favorable response to SRI treatment of depression in Caucasian populations, although the relationship is more ambiguous in other populations (see Mancama & Kerwin, 2003 for a review). There have been no studies, to date, specifically exploring the relationship between this 5-HTT polymorphism and primary or SRI-induced sexual dysfunction. However, given the pharmacological evidence linking increased 5-HT activity to the inhibition of male sexual behavior, it seems plausible that the (s) allele may have a main effect on inhibitory sexual dysfunctions such as hypoactive sexual desire disorder or orgasmic disorder. The possibility of a main effect of the (s) allele on sexual dysfunction highlights the importance of documenting the main effects of genotype on sexual response before considering 'genotype by drug' interactions. Without identifying such main effects, it is possible that a study that identifies a 'gene by drug' interaction is in reality detecting a main effect of genotype, especially if pre-treatment sexual dysfunction is not controlled for. This possibility attests to the need for more basic research on the role genes play in individual differences in sexuality.

4.1.2. Dopamine

DA has long been viewed as a key neurotransmitter for reward, thus it has been a major focus of behavior genetic studies of appetitive and addictive behaviors. For example, controversial evidence links the gene encoding the presynaptic D2 receptor with alcohol dependence (see Dick & Faroud, 2003 for discussion). The DRD4 and DAT genes have been associated with ADHD (Biederman & Faraone, 2002). Most relevant to this discussion, the DRD2 gene has been associated with age at first sexual intercourse in a sample of 414 European-Americans (Miller et al., 1999). Unfortunately, pharmacogenetic

research on antipsychotic medications that act on the DA system provide little information relevant to sexual dysfunctions because the only side-effects that have been explored in these studies are weight gain and tardive dyskinesia. Given the well documented link between DA and sexuality, and the accumulating evidence of dopaminergic genes, pharmacogenetic researchers attempting to predict the occurrence of sexual dysfunction would be well advised to consider the inclusion of genes encoding or regulating the DA receptors and transporter.

4.1.3. Nitric Oxide Synthase

Because of the central role of nitric oxide (NO) in the erectile process and the role of SRIs in reducing the production of NO by NOS (Finkel et al., 1996), genes encoding the three isoforms of NOS make viable candidates for pharmacogenetic investigations of SRI-induced sexual dysfunction. Several studies in rat models have documented that increasing NOS gene expression, using gene therapy techniques, results in a potentiation of the erectile response (Christ, 2002). Another study demonstrated that paroxetine inhibits erectile responses in rats, with this effect ascribed to reduced NO production and NOS expression (Angulo et al., 2001). Despite this compelling animal research, pharmacogenetic investigations into SRI-induced sexual dysfunction, including NOS genotype, have yet to be conducted.

4.2. Pharmacokinetics

One way in which genetic information may help to explain pharmacological side-effects is by accounting for individual differences in the duration of drug effect. Genetic polymorphisms in the genes encoding drug-metabolizing enzymes could account for such differences. Interestingly, SRIs have been found to have a complex relationship with the drug metabolizing CYP isoenzymes, acting as both substrates and inhibitors of these molecules (Harvey & Preskorn, 1996). This complexity increases because some antidepressants can inhibit specific CYP isoenzymes to such an extent that the phenotypic difference between being a genetically-based poor metabolizer (PM) and an antidepressant-induced PM (the phenocopy) becomes insignificant (Nemeroff, DeVane, & Pollock, 1996).

Of the genes encoding various CYP isoenzymes, the CYP2D6 gene has been most actively studied, with at least 15 allelic variants identified that produce nonfunctional gene products (Mancama & Kerwin, 2003). Two studies have specifically attempted to explore what role genetic variation in CYP2D6 may play in sexual dysfunction resulting from SRIs. The first study, by Zourkova and Hadasova (2002), included 30 outpatients diagnosed with depressive disorder taking paroxetine (a known CYP2D6 inhibitor) for an average of 131 days. Participants were genotyped for CYP2D6 and their metabolic phenotype was assessed using dextromethorphan (DEM) as a testing substance. Sexual dysfunction was measured using the ASEX scale (McGahuey et al., 2000). Levels of sexual dysfunction in PM's were significantly higher.

However these results must be interpreted with caution, vis-à-vis genetic effects, because none of the PM participants had a PM genotype–all had phenotypically converted to PM as a result of paroxetine administration. Despite this limitation, the fact that the PM group was significantly more likely to experience erection/lubrication difficulties suggests this line of research is promising.

In the second study, 124 elderly depressed patients were given paroxetine in the context of a double-blind, randomized study comparing paroxetine to a non-SRI antidepressant (Murphy et al., 2003). There were 42 patients (17.4%) with genotypes encoding poor and intermediate metabolism (PM), but they did not significantly differ in their rate of side-effects, including sexual side-effects. Unfortunately, sexual side-effects were not distinguishable from other side-effects in the reported results. Furthermore, the fact that an elderly sample was used may limit generalizability to other age groups, especially in the case of sexual dysfunction. Given the limitations of both of these CYP2D6 studies, further pharmacogenetics research is necessary to resolve the role of CYP2D6 in predicting sexual dysfunction secondary to SRI administration.

5. CONCLUSION

In the emerging and promising area of pharmacogenetic research, single-gene studies have so far only explained a moderate amount of the variance in drug responding, or the occurrence of side-effects. In regards to predicting the occurrence of sexual sexual side-effects, future studies should explore the role of the three monoamine receptors mainly involved in sexual response, and their transporters, as well as NOS, and genes involved in drug metabolism. Given the convincing evidence of interaction between various neurotransmitter systems, a prudent course of research would include genetic information from all of these domains, especially given the decreasing costs of genotyping. The inclusion of data on genes involved in multiple neurotransmitter systems will allow for modeling of ‘gene by gene’ interactions. Before pursuing such a course of research, however, it is essential to document main effects of genes on sexuality. Without doing so, it would be difficult to disentangle the main effect from the interaction, especially without pre-treatment assessment of sexual functioning. Despite the difficulties and complexities in conducting such a course of research, the payoff will be large because it will not only help to limit drug side-effects that can lead to treatment noncompliance, but also help to elucidate the neurobiology of sexual functioning.

6. ACKNOWLEDGEMENTS

Preparation of this article was supported by a National Science Foundation Graduate Research Fellowship awarded to Brian Mustanski.

7. REFERENCES

Abdel-Hamid, I. A., 2004, Phosphodiesterase 5 inhibitors in rapid ejaculation: Potential use and possible mechanisms of action, *Drug.* **64:**13-26.

Angst, J., 1998, Sexual problems in healthy and depressed persons, *Int. Clin. Psychopharmacol.* **13:**S1-S4.

Angulo, J., Peiro, C., Sanchez-Ferrer, C. F., Gabancho, S., Cuevas, P., Gupta, S., & Saenz de Tejada, I., 2001, Differential effects of serotonin reuptake inhibitors on erectile responses, NO-production, and neuronal NO synthase expression in rat corpus cavernosum tissue, *Brit. J. Pharmacol.* **134:**1190-1194.

Araujo, A. B., Durante, R., Feldman, H. A., Boldstein, I., & McKinlay, J. B., 1998, The relationship between depressive symptoms and male erectile dysfunction: Cross-sectional results from the Massachusetts Male Aging Study, *Psychosom. Med.* **60:**458-465.

Arranz, M. J., Munro, J., Sham, P., Kirov, G., Murray, R. M., Collier, D. A., & Kerwin, R. W., 1998, Meta-analysis of studies on genetic variation in 5-HT2A receptors and clozapine response, *Schiz. Res.* **32:**93-99.

Bancroft, J., 1995, Are the effects of androgens on male sexuality noradrenergically mediated? Some consideration of the human, *Neurosci. Biobehav. Rev.* **19:**325-330.

Bancroft, J., 1999, Central inhibition of sexual response in the male: A theoretical perspective, *Neurosci. Biobehav. Rev.* **23:**763-784.

Bancroft, J., Janssen, E., Strong, D., Carnes, L., Vukadinovic, Z., & Long, J. S., 2003a, The relation between mood and sexuality in heterosexual men, *Arch. Sex. Beh.* **32:**217-230.

Bancroft, J., Janssen, E. Strong, D., & Vukadinovic, Z., 2003b, The relation between mood and sexuality in gay men, *Arch. Sex. Beh.* **32:**231-242.

Bancroft, J., Loftus, J., & Long, J. S., 2003, Distress about sex: A national survey of women in heterosexual Relationships, *Arch. Sex. Beh.* **32:**193-208.

Beck, A. T., 1967, *Depression: Clinical, Experimental and Theoretical Aspects,* Staples Press, London.

Biederman, J., & Faraone, S. V., 2002, Current concepts on the neurobiology of Attention-Deficit/Hyperactivity Disorder, *J. Atten. Dis.* **6:**S7-16.

Bitran, D., & Hull, E. M., 1987, Pharmacological analysis of male rat sexual behavior, *Neurosci. Biobehav. Rev.***11:**365-389.

Blier, P., Bergeron, R., & de Montigny, C., 1997, Selective activation of postsynaptic 5-HT1A receptors induces rapid antidepressant response, *Neuropsychopharmacol,* **16:**333-338.

Christ, G. J., 2002, Gene therapy for erectile dysfunction: Where is it going?, *Cur. Opin. Urol.* **12:**497-501.

Croft, H., Settle, E., Jr., Houser, T., Batey, S. R., Donahue, R. M., & Ascher, J. A., 1999, A placebo-controlled comparison of the antidepressant efficacy and effects on sexual functioning of sustained-release bupropion and sertraline, *Clin. Ther.* **21:**643-658.

Cutler, A. J., 2003, Sexual dysfunction and antipsychotic treatment, *Psychoneuroendocrinol.* **28:**69-82.

Dick, D. M., & Faroud, T., 2003, Candidate genes for alcohol dependence: A review of genetic evidence from human studies, *Alcohol. Clin. Exp. Res.* **27:**868-879.

Finkel, M. S., Laghrissi-Thode, F., Pollock, B. G., & Rong, J., 1996, Paroxetine is a novel nitric oxide synthase inhibitor, *Psychopharmacol. Bull.* **32:**653-658.

Gittleson, N. L., & Dawson-Butterworth, K., 1967, Subjective ideas of sexual change in female schizophrenics, *Br. J. Psychiatry.* **113:**491-494.

Gittleson, N. L., & Levine, S., 1966, Subjective ideas of sexual change in male schizophrenics, *Br. J.Psychiatry.* **112:**779-782.

Giuliano, F., & Rampin, O., 2000, Central neural regulation of penile erection, *Neurosci. Biobehav. Rev.* **24:**517-533.

Gorzalka, B. B., Mendelson, S. D., & Watson, N. V., 1990, Serotonin receptor subtypes and sexual behavior, *Ann. N .Y. Acad. Sci.* **600:**435-444.

Gray, J. A., 1987, *The Psychology of Fear and Stress*, Cambridge University Press, Cambridge.

Gregorian, R. S., Jr., Golden, K. A., Bahce, A., Goodman, C., Kwong, W. J., & Khan, Z. M., 2002, Antidepressant-induced sexual dysfunction, *Ann. Pharmacother.* **36:**1577-1589.

Halbreich, U., Kinon, B.J., Gilmore, J.A., & Kahn, L.S., 2003, Elevated prolactin levels in patients with schizophrenia: Mechanisms and related adverse effects, *Psychoneuroendocrinol.* **28:**53-67.

Harvey, A. T., & Preskorn, S. H., 1996, Cytochrome P450 enzymes: Interpretation of their interactions with selective serotonin reuptake inhibitors. Part I, *J. Clin. Psychopharmacol.* **16:**273-285.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., & Lesch, K. P., 1996, Allelic variation of human serotonin transporter gene expression, *J. Neurochem.* **66:**2621-2624.

Hull, E. M., Lorrain, D. S., Du, J., Matuszewich, L., Bitran, D., Nishita, J. K., & Scaletta, L. L., 1998, Organizational and activational effects of dopamine on male sexual behavior, in: *Male, Females, and Behavior: Toward Biological Understanding,* L. Ellis & L. Eberty eds., Greenwood Press, New York, NY, pp.79-96.

Knegtering, R., Castelein, S., Bous, H., Van Der Linde, J., Bruggeman, R., Kluiter, H., & van den Bosch, R. J., 2004, A randomized open-label study of the impact of quetiapine versus risperidone on sexual Functioning, *J. Clin. Psychopharmacol.* **24:**56-61.

Kruger, T. H., Haake, P., Hartmann, U., Schedlowski, M., & Exton, M. S., 2002, Orgasm-induced prolactin secretion: Feedback control of sexual drive?, *Neurosci. Biobehav. Rev.* **26:**31-44.

Larsson, K., & Ahlenius, S., 1999, Brain and sexual behavior, *Ann. N .Y. Acad. Sci.* **877:**292-308.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., & Murphy, D. L., 1996, Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region, *Science.* **274:**1527-1531.

Lilleleht, E., & Leiblum, S. R., 1993, Schizophrenia and sexuality: A critical review of the literature, *Ann. Rev. Sex Res.* **4:**247-276.

Lorrain, D. S., Matuszewich, L., Friedman, R., & Hull, E. M., 1997, Extracellular serotonin in the lateral hypothalamic area increases during postejaculatory interval and impairs copulation in male rats, *J. Neurosci.* **17:**9361-9366.

Mancama, D., & Kerwin, R. W., 2003, Role of pharmacogenomics in individualizing treatment with SSRIs, *CNS Drugs.* **17:**143-151.

Mas, M., Fumero, B., Rodriguez-Perez, I., & Gonzalez-Mora, J. L., 1995, The neurochemistry of sexual satiety: An experimental model of inhibited desire, in: *The Pharmacology of Sexual Function and Dysfunction,* J. Bancroft, ed., Elsevier Science, Amsterdam, pp.115-126.

Masand, P. S., Ashton, A. K., Grupa, S., & Frank, B., 2001, Sustained-release bupropion for selective serotonin reuptake inhibitor-induced sexual dysfunction: A randomized, placebo-controlled study of pharmacologic intervention, *Am. J. Psychiatry.* **157:**239-243.

Mathew, R. J., & Weinman, M. L., 1982, Sexual dysfunction in depression, *Arch. Sex. Beh.* **11:**323-328.

McGahuey, C. A., Gelenberg, A. J., Laukes, C. A., Moreno, F. A., Delgado, P. L., McKnight, K. M., & Manber, R., 2000, The Arizona Sexual Experience Scale (ASEX): Reliability and validity,
J. Sex. Marital Ther. **26:**25-40.

McKenna, K. E., 2000, Some proposals regarding the organization of the central nervous system control of penile erection, *Neurosci. Biobehav. Rev.* **24:**535-540.

Meston, C. M., 2004, A randomized, placebo-controlled, crossover study of ephedrine for SSRI-induced female sexual dysfunction, *J. Sex. Marital Ther.* **30**:57-68.

Michelson, D., Bancroft, J., Targum, S., Yongman, K., & Tepner, R., 2000, Female sexual dysfunction associated with antidepressant administration: A randomized, placebo-controlled study of pharmacologic intervention, *Am. J. Psychiatry.* **157**:239-243.

Michelson, D., Kociban, K., Tamura, R., & Morrison, M. F., 2002, Mirtazapine, yohimbine or olansapine augmentation therapy for serotonin reuptake-associated female sexual dysfunction: A randomized, placebo controlled trial, *J. Psychiatr. Res.* **36:**147-152.

Miller, W. B., Pasta, D. J., MacMurray, J., Chiu, C., Wu, H., & Comings, D. E., 1999, Dopamine receptor genes are associated with age at first sexual intercourse, *J. Biosoc. Sci.* **31:**43-54.

Modell, J. G., Katholi, C. R., Modell, J. D., & DePalma, R. L., 1997, Comparative sexual side effects of bupropion, fluoxetine, paroxetine, and sertraline, *Clin. Pharmacol. Ther.* **61:**476-487.

Monteiro, W. O., Noshirvani, H. F., Marks, I. M., & Leliott, P. T., 1987, Anorgasmia from clomipramine in obsessive-compulsive disorder: A controlled trial. *Brit. J. Psychiatry.* **151:**107-112.

Montgomery, S. A., Baldwin, D. S., & Riley, A., 2002, Antidepressant medications: A review of the evidence for drug-induced sexual dysfunction, *J. Affect. Dis.* **69:**119-140.

Murphy, G. M., Jr., Kremer, C., Rodrigues, H. E., & Schatzberg, A. F., 2003, Pharmacogenetics of antidepressant medication intolerance, *Am. J. Psychiatry.* **160:**1830-1835.

Nemeroff, C. B., DeVane, C. L., & Pollock, B. G., 1996, Newer antidepressants and the cytochrome P450 System, *Am. J. Psychiatry.* **153:**311-320.

Nurnberg, H. G., Hensley, P. L., Gelenberg, A. J., Fava, M., Lauriello, J., & Paine, S., 2003, Treatment of antidepressant-associated sexual dysfunction with Sildenafil: A randomized controlled trial, *JAMA.* **289:**56-64.

Rehman, J., & Melman, A., 2001, Normal anatomy and physiology, in: *Male Sexual Function: A Guide to Clinical Management,* J. J. Mulcahy, ed., Humana Press, Totowa, NJ, pp.1-46.

Rodriguez-Manzo, G., & Fernandez-Guasti, A., 1994, Reversal of sexual exhaustion by serotonergic and noradrenergic agents, *Behav. Brain Res.* **62:**127-134.

Rodriguez-Manzo, G., & Fernandez-Guasti, A., 1995, Participation of the central noradrenergic system in the reestablishment of copulatory behavior of sexually exhausted rats by yohimbine, nalozone, and 8-OH-DPAT, *Brain Res. Bull.* **38:**399-404.

Role, L. W., & Kelly, J. P., 1991, The brain stem: Cranial nervenuclei and the monominergic systems, in: *Principles of neural science, 3rd ed.,* E. R. Kandell, J. H. Schwartz, & T. M. Jessell, eds., Appleton & Lange, Stamford, CT, pp.683-699.

Rosen, R. C., Lane, R. M., & Menza, M., 1999, Effects of SSRIs on sexual function: A critical review, *J. Clin. Psychopharmacol.* **19:**67-85.

Sala, M. Braida, D., Leone, M. P., Calcaterra, P., Monti, S., & Gori, E., 1990, Central effect of yohimbine on sexual behavior in the rat, *Physiol. Beh.* **47:**165-173.

Smith, S. M., O'Keane, V., & Murray, R., 2002, Sexual dysfunction in patients taking conventional antipsychotic medication, *Brit. J. Psychiatry.* **181:**49-55.

Thase, M. E., Reynolds, C. F., Glanz, L. M., Jennings, J. R., Sewitch, D. E., Kupfer, D. J., & Frank, E., 1987, Nocturnal penile tumescence in depressed men, *Am. J. Psychiatry.* **144:**89-92.

Veenstra-VanderWeele, J., Anderson, G. M., & Cook, E. H., Jr., 2000, Pharmacogenetics and the serotonin system: Initial studies and future directions, *Eur. J.Pharmacol.* **410:**165-181.

Veening, J. G., & Coolen, L. M., 1998, Neural activation following sexual behavior in the male and female rat brain, *Behav. Brain Res.* **92:**181-193.

Wu, S., & Comings, D. E., 1999, A common C-1018G polymorphism in the human 5-HT1A receptor gene, *Psychiatr. Genet.* **9:**105-106.

Zourkova, A., & Hadasova, E., 2002, Relationship between CYP 2D6 metabolic status and sexual dysfunction in paroxetine treatment, *J. Sex. Marital Ther.* **28:**451-461.

18. DRUG-INDUCED MOVEMENT DISORDERS

Christopher G. Goetz*

1. INTRODUCTION

Movement disorders constitute a group of neurological diagnoses characterized by changes in muscle tone, the presence of inappropriate movements, the impairment in timing and sequencing of normal movements, and the absence of weakness. As a group, these disorders have their origin in disruption of brainstem and subcortical circuits, known as the basal ganglia system, that involves several neurotransmitters, primarily dopamine and acetylcholine, but also serotonin, norepinephrine, gamma amino butyric acid, and glutamate (Jankovic 2003).

Some movement disorders relate to primary neurodegenerative diseases like Parkinson's disease or various parkinsonism-plus syndromes. In these cases, neurodegeneration occurs in selective, but often multiple brain regions within, and sometimes beyond the basal gangliar system. Other movement disorders are considered as non-degenerative conditions associated with central nervous system neurotransmitter imbalances, like primary dystonia and Gilles de la Tourette syndrome. Still others are clear genetic conditions, like Huntington's disease and familial tremor.

In addition to these primary movement disorders, similar movement impairments can occur as a reflection of metabolic disturbances, infectious diseases, and cerebrovascular accidents. A final category of secondary movement disorders is composed of the syndromes directly related to medication side effects, collectively termed drug-induced movement disorders. In this latter category, psychiatric medications used to treat psychosis and mood disorders are particularly notable for their associations with movement disorders. Often collectively termed "extrapyramidal symptoms" or "EPS", these disorders are variable phenomenologically and include tremors, chorea, dystonia, tics, myoclonus, akathisia, chorea and parkinsonism (Gershanik 1993). This chapter focuses on clinical syndromes associated with dopamine-receptor blocking agents

* Christopher G. Goetz, Rush University Medical Center, Chicago, Illinois, 60612

primarily used to treat psychosis, and agents used in the treatment of mood disorders, specifically antidepressants and lithium carbonate. Because anxiolytics are not associated with movement disorders except during drug withdrawal, they are not discussed. Likewise, drugs that are used in psychiatry but more typically prescribed in other medical arenas or in general medical care, like anticonvulsants or sleeping medications, are not discussed. Each section presents the clinical disorders associated with these psychiatric drugs in terms of phenomenology and temporal development relative to medication exposure. Then, a discussion of data related to biological mechanisms and treatment follows. The final focus concerns available information related to pharmacogenetic research and the search for genetic markers to identify subjects at particular risk for developing movement disorders related to psychotropic medications. Whereas no markers applicable to patient screening have yet been identified, the discussion highlights areas of current research and provides a context for future studies in this domain.

2. MOVEMENT DISORDERS INDUCED BY DOPAMINE RECEPTOR SITE ANTAGONISTS

Numerous movement disorders are associated with the use of drugs that block dopamine receptors. Whereas the majority of such agents are used to treat psychosis, some are predominantly prescribed by internists for nausea, gastrointestinal problems and post-menopausal symptoms (metoclopramide, sulpiride, clebopride, veralipride) (Kompoliti 2003). The agents used for psychosis include phenothiazines, butyro-phenones and other heterocyclic compounds including benzamides and a variety of newer generation antipsychotics, termed "atypical". Some calcium channel blockers (flunarizine and cinnarizine) and selective serotonin reuptake inhibitors may have some antidopaminergic activity as well. Movement disorders are clinically divided into acute, subacute, and chronic disorders depending on their time of onset relative to medication introduction or dosage increase. Acute dystonic reactions occur within hours, akathisia and parkinsonism within days or weeks, and tardive dyskinesia, in its various forms, after at least three months of chronic therapy. Each disorder has a distinct clinical presentation, biological basis and treatment. Pharmacogenomic data are modest for all these drug-induced movement disorders, but the largest number of studies focus on tardive dyskinesia.

2.1. Acute dystonic reactions

2.1.1. Clinical features

Dystonia refers to a movement disorder typified by tonic contractions of muscles, often with a twisting component. Acute neuroleptic-induced dystonias are seen early in the course of neuro-leptic therapy and are often seen following a single parenteral dose. Dystonic manifestations can be diverse, although the most common clinical signs involve the eyes and neck and are termed "oculogyric crises" (Goetz 2001). Patients complain of inability to move the eyes in the vertical

plane and experience double or blurred vision and discomfort or pain on attempted gaze. Most often, the eyes maintain a sustained upward gaze in association with a retrocollic spasm of the neck, termed "opisthotonos." Other muscles, including truncal and limb-related, may be involved in acute drug-induced dystonias, especially in children, but these varieties are uncommon among adults. When laryngeal muscles are affected, respiratory stridor can be life threatening (Gershanik 2002). Dystonic symptoms persist for hours or days after stopping the causative drug, though clinical intensity of symptoms may wax and wane.

Males develop acute dystonic reactions twice as frequently as females, and children and young adults are the highest group at risk for these reactions. In one series, among young males, aged 30 years or less, over 90% experienced some form of acute dystonic reaction when exposed to neuroleptic drugs (Magnuson 1999). An underlying psychiatric diagnosis of mania was formerly considered a higher risk than schizophrenia, but this difference likely relates to the higher peak doses used in managing acute manic and hypomanic episodes (Khanna 1992). The propensity of a given drug to cause acute dystonic reactions parallels the drug's associated prevalence of parkinsonism. The piperazine group of neuroleptics has the highest likelihood of precipitating dystonic reactions, and the piperidine (thioridazine) group and newer generation neuroleptics like clozapine have a low propensity. Clinically, the simultaneous administration of anticholinergic agents at the time that dopamine receptor site antagonists are introduced is thought to decrease the risk of neuroleptic-induced dystonia, and the acute administration of anticholinergic agents typically reverses these dystonias (see below).

2.1.2. Biological Basis and Treatment

Because dystonias in other medical contexts are thought to involve primarily the putamen and its outflow pathways, most of the focus on understanding a biological basis of acute dystonic reactions has been on the dopamine-acetylcholine balance implicit to striatal function and D2 receptor activity. Multiple hypotheses have been offered. Suddenly enhanced dopamine turnover in response to dopamine receptor blockade could theoretically explain this adverse event, but treatment with a dopamine-depleting agent, alpha-methyl-paratyrosine, fails to prevent acute dystonic reactions. These observations, therefore, cast serious doubt on presynaptic dopaminergic release as a primary pathophysiological mechanism (Gershanik 2002; McCann 1990). It is also possible that the dopamine receptor blockers induce other neurochemical changes besides cholinergic disruption, for example, secondary involvement of gamma-amino-butyric acid in the putaminopallidal pathway and resultant enkaphalin enhancement.[9] In support of this hypothesis, dopamine receptor antagonists with a high likelihood of acute dystonic reactions like piperazine-based neuroleptics also have high binding affinities for sigma-1 and sigma-2 receptors (Matsumoto 2000).

Regardless of these observations, the prevailing concept for the biological basis of acute dystonic reactions from dopamine receptor antagonists is a direct disruption of striatal dopamine-acetylcholine homeostasis. As such, with D2 and possibly some D1 receptors blocked, acetylcholine acts in relative, though not absolute, higher striatal concentrations and precipitates the dystonic behaviors seen.

This theory is supported by a low incidence of acute dystonia with neuroleptic agents with high anticholinergic properties, like thioridazine and clozapine. It is further supported by the reduced risk of acute dystonia when neuroleptics are started with an anticholinergic agent. Finally, years of empiric clinical experience demonstrate that successful treatment of acute dystonias occurs with intravenous or intramuscular injections of a centrally active antimuscarinic agent (trihexiphenadyl) or antihistaminic drugs with anticholinergic properities (diphenyhydramine) (Kompoliti 2003). Such treatment usually reverses acute dystonic reactions within minutes. Because these drugs are very short-acting when given intravenously or intramuscularly, oral anticholinergic agents must be prescribed for the next 24 to 48 hours. If the neuroleptic cannot be stopped because of primary psychosis or other reasons, patients must be placed on maintenance anticholinergic treatment for several weeks or switched to another neuroleptic with a lower propensity to cause dystonia. If the dystonic reaction is mild, no intervention is needed and spontaneous resolution should occur if the neuroleptic is stopped. Even if neuroleptic treatment continues in these cases of mild dystonia, usually there is a gradual, spontaneous resolution of dystonic complications after several weeks of continued neuroleptic treatment.

2.1.3. Pharmacogenomics

No studies have established specific molecular biological or genetic attributes of risk for acute dystonic reactions to dopamine receptor antagonists. The repeated reported male predominance of this adverse effect however suggests a possible link to sex chromosomes. Whereas the CYP2D6 genotype or phenotype of poor debrisoquine metabolism can be linked to several acute effects of dopamine receptor antagonists including sedation, postural hypotension and autonomic dysfunction, there is no relationship with risk for acute dystonia (Spina 1992; Armstrong 1997). Because all clinically utilized dopamine antagonists block the D2 receptor system, studies have focused on genetic polymorphisms of this receptor's gene in patients with and without various movement disorder side effects. A comprehensive survey of nine polymorphisms of the D2 receptor site gene did not identify any genetic marker of acute dystonia (Kaisir 2002).

2.2. Subacute akathisia

2.2.1. Clinical features

Akathisia is a severe, subjective sense of restlessness, often associated with pronounced anxiety that provokes a patient to move incessantly (Gershanik 2002). The movement disorder of akathisia usually involves stereotypic and repetitive pacing, marching or shifting leg movements that are purposefully performed in order to relieve the restless discomfort. The patient may also demonstrate repetitive hand movements, truncal rocking, and panting respirations (Sunami 2000). Unlike dystonias, akathitic side effects of dopamine receptor antagonists develop after days of therapy. Akathisia is not a prominent symptom in most medical or neurological disorders, but it is common in Parkinson's disease, in iron deficiency syndromes,

and can be seen in cases with structural putaminal of pallidal lesions (Jankovic 2003; Barton 1990).

Neuroleptic-induced akathisia is a frequently unrecognized and therefore likely underreported side effect of therapy. Because psychiatric patients starting neuroleptics are often mentally and motorically agitated, it may be difficult to ascribe new movements properly to drug-induced restlessness. Frequency estimates of akathisia among patients starting neuroleptics or increasing their dose is approximately 33%. The problem can be so distressful that it has been linked to suicide and medication non-compliance (Hirose 2000; Duncan 2000).

The risk for developing akathisia increases as the dose and antipsychotic potency of neuroleptics increase. Newer generation ("atypical") neuroleptics are not devoid of risk, and respiridone and haloperidol have been reported as having equal propensity for precipitating this adverse effect (Rosebush 1999). Subjects with acute mania may have a higher absolute rate of akathisia than schizophrenics, but this observation is most likely explained by the higher doses used to control the former diagnosis. Though not firmly established, the development of subacute akathisia has been suggested to predict a risk for poor treatment outcome and a higher risk of tardive dyskinesia among schizophrenics (Nair 1999; Eichhammer 2000).

2.2.2. Biological basis and treatment

The biological basis of dopamine receptor antagonist-induced akathisia is poorly understood, but the occurrence of akathisia in other conditions suggests some clues. The frequent association of drug-induced parkinsonism with akathisia and the spontaneous occurrence of akathisia in Parkinson's disease itself suggest that the nigro-stiatal dopaminergic system plays a direct role. Cases with structural subcortical lesions associated with akathisia have most damage in the putamen or outflow tracts to the globus pallidus. Because the movements are predominantly leg stereotypies, subcortical-spinal networks or mesocortical pathways are likely involved. Whereas traditionally, D2 dopaminergic receptor blockade was hypothesized to be at the core of akathisia, this disorder has a less predictable pharmacology than acute dystonia. Noradrenergic and opioid influences play a likely role in the pathophysiology, because beta-noradrenergic blockers and opioid antagonists are sometimes effective therapies if the neuroleptic cannot be withdrawn (Kompoliti 2003). These secondary chemical influences likely involve brainstem nuclei or mesospinal pathways. Serotonergic/dopaminergic imbalance has also been suggested to play a role in akathisia, based on evidence that some of the newly developed neuroleptics with high affinity for 5HT3 receptors have a low index of akathisia (Hirsch 2002). The link of akathisia to iron metabolism has been studied, and at least in some studies, neuroleptic-treated patients with and without akathisia have both lower iron and lower ferritin plasma levels than healthy controls (Kugolu 2003). Those with akathisia tend to have lower levels than non-akathitic subjects, but the iron and ferritin levels still fall within the normal range (Hofmann 2000). Though iron levels influence dopamine D1 and D2 receptor numbers and sensitivity in rats, exact cellular effects caused by dopamine receptor antagonists in humans have not been identified (Erikson 2001).

The anguish experienced by patients with akathisia prompts the need for clinicians to diagnose and treat this condition immediately. Withdrawal of dopamine receptor antagonists will abate symptoms over days. Lowering the dose can also be adequate or a switch to a more low potency neuroleptic may resolve the problem without sacrificing necessary control of psychosis. In the event that these solutions are not successful, the clinician enters into the realm of vague empiricism, and agents that have been used cross several pharmacological systems. Decreasing noradrenergic activity with beta-blockers, like propranalol, or with low dose clonidine that is thought to act to downregulate noradrenergic systems through autoagonism at presynaptic receptors have been used with success in some series of patients (Gershanik 1993). Opioid antagonists like propoxyphene may be effective, and some serotonin receptor antagonists, specifically those preferentially blocking 5HT2 receptors like mianserin, are reported to abate akathisia in subjects on neuroleptics (Poyurovsky 1999). Recent reports that ziprasidone, has a very low propensity to provoke akathisia and yet is effective in managing psychotic agitation provides additional evidence that serotonergic blockade may be beneficial in avoiding neuroleptic-induced akathisia (Hirsch 2002). Finally, it is important to determine if the patient has both akathisia and drug-induced parkinsonism with tremor, rigidity and bradykinesia, because in these cases, amantadine or anticholinergic agents may treat both syndromes (Gershanik 1993).

2.2.3. Pharmacogenomics

Because all clinically used antipsychotic agents have activity one the D2 dopaminergic receptor system, genetic variants that might modulate therapeutic and adverse effect profiles have been examined. In the comprehensive survey of nine known polymorphisms of the D2 dopamine receptor cited above, no genetic pattern could detect subjects with akathisia. As will be discussed further in the section on tardive dyskinesia, particular emphasis has been placed on the dopamine D3 receptor gene in regards to side effects of dopamine receptor antagonists. Subjects whose alleles are homozygous for the Ser9Gly polymorphism of this gene are twice as likely to develop akathisia as subjects with other polymorphisms (Eichhammer 2000). This same pattern has been reported in several studies of tardive dyskinesia (see below), suggesting a molecular biological link between the two conditions and reinforcing clinical observations that akathisia is a predictor of tardive dyskinesia risk.[19,20] No relationship has been established between CYP2D6 polymorphisms or phenotype of debrisoquine metabolic patterns and akathisia (Dahl 2002; Andreassen 1997).

2.3. Subacute parkinsonism

2.3.1. Clinical features

Days or weeks after starting or increasing the daily dose of dopamine receptor antagonists, patients may experience the gradual development of bradykinesia, rigidity, gait, and balance instability and resting tremor. When these signs appear in unison, the diagnosis of parkinsonism is not difficult, but often the early signs are

subtle with more vague symptoms of increasing slowness and difficulty with movement (Kompoliti 2003). In these cases where tremor is not prominent, the clinician, family and patient may misinterpret drug-induced parkinsonism as a global overmedication effect or as a sign of impending depression, especially in subjects with bipolar affective disorders. The signs of drug-induced parkinsonism are not different from Parkinson's disease itself, although the latter is usually dominated by less symmetric signs. Tremor is seen when the patient's hands are relaxed in the lap, and the movement disorder abates as the patient moves and executes tasks of daily living (resting tremor). Fine motor movements like buttoning, opening envelopes, manipulating an eating utensil become slow and difficult, although there is no weakness (bradykinesia). On examination, while the patient remains relaxed, the clinician can detect hypertonicity with a ratchet-like quality (cogwheel rigidity). Difficulties rising from a chair, initiating walking, pivoting smoothly to turn, and resisting a postural threat, usually tested by pulling abruptly on the shoulders to assess if patients can right themselves without falling backwards, all are indicative of gait and postural reflex impairment. All signs may be present simultaneously, but often the syndrome only partially develops, so that the physician must be sensitive to all clinical features. Elderly patients are thought to be at higher risk for this side effect than younger patients, but there is no established gender predominance. Particularly characteristic of drug-induced parkinsonism is rest tremor that predominates in the lips and chin, provoking a rhythmic flutter that has been termed "rabbit syndrome" (Meltzer 1987). The movement disorder is a parkinsonian tremor and must be differentiated from the more choreic lingual buccal movements of tardive dyskinesia (see below).

2.3.2. Biochemical basis and treatment.

Because Parkinson's disease and drug-induced parkinsonism are phenotypically indistinguishable, similar biochemical bases for the two disorders have been proposed (Kawans 1973). In Parkinson's disease, the primary degenerative lesion is loss of the dopaminergic cells projecting from the pars compacta of the substantia nigra in the mesencephalon to the putamen and caudate nucleus (striatum). These cells interact primarily with D2 receptors (Jankovic 2003). The primary site of action for neuroleptics precipitating drug-induced parkinsonism is this same post-synaptic dopamine D2 receptor population within the striatum. Patients with preclinical Parkinson's disease may be at a high risk for drug-induced parkinsonism. One case series identified patients referred for evaluation of neuroleptic-induced parkinsonism (Goetz 1983). Although the parkinsonism resolved over months after neuroleptic withdrawal, patients returned within three years with idiopathic Parkinson's disease. The treatment of drug-induced parkinsonism preferably includes cessation of the provoking agent, but in the case where neuroleptic continuation is needed, anticholinergic drugs or amantadine can be useful (Gershanik 2002). Because neuroleptic metabolism often involves the generation of several intermediate or end products with dopamine antagonist properties, full drug clearance should be not be presumed until three or four months after cessation.

2.3.3. Pharmacogenomics

The phenotypic similarity between Parkinson's disease and dopamine receptor antagonist-induced parkinsonism has prompted researchers to posit hypotheses that may relate the two at a molecular biological level. None of these has proved fruitful at the present time. A number of hereditary forms of familial parkinsonism have been identified and these include abnormalities located on chromosomes 1p, 2p, 4q, 5q, 6q, 8p, 9q, and 17q (DeStefano 2002; West 2002). Defects in pathways involved in intracellular proteolysis or energy metabolism unify these forms of familial parkinsonism. Though these defects are not seen in most cases of non-familial Parkinson's disease, they give clues to the areas of focus for future studies and have not been specifically studied in relation to drug-induced parkinsonism. Testing of polymorphism patterns among subjects with drug-induced parkinsonism may reveal risk factors shared by both disorders. Early reports of links between young-onset Parkinson's disease and P450 metabolism, though later not confirmed, led to searches for different patterns of CYP2D6 polymorphism among subjects with drug-induced parkinsonism. Like the studies in Parkinson's disease, there have been inconsistent results: Several, though not all, show a significant increase or trend towards overrepresentation of mutated CYP2D6 alleles among subjects who develop dopamine receptor antagonist-induced parkinsonism (Lane 1997; Pollock 1995; Arthur 1995).

These studies are methodologically imperfect, and in some series, subjects were taking antipsychotic agents not specifically metabolized by the P450 system or were on multiple drugs with different mechanisms of action. Based on studies of the genetics of Parkinson's disease, polymorphism patterns for several other genes are reasonable research targets, including manganese superoxide dismutase gene, the E2 subunit of the alpha-ketoglutarate complex, the alpha-1-antichymostypsin gene, monoamine oxidase B intron 13, and the catechol-O-methyl transferase gene (Kruger 2000; Mizuta 2000; Goudreau 2002).

2.4. Tardive dyskinesia

2.4.1. Clinical features.

Tardive dyskinesia is a general term that describes movement disorders specifically provoked by chronic exposure to dopamine receptor antagonists (Kompoliti 2003). In a more restrictive context, some authors refer to this entity as "tardive syndrome", reserving tardive dyskinesia to connote the typical lingual-facial-buccal distribution and choreic phenomenology of the most classic form of this dyskinesia (Gershanik 2002). The rapid and repetitive movements include lip smacking, tongue darting, and chewing movements that are unsightly and disruptive to social interactions, dental hygiene, and vocal communication (Joseph 1999). When the diaphragmatic muscles and vocal apparatus are involved, irregular gasping respirations and noises may also occur. Other non-choreic forms of tardive dyskinesia include tardive dystonia, generally typified by back arching and retrocollic neck postures (Burke 1982), tardive tics or repetitive stereotypic eye blinks, facial grimaces and sometimes more complex movements (Klawans 1982),

tardive myoclonus typified by lightning-like jerks (Tominaga 1987), and even tardive tremor (Stacy 1992). In all cases, tardive dyskinesia relates to chronic exposure to dopamine receptor antagonists and should not be diagnosed in the context of recent introduction (less than three months) of medication, after recent increases of medication dosage, or as a spontaneously developing disorder without medication exposure.

It is not clear why some patients develop one tardive syndrome and others develop another. In most instances, however, at least mild lingual-facial-buccal movements are present even when there are more prominent dystonic, tic, or myoclonic features. In fact, from a clinical perspective, the combination of multiple phenomenologies within the same patient should suggest the diagnosis of tardive dyskinesia (Kompoliti 2003).

The time of onset and context of development for tardive dyskinesia are variable. By definition, the exposure time to drug must be a minimum of three months, and the risk appears to increase with exposure duration and cumulative dose. Other proposed risk factors have been age and gender, with elderly women usually being considered the subjects at highest risk. These data are based on estimates that approximately 5% of exposed young adults develop tardive syndromes, whereas in patients over 45, the rate exceeds 30%, and in older institutionalized subjects the prevalence is 60% (Byne 1998). Within the elderly population, a reduced rate of dyskinesia occurs in very old subjects, an observation that be explained by documented late age-related loss of striatal dopamine receptors (Sweet 1992). Overall, an estimated 15-20% risk occurs (Koshino 1992). Often tardive dyskinesia first appears at a time when the clinician is lowering the overall medication dosage (withdrawal dyskinesia). It can also, however, first occur on steady doses (breakthrough dyskinesia). Because many cases are short-lived, especially among children who usually experience rapid and spontaneous remission, these cases are rarely even counted among the tardive dyskinesia statistics.

For adults, however, once tardive dyskinesia develops, it is likely that some form of movement disorder will persist. Some remain stable, but some become more intense over time even if the causative medication is stopped (Crane 1971; Koshino 1991). Remission rates vary depending on the follow-up observation time, and it is clear that some cases resolve as late as five years after cessation of the causative drug (Gershanik 2002).

The choice of neuroleptic drug is likely important to the risk of tardive dyskinesia. Although not extensively studied with carefully controlled trials, traditional neuroleptics of the piperazine group and the butyrophenone, haloperidol, are thought to have the highest risk of causing tardive dyskinesia, followed by the piperidine compounds, like chlorpromazine, and lastly by the piperidine neuroleptics, like thioridazine and the newer generation compounds, including clozapine, olanzapine, quetiapine, ziprasidone, and aripiprazole.[3,28] Even with these latter agents, however, a risk of tardive dyskinesia is present, and risk is thought to be dose and duration related (Glazer 1993).

Besides high neuroleptic dosing, concomitant use of anticholinergic medications has also been implicated as a clinical risk factor. This issue is particularly important, because it is entirely physician controllable and, in many countries, neuroleptic medications are introduced with automatic co-prescriptions of

anticholinergics. Whereas short-term, this type of treatment is likely useful for preventing or minimizing drug-induced acute dystonias, chronic treatment with anticholinergics may not only be unnecessary, but may be promoting a potentially irreversible side effect (Muscettola 1993). Another treatment strategy in managing psychiatric illnesses has been intermittent attempts at "drug holidays" or short, medication-free periods, but one study suggested that neuroleptic interruptions were associated with a threefold increase in tardive dyskinesia risk (Van Harten 1998).

2.4.2. Biochemical basis and treatment

Several explanations have been posited to explain the underlying pathophysiology of tardive dyskinesia. A primary theory has long focused on neuroleptic-induced receptor site hypersensitivity caused by chronic receptor blockade (Klawans 1973). In this way, secondary increases in dopamine turnover occur in dopamine cells projecting to the chemically denervated structures. According to this model, when the dopamine receptor antagonist dose is reduced or the drug withdrawn, dopamine acting at hypersensitive receptors would provoke the various hyperkinetic movement disorders typical of tardive dyskinesia. Each phenomenological variant of tardive dyskinesia could correspond to a different hypersensitized neuroanatomical area, for instance, the striatum for chorea, the putaminal-pallidal system for dystonia, and the mesocortical projections for tics.

This traditional concept has been challenged because the predominant phenomenologies of tardive dyskinesia are not drug-specific and every drug can cause all types of movement disorders. More extensive understanding of the basal gangliar circuitry has also allowed more extensive analyses of neurotransmitter systems that may be directly or indirectly implicated in the biological basis of tardive dyskinesia. In this light, greater emphasis has been placed on the subthalamic nucleus, because it is known to be an important integrative center in basal gangliar function, and alterations in its activity influence signs of parkinsonism, chorea, dystonia and other movement disorders. Chronic neuroleptic use causes loss of gamma-aminobutyric acid-medicated inhibition from this nucleus that normally regulates thalamic activation of the cortex. Unchecked thalamo-cortical activity could theoretically cause a wide variety of movement disorders affecting descending cortico-spinal, cortico-brainstem, and especially cortico-subcortical networks (Mitchell 1992). Excessive activation of thalamo-cortical function primarily involves the neurotransmitter glutamate, and tardive dyskinesia has also been suggested to relate to excitotoxic effects of glutamate and related compounds (Naidu 2001). A complementary hypothesis focuses on the opioid system, because animals treated with chronic neuroleptics have reduced activity of the medial globus pallidus, ventral anterior and ventral lateral thalamic nuclei, as well as markedly increased subcortical metenkephalin levels. Drugs associated with low frequencies of tardive dyskinesia, like clozapine, do not induce metenkephalin-like immunoreactivity responses whereas a more traditional neuroleptic, haloperidol, causes significant enhancement (Auchus 1992). Other neurotransmitters and neuromodulators studied in models of tardive dyskinesia include neurotensin because its striatal concentration increases after chronic neuroleptic exposure, and cellular energy markers such as cAMP, cGMP and nitric

oxide levels, because their levels decrease in anatomical regions with supersensitive D2 receptors (Bester 2000).

New interest has focused on neuroleptic effects on D1 receptors, those linked to adenyl cyclase enzymatic activity. Whereas typical neuroleptics primary block D2 receptors, leaving D1 variably affected, high turnover rates of dopamine consequent to the D2 receptor blockade could abnormally activate D1 receptors. In an animal model of tardive dyskinesia, rats with genetically mediated inactivated D1 receptor expression developed fewer abnormal movements when treated with chronic neuroleptics than those with full D1 function (Van Kampen 2000). The D3 receptor system, important to the antipsychotic efficacy of management of psychosis, has been studied in terms of genetic polymorphisms that might affect the risk of developing tardive dyskinesias among subjects exposed to dopamine receptor antagonists. (See below).

Based on these multidimensional theories, a single therapeutic target for the treatment of tardive dyskinesia has not been established. Because the dopamine antagonists are the causative agents, these drugs should only be used when needed. By definition, the disorder occurs in subjects exposed to at least 3 months of therapy, so short-term treatment can potentially eliminate risk. If involuntary movements develop, withdrawal or reduction in dose may lead to a transient increase in intensity, but over time movements stabilize or improve in most patients. In some patients, however, tardive dyskinesia can increase in intensity and spread to new body regions even after full withdrawal of medications. In patients who need to remain on dopamine antagonists for primary psychiatric or medical disease, switching to agents associated with a lower perceived risk of tardive dyskinesia, clozapine, quetiapine, risperidone, olanzapine, ziprasidone, aripiprazole can be effected.

Dopamine-depleting drugs, like reserpine and tetrabenazine have been used with some success in treating tardive dyskinesia, but these agents are associated with drug-induced parkinsonism and depression, so they may not be feasible treatments. Very low dopamine agonists, felt to activate dopaminergic presynaptic autoreceptors and thereby decrease dopamine release have been advocated from small observational studies. Calcium channel blockers and GM1 ganglioside, aimed at promoting neuronal repair, have been used, as well as vitamin E, but results have not been consistent enough to advocate wide usage (Lerner 2001).

In terms of treating individual movement disorders, because the most frequent manifestation of tardive dyskinesia is choreic or stereotypic and repetitive, anticholinergics should generally be slowly withdrawn and stopped if possible. For cases of marked tardive dystonia, anticholinergic agents may be symptomatically helpful for the tonic spasms, though the faster, clonic movements of other phenomena may increase.

In severe cases, neurosurgical procedures to target the globus pallidus or subthalamic nucleus may be warranted.

2.4.3. Pharmacogenomics

CYP2D6 polymorphisms have been examined in relation to tardive dyskinesia, but there is no consistent relationship (Dahl 2002). Other reports suggest that an

intronic polymorphism in the CYP1A2 gene may contribute to tardive dyskinesias risk, but these studies have not involved large numbers of subjects (Ozdemir 2001). Nine polymorphisms of the D2 dopamine receptor have been examined in a series of over 600 schizophrenic patients and no relationship with tardive dyskinesias has been established with any of them (Steen 1997). In regards to the D3 system that is felt to be related to psychotic and some locomotor behaviors, mutations in genes related to the D3 dopamine receptor itself have been examined. In several studies, including a combined analysis of 780 patients, Ser9Gly polymorphism has been associated with increased risk for tardive dyskinesia (Steen 1997; Basile 1999; Segman 1999). Other studies, however, have failed to reproduce these observations (Reitschel 2000). When an association is found, the increased risk conferred by the D3 polymorphism is independent of age and gender (Segman 2000). Even if the association exists, the positive predictive value of D3 receptor glycine alleles for tardive dyskinesia, is quite low, and therefore testing of subjects prior to neuroleptic exposure has no real practical utility at the present time. In China, where tardive dyskinesia is reported to be infrequent, no association between this polymorphism and tardive dyskinesia has been found (Garcia-Barcelo 2001). Other genes that have been associated with an increased risk of tardive dyskinesia in at least one report include the 5HT2C receptor gene (Segman 2000), 5HT2A receptor gene (Segman 2001), and the manganese superoxide dismutase gene (Hori 2000). Given the complexity of phenotypic expressions of tardive dyskinesias, it is feasible to search for gene interactions or additive gene contributions. An additive effect between the D3 polymorphisms and the 5HT2C receptor gene profile has been reported to influence severity of abnormal involuntary movements in one analysis, though no statistical interaction was documented (Segman 2000). The same team suggested an interaction between the Ser9Gly polymorphism of the D3 receptor and the cytochrome P450 17-alpha-hydroxylase gene (CYP-17) in determining tardive dyskinesia severity. They found CYP-17 allelic status to influence subjects homologous for the glycine allele of the D3 receptor gene (Segman 2002). Clearly such observations require confirmation by independent groups, but with developing technological tools and large patient cohorts already identified, testing can be performed with efficiency. Smaller studies have subdivided patients with tardive dyskinesias by phenomenological presentation and focused on tardive dystonia only, but without finding relationships to the cytochrome P450 enzyme or to polymorphisms of the D2 receptor (TaqIA and -141C Ins/Del) or the D3 receptor (Ser9Gly) (Mihara 2002).

3. TRICYCLIC ANTIDEPRESSANTS

3.1. Tremor and myoclonus

3.1.1. Clinical features

Subjects treated with amytriptyline, imipramine and other tricyclic compounds can develop a fine, rapid postural tremor that is maximally apparent when the patient holds his hands out or tries to execute a task. This movement disorder may

occur in as high as 10% of exposed subjects (Kompoliti 2003). Fine motor control is impaired because of the shaking, and typically handwriting is jerky or the subject spills food when trying to use a soup spoon or other utensil to transport food from the plate to the mouth. Occasionally more rapid and high amplitude jerks, termed myoclonus, can develop, and this movement is more disabling. Tremors can occur in the context of normal cognitive function, but when myoclonus occurs with tricyclic drugs, it is usually part of a generalized delirium, indicative of acute or sub-acute drug intoxication.

3.1.2. Biological basis and treatment

Postural tremors are generally believed to relate to high noradrenergic activity in subcortical pathways, possibly also involving inputs from the cerebellum to the ventral-anterior and ventral-lateral nuclei of the thalamus. The known blockade of noradrenergic presynaptic reuptake that is inherent to tricyclic antipressant activity is considered the origin of tricyclic-related tremor. Myoclonus, on the other hand, is generally linked to serotonergic dysfunction, and in different clinical settings, both over- and under-activity of serotonergic systems can be associated with myoclonus. In the case of tricyclic antidepressant overdose, myoclonus is presumed to relate to heightened serotonergic activation at reticular-spinal, and reticular-subcortical and reticulo-cortical systems. The serotonergic reuptake mechanism of tricyclic antidepressants is thought to play at least a partial pathophysiological role in tricyclic-induced myoclonus, but anticholinergic intoxication and a more generalized encephalopathy unlinked to a single neurotransmitter has also been suggested. Based on these observations, treatment involves the cessation of tricyclic drugs, hydration, and in the case of severe myoclonus, physical protection from self-injury. Resolution of tremor and myoclonus is anticipated shortly after drug cessation. In cases where these signs occur in the context of suicide attempts, reversal of toxic signs including the movement disorder, can be hastened by giving cholinesterase inhibitors aimed at reversing anticholinergic intoxication.

3.1.3. Pharmacogenomics

Because the field of pharmacogenomics is more modern than the era of wide usage of tricyclic compounds, these agents have not been studied extensively in relation to genetic markers of efficacy and no study has focused on movement disorder side effects. Because chronic administration of tricyclic compounds results in down regulation of the 5HT2 receptor, individual patient genetic profiles for the 5HT2 receptor may be a reasonable target for studying relative risks of myoclonus (Glennon 1995). CPY2D6 polymorphisms have been studied in relation to tricyclic efficacy, but without a specific focus on drug-induced movement disorders (Bertilsson 1997; Lerer 2002). One study examined whether antidepressant-induced myoclonus was associated with CYP2D6 or CYP2C19 polymorphisms reflective of poor psychotropic drug metabolism. The study sample was small and comprised of cases exposed to numerous antidepressants and not just tricyclics. Myoclonus, however, was not associated with a slow metabolizing P450 enzyme

system (Spigset 1997). Other studies have considered an overrepresentation of slow metabolizer-related genotypes of the P450 enzyme system among antidepressant-treated patients and movement disorders, but these case series have a wide variety of drug exposures, different types of antidepressants, and often concomitant neuroleptic exposure (Vandel 1999 and 2000).

3.2. Other reported movement disorders

There are rare reports of chorea, choreoathetosis, and akathisia in association with tricyclic compounds, but the documentation is sketchy and the clinical characteristics poorly characterized. If such conditions developed, treatment would involve withdrawal of the antidepressant. In subjects with long-term treatment with neuroleptics, a concern that the anticholinergic properties of tricyclics could increase the risk of tardive dyskinesia or unveil latent dyskinesia has not been extensively studied. No pharmacogenetic studies are available for these types of movement disorders, though one study compared a group of patients who developed a variety of complications including dystonia, parkinsonism, chorea, and akathisia while on neuroleptics and/or antidepressants. They compared CYP2D6 polymorphisms in the movement disorder group with similarly exposed cases without movement disorders and found a more frequent representation of poor metabolizers in those with movement disorders. The multiple drugs of different mechanisms of action and the multiple movement disorders described makes this report difficult to interpret specifically in the context of tricyclic antidepressants (Vandel 2000).

4. SELECTIVE SEROTONERGIC RE-UPTAKE INHIBITORS (SSRIs)

4.1. Movement disorders

4.1.1. Clinical features

Whereas SSRIs are frequently associated with a vague sense of nervousness, akathisia has also been reported as a specific neurological syndrome of restlessness accompanied by volitional movements, usually, legs and trunk, that are performed by the subject in order to abate the restless feelings. Drug-induced parkinsonsonism or exacerbation of parkinsonism in patients with Parkinson's disease has been reported in small series of patients receiving SSRI treatment, but the frequency of such adverse effects has not been studied in detail and some reports found parkinsonian bradykinesia improved with SSRIs (No Authors Listed, Extrapyramidal effects of SSRI antidepressants 2001; Vand de Vijver 2002: Rampello 2002). Acute dystonic reactions when SSRIs are introduced or when the daily dose is increased have been rarely reported in association with SSRI treatment. Myoclonus can occur as well and develop in isolation or in the context of the "serotonin syndrome", characterized by high fever, myoclonus, rigidity, agitation, autonomic instability and a high risk of rhabdomyolysis and death

(Charbone 2000). In a review of 127 published reports of SSRI-associated movement disorders, Gerber and Lynd documented 30 cases of akathisia, 25 of parkinsonism, and 19 of dystonia (Gerber 1998). Additionally, 12 cases, termed dyskinesia, six cases of tardive dyskinesia and 15 with "mixed" movement disorders were cataloged, although their characterization was less clear. These investigators also emphasized the possibility of bruxism developing in the context of SSRI-treatment, although whether this complication represented a form of dystonia is difficult to ascertain from the clinical descriptions.

4.1.2. Biological basis and treatment

SSRI-related movement disorders are felt to relate either to enhanced serotonergic activity (myoclonus) or to indirect effects on other amine systems, specifically dopaminergic. Electrophysiological and biochemical evidence suggest that serotoneric neurons in the raphe nucleus inhibit dopaminergic neurons in the substantia nigra (Dray 1976). Furthermore, large doses of fluoxetine inhibit dopaminergic synthesis (Baldessarini 1990). These effects could underlie aggravated parkinsonism, acute dystonia, and akathisia, conditions usually associated with hypodopaminergic states. To treat all movement disorders induced by SSRI's lower doses or discontinuation will resolve the clinical problem.

4.1.3. Pharmacogenomics

As with other drugs, the main pharmacogenetic focus has been the P450 metabolic pathways and genetic determinants of rapid and slow drug metabolizers. None of the SSRI-associated extrapyramidal side effects has yet to be associated with specific genotoypes (Vandel 1996). Because SSRIs inhibit cytochrome P450, blood levels of drugs that are substrates for this enzyme system would be expected to rise during SSRI treatment. Such drugs include both tricyclic antidepressants and monoamine oxidase inhibitors. A specific search for allelic patterns related to CYP2D6 would be reasonable. Because of the clinical reports that Parkinson's disease can be exacerbated by SSRI's in some patients (No Authors Listed, Extrapyramidal effects of SSRI antidepressants 2001; Van de Vijver 2002), focused genetic studies on biomarkers currently identified to be associated with some forms of genetic Parkinson's disease would be reasonable to test in SSRI-sensitive subjects. No specific studies have examined serotonergic or dopaminergic receptor polymorphisms among subjects with SSRI-related movement disorders.

5. LITHIUM CARBONATE

5.1. Movement disorders

5.1.1. Clinical features

Two specific movement disorders of clinical importance are associated with lithium treatment, a fine postural tremor seen when patients have normal therapeutic

drug levels and a course irregular tremor, often associated with myoclonus that is seen with toxic levels of the drug (Gershanik 1993). In the former case, the tremor is small amplitude with the characteristics of enhanced physiological tremor, occurring with the hands outstretched or when the subjects tries to stabilize the extremity during eating or writing. Typically patients spill soup when bringing the hand to the lips or tremble as they write. When plasma levels of lithium increase beyond the therapeutic range, the tremor becomes large amplitude and disruptive of all coordinated activities. Often, superimposed lightning-like myoclonic jerks cause abrupt movements and can cause sudden spilling of food or even dropping of objects. Dyssenergia and ataxia with poor coordination and stumbling gait can also occur, and these movement disorders may be accompanied by loss of consciousness and seizures (Kompoliti 2003).

Lithium treatment has been occasionally reported to exacerbate Parkinson's disease, though the validity of this claim is suspect in several instances (Lecamwasam 1994). Most reports of this effect document enhanced tremor, but parkinsonian tremor is generally a rest-predominant tremor and distinct from the postural tremor associated with lithium. Because some Parkinson's disease patients have postural tremor as part of their clinical disorder, the lithium tremor should not be considered in itself an exacerbation of parkinsonism. Convincing reports of actual changes in bradykinesia, as distinct from ataxia, enhanced rigidity, and postural reflex impairment increases outside of the context of ataxia are not well-established.

5.1.2. Biological basis and treatment.

The neurochemical basis of tremorgenic effects due to lithium carbonate is not completely understood. Most agents that cause or exacerbate postural tremors predominantly affect the central noradrenergic system, and it has been largely presumed that effects on the cerebellar outflow system or the motor integration paths leading to the ventrolateral and ventroanterior thalamic areas are responsible for lithium-induced tremors (Kompoliti 2003). Whereas the two tremor syndromes are clinically distinct, there is likely a common mechanism for both, the latter syndrome representing a more severe noradrenergic disruption than the former. Because lithium carbonate is a simple salt compound, ion channels are likely the subcellular site of action, and more than one neurotransmitter system may be involved. Treatment involves cessation of the drug or lowering of daily dose. Drugs typically used to abate postural tremor, like β-noradrenergic antagonists, can provoke depression and must be used with caution. Primidone and the antidepressant, mirtazapine, also can abate postural tremor.

5.1.3. Pharmacogenomics

No studies have examined genotypes of subjects receiving lithium therapy to determine risk factors for postural tremor or the toxic tremor/ataxia syndrome. Because patients who need lithium therapy are most commonly afflicted with bipolar affective disorder, they represent significant clinical challenges for drug compliance and dosage management. If subjects at risk could be identified,

enhanced vigilance and more frequent monitoring could be appropriately incorporated into their clinical care. Because lithium treatment regularly involves patients providing blood samples for medication monitoring, these same samples, if accompanied with clinical data on the presence or absence of tremor, could be easily used to develop a large bank of material for pharmacogenetic studies.

6. FUTURE PERSPECTIVES

The traditional clinical separation between neurology and psychiatry has impeded progress in the study of drug-related movement disorders. Multispeciality groups of psychiatrists, neurologists, pharmacologists and molecular biologists are well-positioned to develop programs that break down this traditional separation and will potentially address pivotal scientific questions of clinical import. These questions include: which subjects are likely to develop movement disorders when exposed to various medications to treat underlying psychiatric diseases?; is the risk of specific movement disorders related to certain genotypes?; is the risk related to or independent of the genetic risk factors for the underlying psychiatric illness?; is the risk of one type of drug-induced movement disorder more genetically linked than others?; is the risk of drug-induced movement disorders linked to the genetic markers of the same phenomenology seen in conditions unrelated to drug exposures (e.g., is drug-induced parkinsonism related to genetic risk factors for Parkinson's disease)?

The goal of such research is the identification of subjects likely to develop drug-induced movement disorders prior to their exposure and thereby avoid these disorders as common adverse effects of therapy. In concert with similar research efforts to identify the factors that accurately predict efficacy of psychotropic treatments, clinicians and patients may look forward to the selection of a single best treatment that is both safe and rationally chosen to have maximal benefit. This goal remains unrealized at the present time, but the research outlined in this chapter demonstrates the concerted focus on moving from conceptual frameworks to practical application. Because very large sample sizes are needed for this area of research, enhanced government, foundation, and pharmaceutical sponsorship as well as concerted collaborations between large teams of clinical and laboratory scientists will be essential to the achieve the needed advances in pharmacogenetics.

7. REFERENCES

Andreassen, O.A., MacEwan, T., and Gulbrandsen, A.K., 1997, Non-function CYP2D6 alleles and risk for neuroleptic-induced movement disorders in schizophrenic patients, Psychopharmacology (Berlin) **131**, 174-179.

Armstrong, M., Daly, A.K., and Blennerhassett, R., 1997, Antipsychotic drug-induced movement disorders in schizophrenics in relation to CYP2D6 genotype, Br. J. Psychiatry **170**, 23-26.

Arthur, H., Dahl, M.L., Siwers, B., et al., 1995, Polymorphic drug metabolism in schizophrenic patients with tardive dyskinesia, J. Clin. Psychopharmacol. **15**, 211-216.

Auchus A.P., and Pickel, V.M., 1992, Quantitative light microscopic demonstration of increased pallidal and striatal met5-enkaphalin-like immunoreactivity in rats following chronic treatment with haloperidol but not with clozapine, Exp. Neurol. **117**, 17-27.

Baldessarini, R.J., and Marsh, E., 1990, Fluoxetine and side effects, Arch. Gen. Psychiatry **47**, 191-192.
Barton, A., Bowie, J., and Ebmeier, K., 1990, Low plasma iron status and akathisia, J. Neurol. Neurosurg. Psychiatry **53**, 671-674.
Basile, V.S., Masellis, M., and Badri, F., 1999, Association of the MscI polymorphisms of type dopamine D3 receptor gene with tardive dyskinesias in schizophrenia, Neurospsycho-pharmacology **21**, 17-21.
Bertilsson, L., Dahl, M.L., and Tybring, G., 1997, Pharmacogenetics of antidepressants: clinical aspects, Acta Psychiatr. Scand. **391**, 14-21.
Bester, A.M., and Harvey, B.H., 2000, Early suppression of striatal cyclic GMP may predetermine the induction and severity of chronic haloperidol-induced vacuous chewing movements, Metab. Brain Dis. **15**, 275-285.
Burke, R.E., Fahn, S., and Jankovic, J., 1982, Tardive dystonia: late-onset and persistent dystonia caused by anti-psychotic drugs, Neurology **32**, 1335-1346.
Byne, W., White, L., and Parella, M., 1998, Tardive dyskinesias in a chronically institutionalized population of elderly schizophrenic patients, Int. J. Geriatr. Psychiatry **13**, 473-479.
Charbone, J.R., 2000, The neuroleptic malignant and serotonin syndromes, Emerg. Med. Clin. North Am. **18**, 317-325.
Crane, G.E., and Naranjo, E.R., 1971, Motor disorders induced by neuroleptics, Arch. Gen. Psychiatry **24**, 179-184.
Dahl, M.L., 2002, Cytochrome P450 phenotyping/genotyping in patients receiving antipsychotics, Clin. Pharmacokinet. **41**, 453-470.
DeStefano, A.L., Lew, M.R., and Golbe, L.I., 2002, PARK3 influences age at onset of Parkinson's disease, Am. J. Hum. Genet. **70**, 1089-1095.
Dray, A., Gonyee, T.J., and Oakley, N.R., 1976, Evidence for the existence of a raphe projection to the substantia nigra in rat, Brain Res. **113**, 45-57.
Duncan, E.J., Adler, L.A., and Stephanides, M., 2000, Akathisia and exacerbation of psychopathology, Clin. Neuropharm. **23**, 169-173.
Eichhammer, P., Albus, M., and Borrmann-Hassenbach, M., 2000, Association of dopamine D3-recetpor gene variants with neuroleptic induced akathisia in schizophrenic patients, Am. J. Med. Gene **96**, 187-191.
Erikson, K.M., Jones, B.C., and Hess, E.J., 2001, Iron deficiency decreases dopamine D1 and D2 receptors in rat brain, Pharmacol. Biochem. Behav. **69**, 409-418.
Garcia-Barcelo, M.M., Lam, L.C., and Ungvari, G.S., 2001, Dopamine D3 receptor gene and tardive dyskinesia in Chinese schizophrenic patients, J. Neural Transm. **108**, 671-677.
Gerber, P.E., and Lynd, L.D., 1998, Selective serotonin-reuptake inhibitor-induced movement disorders, Ann. Pharmacother. **32**, 692-698.
Gershanik, O., 1993, Drug-induced movement disorders, *Curr. Opin. Neurol. Neurosurg.* **6**, 369-376.
Gershanik, O.S., in: *Parkinson's Disease and Movement Disorders, 4th Edition*, edited by J. Jankovic and E. Tolosa (Lippincott, Williams and Wilkins, Philadelphia, 2002), pp. 365-379.
Glazer, W.M., Morgenstern, H., and Doucette, J.T., 1993, Predicting the long-term risk of tardive dyskinesias in outpatients maintained on neuroleptic medications, J. Clin. Psychiatry **54**, 4-6.
Glennon, R.A., and Dukat, M., in: *Psychopharmacology: The Fourth Generation of Progress*, edited by F.E. Bloom and D.J. Kupfer (Raven Press, New York, 1995).
Goetz, C.G., 1983, Drug-induced parkinsonism and idiopathic Parkinson's disease, Arch. Neurol. **40**, 325-326.
Goetz, C.G., Kompoliti, K., and Washburn, K., in: *Clinical Neurology*, edited by R.J. Joynt (Lippincott-Raven, Philadelphia, 2001), **20**, 1-112.
Goudreau, J.L., Maraganore, D.M., and Farrer, M.J., 2002, Case-control study of dopamine transporter-1, monoamine oxidase-B, and catechol-O-methyltransferase polymorphisms in Parkinson's disease, Mov. Disord. **17**m 1305-1311.
Hirose, S., 2000, Restlessness of respiration as a manifestation of akathisia, J. Clin. Psychiatry **61**, 737-741.
Hirsch, S.R., Kissling, W., and Bauml, J., 2002, A 28-week comparison of ziprasidone and haloperidol in outpatients with stable schizophrenia, J. Clin. Psychiatry **63**, 516-523.
Hofmann, M., Seifritz, E., and Botschev, C., 2000, Serum iron and ferritin in acute neuroleptic akathisia, Psychiatry Res. **93**, 201-207.

Hori, H., Ohmori, O., and Shinkai, T., 2000, Manganese superoxide dismutase gene polymorphism and schizophrenia: relation to tardive dyskinesia, Neuropsychopharmacology **23**, 170-177.

Jankovic, J., in: *Textbook of Clinical Neurology, 2nd Edition,* edited by C.G. Goetz (W.B. Saunders, Philadelphia, 2003), pp. 713-740.

Joseph, A.B., and Young, R.R., *Movement Disorders in Neurology and Neuropsychiatry* (Blackwell Science, Oxford UK, 1999).

Kaisir, R., Tremblay, P.B., and Klufmoller, F., 2002, Relationship between adverse effects of antipsychotic treatment and dopamine D2 receptor polymorphisms in patients with schizophrenia, Mol. Psychiatry **7**, 695-705.

Khanna, R., Das, A., and Damodaran, S.S., 1992, Prospective study of neuroleptic-induced dystonia in mania and schizophrenia, Am. J. Psychiatry **149**, 511-513.

Klawans, H.L., Nausieda, P.A., and Goetz, C.G, 1982, Tourette-like symptoms following chronic neuroleptic therapy, Adv. Neurol. **35**, 415-418.

Klawans, H.L., *The Pharmacology of Extrapyramidal Movement Disorders* (S. Karger, Basal, 1973).

Kompoliti, K., and Horn, S.S., in: *Textbook of Clinical Neurology, 2nd Edition,* edited by C.G. Goetz (W.B. Saunders, Philadelphia, 2003), pp. 1225-1254.

Koshino, Y., Madokoro, S., and Ito, T., 1992, A survey of tardive dyskinesias in psychiatric inpatients in Japan, Clin. Neuropharm. **15**, 34-43.

Koshino, Y., Wada, Y., and Isaki, K., 1991, A long-term outcome study of tardive dyskinesia in patients on antipsychotic medication, Clin. Neuropharm. **14**, 537-546.

Kruger, R., Menezes-Saecker, A.M., and Schols, L., 2000, Genetic analysis of the alpha2-macroblobulin gene in early- and late-onset Parkinson's disease, Neuroreport **11**, 2439-2442.

Kuloglu, M., Atmaca, M., and Ustundag, B., 2003, Serum iron levels in schizophrenic patients with or without akathisia, Eur. Neuropsycholpharmacol. **13**, 67-71.

Lane, H.Y., Hu, O.Y.P., Jann, M.W., et al., 1997, Dextromethorphan phenotyping and haloperidol disposition in schizophrenic patients, Psychiatry Res. **69**, 105-111.

Lecamwasam, D., Synek, B., Moyles, K., and Ghose, K., 1994, Chronic lithium neurotoxicity presenting as Parkinson's disease, Int. Clin. Psychopharmacol. **9**, 127-129.

Lerer, B., and Macciardi, F., 2002, Pharmacogenetics of antidepressant and mood-stabilizing drugs: a review of candidate-gene studies and future research directions, Int. J. Neuropsychopharmacol. **5**, 255-275.

Lerner, V., Miodownik, C., and Kaptsan, A., 2001, Vitamin B6 in the treatment of tardive dyskinesias, Am. J. Psychiatry **158**, 1511-1514.

Magnuson, T.M., Roccaforte, W.H., and Wengel, S.P., 1999, Medication-induced dystonias in nine patients with dementia, J. Neuropsychopharmacol. **19**, 219-225.

Matsumoto, R.R., and Pouw, B., 2000, Correlation between neuroleptic binding to sigma 1 and sigma 2 receptors and acute dystonic reactions, Eur. J. Pharmacol. **401**, 155-160.

McCann, U.D., Penetar, D.M., and Belenky, G., 1990, Acute dystonic reaction in normal humans caused by catecholamine depletion, Clin. Neuropharm. **13**, 565-568.

Meltzer, H.Y., *Psychopharmacology: The Third Generation of Progress* (Raven Press, New York, 1987).

Mihara, K., Kondo, T., and Higuchi, H., 2002, Tardive dystonia and genetic polymorphisms of cytochrome P4502D6 and dopamine D2 and D3 receptors: preliminary findings, Am. J. Med. Genet. **114**, 693-695.

Mitchell, I.J., Crossman, A.R., and Limigna, U., 1992, Regional changes in 2-deoxyglucose uptake associated with neuroleptic-induced tardive dyskinesias in the Cebus monkey, Mov. Disord. **1**, 32-37.

Mizuta, I., Mizuta, E., and Yamasaki, S., 2000, Meta-analysis of polymorphism of the catechol-O-methyltransferase gene in relation to the etiology of Parkinson's disease in Japan, Mov. Disord. **15**, 1013-1014.

Muscettola, G., Pampallona, S., and Barbato, G., 1993, Persistent tardive dyskinesias: demographic and pharmacological risk factors, Acta Psychiatr. Scand. **87**, 1-14.

Naidu P.S., and Kulkarni, S.K., 2001, Excitatory mechanisms in neuroleptic-induced vacuous chewing movements, Behav. Pharmacol. **12**, 209-216.

Nair, C.J., Josiassen, R.C., and Abraham, G., 1999, Does akathisia influence psychopathology in psychotic patients treated with clozapine? Biol. Psychiatry **45**, 1376-1383.

No Authors Listed, Extrapyramidal effects of SSRI antidepressants, Prescrire Int. **10**, 118-119 (2001).

Ozdemir, V.V., Basile, E., and Masellis, M., 2001, Pharmacogenetic assessment of antipsychotic-induced movement disorders: contribution of the dopamine D3 receptor and cytochrome P450 1A2 genes, J. Biochem. Biophys. Meth. **30**, 151-157.

Pollock, B.G., Mulsant, B.H., Sweet, R.A., et al., 1995, Prospective cytochrome P450 phenotyping for neuroleptic treatment in dementia. Pscyhopharmacol. Bull. **31**, 327-331.

Poyurovsky, M., Shardorosdsky, M., and Fuchs, C., 1999, Treatment of neuroleptic-induced akathisia with the 5-HT2 antagonist mianserin: double blind, placebo controlled study, Br. J. Psychiatry **174**, 238-242.

Rampello, L., Chiechio, S., Raffaele, R., Vecchio, I., and Nicoletti, F., 2002, The SSRI, citalopram, improves bradykinesia in patients with Parkinson's disease treated with L-dopa, Clin. Neuropharmacol. **25**, 21-24.

Reitschel, M., Krauss, H., and Muller, D.J., 2000, Dopamine D3 receptor variant and tardive dyskinesias, Eur. Arch. Psychiatry Clin. Neurosci. **250**, 31-35.

Rosebush, P.I., and Mazurek, M.F., 1999, Neurologic side effects in neuroleptic-naïve patients treated with haloperidol or risperidone, Neurology **52**, 782-785.

Segman, R., Neeman, T., and Heresco-Levy, U., 1999, Genotypic association between the dopamine D3 receptor and tardive dyskinesias in chronic schizophrenia, Mol. Psychaitry **4**, 247-253.

Segman, R.H., Heresco-Levy, U., and Yakir, A., 2002, Interactive effect of cytochrome P450 17a-hydroxylase and dopamine D3 receptor gene polymorphisms on abnormal involuntary movements in chronic schizophrenia, Biol. Psychiatry **51**, 261-263.

Segman, R.H., Heresco-Levy, U., and Finkel, B., 2000, Association between the serotonin 2C receptor gene and tardive dyskinesia in chronic schizophrenia, Psychopharmacology **152**, 408-413.

Segman, R.H., Heresco-Levy, U., and Finkel, B., 2001, Association between the serotonin 2A receptor gene and tardive dyskinesia in chronic schizophrenia, Mol. Psychiatry **6**, 225-229.

Spigset, O., Hedenmalm, K., Dahl, M.L., and Wiholm, B.E., 1997, Seizures and myoclonus associated with antidepressant treatment, Acta Psychiatr. Scand. **96**, 379-384.

Spina, E., Ancione, M., and Di Rosa, A.E., 1992, Polymorphic debrisoquine oxidation and acute neuroleptic-induced adverse effects, Eur. J. Clin. Pharmacol. **42**, 347-348.

Spina, E., Sturiale, V., and Valvo, S., 1992, Debrisoquine oxidation phenotype and neuroleptic induced dystonic reactions, Acta Psychiatr. Scand. **42**, 347-348.

Stacy, M., and Jankovic, J., 1992, Tardive tremor, Mov. Disord. **7**, 53-57.

Steen, V.M., Lovlie, R., and MacEvan, T., 1997, Dopamine D3-receptor gene variant and susceptibility to tardive dyskinesias in schizophrenia, Mol. Psychiatry **2**, 139-145.

Sunami, M., Nishikawa, T., and Yorogi, A., 2000, Intravenous administration of levodopa ameliorated a refractory akathisia: case induced by interferon-alpha, Clin. Neuropharm. **23**, 59-61.

Sweet, R.A., Mulsant, B.H., and Rifai, A. H., 1992, Relation of age to prevalence of tardive dyskinesias, Am. J. Psychiatry **149**, 141-145.

Tominaga, H., Fukuzako, H., and Izumi, K., 1987, Tardive myoclonus, Lancet, **1**, 322.

Van de Vijver, D.A., Roos, R.A., Jansen, P.A., Porsius, A.J., and de Boer, A., 2002, Start of a selective serotonin reuptake inhibitor (SSRI) and increase of antiparkinsonian drug treatment in patients on levodopa, Br. J. Clin. Pharmacol. **54**, 168-170.

Van Harten, P.N., Hoek, H.W., and Matroos, G.E., 1998, Intermittent neuroleptic treatment and risk for tardive dyskinesias, Am. J. Psychiatry **155**, 565-567.

Van Kampen J.M., and Stoessl, A.J., 2000, Dopamine D1A receptor function in a rodent model of tardive dyskinesias, Neuroscience **101** 629-636.

Vandel, P., Bonin, B., Vandel, S., and Sechter, D., 1996, CYP2D6 PM phenotype hypothesis of antidepressant extrapyramidal side-effects, Med. Hypoth. **47**, 439-442.

Vandel, P., Haffen, E., Vandel, S., and Bonin, B., 1999, Drug extrapyramidal side effects: CYP2D6 genotypes and phenotypes, Eur. J. Clin. Pharmachol. **55**, 659-665.

Vandel, P., Haffen, E., Vandel, S., Bonin, B., and Sechter, D., 2000, Drug extrapyramidal side-effects or not: is there a dextromethorphan phenotype difference? Thérapie **55**, 349-353.

West, A.B., Maraganore, D., and Crook, J., 2002, Functional association of the parkin gene promoter with idiopathic Parkinson's disease, Hum. Mol. Genet. **11**, 2787-2792.

19. CARDIAC SIDE EFFECTS OF PSYCHOTROPIC MEDICATION:

Focus on QTc Prolongation and Toriade de Pantes

Cherry Lewin and Peter M. Haddad*

1. INTRODUCTION

A wide range of cardiac side effects can occur with psychiatric drugs with antidepressant and antipsychotic drugs being particularly implicated (table 1). In this chapter we focus on drug-induced QTc prolongation and torsade de pointes (TdP). QTc prolongation is a marker for the occurrence of TdP, a polymorphic ventricular arrhythmia. There are several reasons for this focus:

- TdP is a serious side effect. It is associated with a range of clinical manifestations including palpitations, syncope and sudden death. The overall mortality of TdP is between 10 and 17% (Salle et al 1985; Fung et al 2000).
- QTc prolongation and TdP occur with many drugs and so the phenomenon has relevance to a wide range of patients as well as to clinicians practising in different specialities.
- Genetic factors are relevant to drug-induced QTc prolongation and TdP.

With regard to the last point, several cytochrome enzymes that metabolise QTc prolonging drugs display genetic polymorphisms. These can make individuals more susceptible to develop QT prolongation/TdP particularly when drugs are co-prescribed with the potential for pharmacokinetic interactions. In addition, a small minority of patients with drug induced QTc prolongation have a genetic predisposition in the form of a mutation in one of the genes that code for potassium channels involved in cardiac repolarisation. However this must be put into context, as at present the majority of patients with drug induced QTc prolongation do not have an indentifiable genetic vulnerability.

* Peter M. Haddad, Cromwell House Community Mental Health Centre, Bolton, Salford & Trafford Mental Health NHS Trust, Manchester, M30 OGT, UK
Cherry Lewin, Cromwell House Community Mental Health Centre, Bolton, Salford & Trafford Mental Health NHS Trust, Manchester, M30 OGT, UK

Table 1. Examples of cardiac side effects caused by psychiatric drugs

Cardiac side effect	Examples of causal drug	Mechanism
Postural hypotension	• Dosulepin (tricyclic antidepressant) • Chlorpromazine (conventional antipsychotic) • Quetiapine (atypical antipsychotic)	Blockage of adrenergic (alpha-1) receptors
Hypertension	• Venlafaxine (antidepressant: serotonin and noradrenalin reuptake blocker, SNRI)	Noradrenergic stimulation
QTc prolongation and/or TdP	• Thioridazine (conventional antipsychotic) • Ziprasidone (atypical antipsychotic) • Lithium (mood stabiliser)	Blockade of cardiac potassium channels
Cardiomyopathy	• Clozapine (atypical antipsychotic)	Hypersensitivity?
Myocarditis	• Clozapine (atypical antipsychotic)	Hypersensitivity?
Tachycardia	• Clozapine (atypical antipsychotic) • Dosulepin (tricyclic antidepressant)	Antimuscarinic blockade

2. QTc PROLONGATION

The QT interval on the electrocardiogram (ECG) is the time from the onset of ventricular depolarisation to completion of repolarisation, that is it reflects the duration of individual action potentials in the cardiac myocytes. The QT interval shortens with increasing heart rate and correcting for this variation gives the QTc or rate-corrected value. There are various formulae that can be used to calculate this correction and opinion is divided as to which is the most accurate.

Bazett's correction (QTc = QT/RR ½) is the most commonly used, but has the disadvantage of tending to overestimate the QTc at higher heart rates, and conversely to underestimate the QTc when applied to drugs (e.g. beta-blockers) which cause slowing of the heart rate. The Fridericia formula (QTc = QT/RR

1/3) is less sensitive to this effect. Over or under correction is a disadvantage of all universal correction formulae when the heart rate extends beyond a narrow physiological range. The most reliable method of correction is to devise an individual formula for each individual, but this is expensive and requires large numbers of ECGs from each person. There is no evidence that any particular method is a better predictor of sudden death (de Bruyne et al.1999).

The QT interval varies with gender (females have longer QT intervals) (Stramba-Badiale et al.1997) and time of day (it increases during sleep). It increases after a meal (Nagy et al.1997). Obesity has been associated with QTc prolongation (Carella et al.1996). The QTc interval also varies according to the positioning of ECG leads (Higham and Campbell1994) as it is subject to variation within the myocardium, a phenomenon known as QT dispersion.

In healthy un-medicated subjects several studies have linked QTc prolongation to excess cardiovascular mortality (e.g. Schoeten et al 1991, Algra et al 1991) though other studies have failed to demonstrate such a link (Goldberg et al 1991). The marked variability between individuals makes it difficult to strictly define normal from abnormal QTc values. In 1997 the Committee for Proprietary Medicinal Products (CPMP) published a report on the assessment of QT interval prolongation by non-cardiovascular drugs. This used Bazett's correction to suggest normal, borderline and prolonged values for both males and females (table 2). It is important to emphasise that there is no direct relationship between QTc interval and arrhythmic risk; raised QTc is simply a marker for increased risk.

Table 2. Normal, borderline and prolonged QTc values (Bazett corrected; in the absence of any drug or disease) suggested by the Committee For Proprietary Medicinal Products (1997)

	Normal msec	**Borderline msec**	**Prolonged msec**
Males	<430	431 - 450	>450
Females	<450	451 - 470	>470

3. TORSADE DE POINTES

First described in the mid1960s, Torsade de Pointes, literally 'twisting of the points', is a polymorphic ventricular arrythmia that can progress to ventricular fibrillation and sudden death. The 'twisting' refers to the movement of QRS complexes around the isoelectric line of the ECG trace. Any delay in completing the repolarisation phase appears to allow the development of spontaneous depolarisations of non-pacemaker myocytes, so-called early after-de-polarisations. The occurrence of early after-depolarisations in combination with increased heterogeneity of cardiac repolarisation appears to act as a trigger for TdP. The relationship between increasing QT interval and risk of arrythmia

however is not linear, and some drugs (e.g. Amiodarone) cause QTc prolongation but rarely cause TdP.

TdP can be asymptomatic or present with a variety of symptoms including palpitations. When TdP is prolonged symptoms arise from impaired cerebral circulation and include dizziness, syncope and seizures. In about 20% of cases TdP progresses to ventricular fibrillation and often sudden death will result (Salle et al 1985). The overall mortality of TdP is between 10 and 17% (Salle et al 1985; Fung et al 2000).

4. DRUGS ASSOCIATED WITH QTc PROLONGATION AND TdP

When first described TdP was regarded as a side effect of cardiac drugs alone, or as being due to congenital long QT syndrome (see next section). It is now recognised that TdP can occur with a wide range of drugs including psycho-tropic and non-psychotropic drugs (Table 3). Among psychiatric drugs most attention has focused on antipsychotics, though other drugs can cause QTc prolongation including tricyclic antidepressants (TCAs), lithium and chloral hydrate (Haverkamp et al 2000). Selective serotonin reuptake inhibitors (SSRIs) have been studied and although occasional cases of QTc prolongation have been recorded they are generally felt to be less arrhythmogenic than the TCAs, even in overdose. A case TdP was recorded in a patient on citalopram (Meuleman et al 2001).

In 1988 prenylamine, an antianginal drug, became the first drug to be withdrawn from the market because of concerns about is ability to cause QTc prolongation and TdP. Since that time at least 8 other drugs have been withdrawn from various markets for this reason. These include terodiline, terfenadine, astemizole, grepafloxacin, cisapride, levacetylmethadol and two antipsychotics, sertindole and droperidol. Identical concerns have led to prescribing restriction being introduced for many drugs including the antipsychotics pimozide and thioridazine. Prior to licensing, various drugs have had their development abandoned due to QTc concerns. Assessment of the proarrhythmic risk of drugs is now an important part of drug development and regulatory approval.

Table 3. Examples of drugs associated with QTc prolongation and /or TdP (data from Haverkamp et al (2000) and FDA (2000): reproduced in modified form from Haddad and Anderson (2002) with permission)

Antiarrhythmic Drugs	Class 1a
	Disopyramide
	Procainamide
	Quinidine
	Class 3
	Amiodarone
	Bretylium
	Dofetlidine
	Sotalol
Antihistamines	Astemizole
	Terfenadine (withdrawn)
Antimicrobials and Antimalarials	Fluoroquinolone antibiotics
	Grepafloxacin (withdrawn)
	Levofloxacin
	Sparfloxacine
	Macrolide antibiotics
	Clarithromycin
	Erythromycin
	Imidazole antifungals
	Ketoconazole
	Antimalarials
	Chloroquine
	Halofantrine
	Quinine
	Miscellaneous antimicrobials
	Co-trimoxazole
	Spiramycine
	Pentamidine
Calcium Channel Bockers	Prenylamine (withdrawn)
	Terodiline (withdrawn)
Miscellaneous Non-Psychotropics	Cisapride (withdrawn)
	Probucol
Tricyclic and Related Antidepressants	Amitriptyline
	Clomipramine
	Desipramine

	Doxepin Imipramine Maprotiline Nortriptyline
Typical Antipsychotics	Chlorpromazine Droperidol (withdrawn) Fluphenazine Haloperidol Mesoridazine Pimozide Sulpiride Thioridazine Trifluoperazine
Atypical Antipsychotics	Sertindole (withdrawn) Ziprasidone
Miscellaneous Psychotropics	Lithium Chloral hydrate

NB: This list is not comprehensive and is given to indicate the diversity of drugs involved. The drugs listed differ with regard to their effect on QT prolongation. Some have marked effects while others have minor effects.

In 1997 the Committee for Proprietary Medicinal Products (CPMP) provided guidance on signal values for QTc measurements (after Bazett's correction) that suggest a risk of drug-induced TdP. Two changes were observed as significant in raising the potential risk:

- An individual change in QTc of > 60 msec relative to drug-free baseline measurements
- An absolute QTc value (or at a lower heart rate an uncorrected QT value) > 500msec while prescribed the drug.

A further two were highlighted as raising 'concern'. These referred to the QTc dispersion across a 12 lead ECG (dispersion being the heterogenous as opposed to homogenous prolongation of QT across the myocardium):

- Dispersion > 100msec
- Change in dispersion of >100%.

5. MECHANISM OF DRUG-INDUCED QTc PROLONGATION

As mentioned previously, the QT interval is an ECG measure that includes both depolarisation and repolarisation of cardiac myocytes. It begins with the onset of ventricular depolarisation (Q wave) and ends with completion of

repolarisation (T wave). The trans-membrane movement of various ion currents in cardiac cells, including sodium, calcium and potassium, generates the cardiac action potential. Depolarisation of ventricular cells is primarily the result of a rapid influx of sodium ions through selective sodium channels, and its duration is measured by the QRS interval. The repolarisation phase involves an outward flux of calcium, sodium and potassium ions from the myocardial cells, though of these it is potassium that is the predominant outward current. The delayed rectifier potassium current (Ik) is the sum of 2 different potassium currents, a rapid activating (Ikr) and a slow-activating current (Iks). Drug-induced blockade of the Ikr channel hinders the outward movement of potassium and is the key mechanism by which drugs cause QTc prolongation and TdP. The ion channel associated with Ikr is coded for by the HERG gene (human ether-a-go-go related gene) (De Ponti et al 2000).

The differential effect of drugs on the QTc interval partly reflects their differential blockade of the Ikr channel. This can be investigated during drug development using cloned channels in vitro tissue preparations as well as in vivo animal models. Other factors influence the arrhythmic potential of drugs. For example drugs that cause homogeneous QT prolongation across the myocardium may be associated with less risk that those that produce heterogeneous prolongation i.e. those that increase QT dispersion (Hii et al 1992). Drugs that are associated with bradycardia may be more likely to cause TdP while the risk may be lower with those associated with tachycardia. At any degree QTc interval other properties of a drug (e.g. degree of blockade at alpha and beta adrenoceptors and calcium channel blocking activity) can modify the risk of TdP.

Several studies have shown that prolonged QTc is more prevalent within the psychiatric patient population. For example, Warner et al (1996) found prolongation in 23% of inpatients with chronic schizophrenia compared with 2% of drug-free and age-matched controls, and it seems likely that the prescription of antipsychotics is implicated in this. Sudden death and even non-fatal arrhythmias remain however extremely rare, and it appears that other risk factors must play a part in precipitating arrhythmias within an individual with QTc prolongation. There are several methods of classifying these other risk factors. Perhaps the most simplistic is to divide them into pharmacological risk factors and non-pharmacological risk factors. An alternative system, and the one adopted in this chapter, is to divide risk factors for drug-induced TdP into genetic and non-genetic factors.

Genetically determined factors include gender, the presence of congenital long QT syndrome, and mutations affecting cardiac ion channels and cytochrome P450 enzymes that determine the pharmacokinetics of drugs associated with QTc prolongation. Non-genetic factors include cardiac disease, renal impairment, hepatic impairment, drug dose, pharmacodynamic and kinetic drug interactions, and alcohol and substance misuse. Genetic and non-genetic risk factors are discussed further in the following two sections.

6. GENETIC RISK FACTORS

6.1. Congenital long QT syndrome

Congenital long QT syndrome (LQTS) is a rare disorder with an estimated frequency of 1 in 5000 individuals in the Unites States (Website). It commonly presents in childhood often with syncopal episodes triggered by physiological or psychological stress. However initial presentation can be as serious as cardiac arrest and even death (Viskin 1991). Two specific syndromes have been identified: Romano-Ward (autosomal dominant) and Jervell and Lange-Nielsen, which is extremely rare and is also associated with congenital deafness. Patients with congenital LQTS are at greater risk of developing drug-induced LQTS.

In the last 10 years great strides have been made in understanding the genetic basis of congenital LQTS. On the basis of genetic linkage studies five key loci have been identified on chromosomes 3, 4, 7, 11 and 21 enabling congenital LQTS to be divided into 5 genetic subtypes (table 4). Each gene codes for a subunit of either a potassium or sodium channel. More than 200 mutations have been identified at these sites in LQTS patients with the KCNQ1 and HERG genes being those most commonly involved. However up to 50% of patients with LQTS have no identifiable mutation. This suggests that there are further undiscovered genes or mutations underlying this syndrome.

Some family members of patients with congenital LQTS have normal ECGs, but are known from genetic studies to carry mutations of LQTS genes. The frequency of these 'silent carriers' may be a high as 33% in the family members of patients with LQTS (Priori et al 1999). These patients can be regarded as representing cases of *forme frust* of the congenital LQTS.

Table 4. Subtypes of congenital LQTS

Subtype	Gene	Chromosome	Ion channel
Long QT-1	KCNQ1	11	I_{Ks} (alpha subunit)
Long QT-2	KCNH2 (HERG)	7	I_{Kr} (alpha subunit)
Long QT-3	SCN5A	3	I_{Na}(alpha subunit)
Long QT-4	ANK2	4	?
Long QT-5	KCNE1	21	I_{Ks} (beta subunit)
Long QT-7	KCNE2	21	I_{Kr} (beta subunit)

6.2. Mutations of cardiac ion channels

Indirect evidence of a genetic susceptibility to drug induced LQTS comes from several sources. A study of patients prescribed class III anti-arrhythmics

(Houltz et al 1998) found greater QT prolongation in those who went on to develop TdP than those who did not. This appeared unrelated to individual drug dosage and suggested that these individuals were responding differently to the drug. Another study showed that individuals who developed drug-induced TdP were more likely to experience a drug-induced arrhythmia when prescribed a different drug at a later time point, again suggesting an underlying susceptibility QTc prolonging drugs.

Recent research has indicated that a small proportion of patients with acquired LQTS have mutations that involve some of the genes (HERG, KCNQ1, KCNE1, KCNE2) that are mutated in congenital LQTS suggesting that acquired and congenital LQTS are related, at least in these patients (e.g. Napolitano et al 2000). These mutations reduce ion channel function or make the channels more sensitive to blockade by certain drugs. The genetic abnormality means that these patients have a reduced '*repolarisation reserve*' and will develop marked QT prolongation in the presence of environmental factors that prolong repolarisation (e.g. drugs that block potassium channels, hypokalaemia, bradycardia). Other patients with drug-induced LQTS have been identified with mutations in genes entirely unrelated to the congenital LQTS. However it must be emphasised that mutations, irrespective of site, have only been identified in a small proportion of patients with drug induced LQTS. In contrast most cases of drug-induced TdP appear to be caused by drug interactions.

6.3. Polymorphisms of the cytochrome system

Genetic polymorphisms refer to variant or mutant genes that exist at a frequency of more than 1% in the general population (Meyer 1991). The hepatic cytochrome P450 (CYP) system is involved in the metabolism of many drugs. Several CYP enzymes show polymorphisms with the result that individuals can differ markedly in their ability to metabolise drugs and in their susceptibility to pharmacokinetic interactions. Three cytochrome enzymes (CYP2C9, CYP2C19, CYP2D6) involved in the metabolism of QTc prolonging drugs show well characterised genetic polymorphisms. Of these CYP2D6 is the most studied and the most clinically relevant as it is responsible for the oxidative biotransformation of over 60 drugs including several drugs known to prolong the QT interval e.g. nortipytline, sertindole and thioridazine.

More than 70 variant alleles of the CYP2D6 locus on chromosome 22 have been described. These mutations can have no effect on enzyme activity or can code for enzymes with decreased or absent activity. Gene duplications can lead to increased enzyme activity. Three main population CYP2D6 phenotypes exist; ultra-rapid metabolisers, extensive metabolisers and poor metabolisers. Individuals carrying two decreased-activity or loss-of-function alleles are poor metabolisers, those with at least one normal functional capacity allele (wild-type gene) are extensive metabolisers, and patients with multiple copies of normal metabolic capacity alleles are ultra-rapid metabolisers. Between 7-10% of the Caucasian population are CYP2D6 poor metabolisers (Kroemer and Eichelbaum 1995) while in Japan the figure is less than 1% (Tateishi et al (1999)).

Poor metabolisers experience higher plasma levels of drugs metabolised by CYP2D6 than extensive or ultra-rapid metabolisers and so are more at risk of adverse effects of the parent drug. Conversely poor metabolisers experience lower levels of metabolites of the parent drug while extensive and ultra-rapid metabolisers are exposed to higher levels of metabolites. Consequently extensive metabolisers and ultra-rapid metabolisers are at greater risk of adverse effects of the metabolites but at lesser risk of adverse effects of the parent drug.

The occurrence of QTc prolongation and TdP with the conventional antipsychotic thioridazine illustrates the relevance of CYP2D6 polymorphisms. A range of evidence has accumulated to link thioridazine with QTc prolongation and TdP (see Haddad and Anderson 2003 for a review) and in 2000 both the Medicine Controls Agency in the UK and the Food and Drugs Administration in the USA took action to restrict the use of thioridazine. QTc prolongation with thioridazine is largely due to the parent compound, though metabolites do contribute, and is dose related. CYP2D6 poor metabolisers achieve a peak plasma concentration (Cmax) with thioridazine that is 2.4 times higher than that seen in extensive metabolisers (von Bahr et al 1991). Consequently, in theory at least, poor metabolisers are at higher risk of TdP with thioridazine though evidence that this is actually the case is contradictory (Llerena et al 2002; Thanoccody et al 2003). Irrespective of this in the UK the Summary of Product Characteristics (SPC) for thioridazine advises that the drug is contraindicated in those with decreased CYP2D6 activity and in those prescribed a substrate or inhibitor of CYP2D6.

6.4. Gender

Two thirds of cases of TdP occur in females (Makkar et al 1993), a difference that cannot be explained in terms differences in drug exposure, prescribed dosages or use of polypharmacy i.e. female gender is an independent risk factors for the occurrence of drug-induced TdP. One reason for this is that, on average, women have a longer QT interval than men (Merri et al 1989; Rautaharju 1992) by about 20 msec (Stramba-Badiale et al 1997). This confers a greater risk of drug-induced TdP that remains irrespective of the presence or absence of other risk factors. In addition women show more marked drug-induced QTc prolongation (Benton et al 2000). These differences appear to result from the regulation of cardiac ion channels by sex steroids (Drici et al 1996).

7. NON-GENETIC RISK FACTORS

7.1. Drug dosage

QTc prolongation is related to the plasma concentration of a drug. The most obvious way of increasing plasma concentration is to increase the prescribed dose and epidemiological studies show an effect between dose and QTc prolongation/ arrhythmia (Warner et al 1996; Reilly et al 2000; Ray et al 2001).

Overdose and renal and hepatic impairment all have effect of increasing plasma concentration of a drug and thereby increasing the risk of QTc prolongation and TdP.

7.2. Pharmacodynamic interactions

There can be an additive effect of prescribing two or more drugs each individually causing QTc prolongation (pharmacodynamic interactions). The wide range of drugs can cause QTc prolongation increase the potential for such interactions (table 3). With regard to psychiatric drugs, ventricular arrhythmias and a case of sudden death have been reported in patients co-prescribed imipramine and thioridazine (Heiman et al 1983; Swanson et al 1997). Sudden death has also been reported with the combination of imipramine and chlorpromazine (Swanson et al 1997).

Wherever possible one should try to avoid co-prescribing QTc prolonging drugs. In the UK, the Summary of Product Characteristics (SPC) for thioridazine contraindicates the prescription of drugs known to prolong the QTc interval, this includes tricyclic antidepressants, maprotiline, phenothiazines, sertindole and various non-psychotropic agents.

7.3. Pharmacokinetic interactions

Increased plasma concentration of a QT prolonging drug can be due to co-prescription of another drug that inhibits its metabolism by a CYP isoenzyme. The presence of such a drug has the effect of converting an extensive metaboliser to a poor metaboliser. Some drugs that are CYP enzyme inhibitors also cause delayed cardiac repolarisation in their own right, increasing the severity of the effect. For example imidazole and macrolide antibiotics inhibit CYP3A4 but also have a direct QTc-prolonging effect.

The clinical importance of pharmacokinetic interactions in causing TdP is highlighted by the experience with cisapride, a motility stimulant, and terfenadine, an antihistamine. The metabolism of both drugs is chiefly by CYP3A4. When prescribed alone cisapride and terfenadine cause minor QTc elevation. In contrast there is a marked rise in QTc values when either drug is co-prescribed with a potent CYP3A4 inhibitor, reflecting an increase in plasma cisapride or terfenadine levels respectively. Between 1993 and 1999 the Food and Drug Administration (FDA) received 341 reports of QT prolongation and ventricular arrhythmia (80 fatal) in association with cisapride (Wysowski et al 2001). 126 (37%) of these cases occurred in patients co-prescribed a drug known to inhibit CYP3A4 making pharmacokinetic interactions the largest single risk factor identified by the FDA. Co-prescription of a CYP3A4 inhibitor was also identified as a major risk factor for sudden deaths reported with terfenadine (Woosley et al 1993).

Pimozide is an antipsychotic associated with QTc prolongation. It is largely metabolised by CYP3A4. A recent case report (Flockhart et al 2000) detailed the sudden death of a 27-year-old male 2 days after he was prescribed pimozide and clarithromycin, a macrolide antibiotic that is a CYP3A4 inhibitor. The UK

Summary of Product Characteristics (SPC) for pimozide contraindicates the concomitant prescription of drugs known to inhibit CYP 3A4 or 2D6.[116] The UK SPC for thioridazine, metabolized by CYP2D6, contraindicates concomitant use of 2D6 inhibitors.

7.4. Electrolyte imbalance

Hypokalaemia increases the risk of TdP due to a reduction in Ikr. Hypocalcaemia and hypomagnesaemia also increase the risk. These electrolyte abnormalities can result from any number of physical disorders that cause vomiting and diarrhoea, and also may be secondary to malabsorption or dehydration. Self-induced vomiting in patients with anorexia nervosa can lead to hypokalaemia. In combination with female sex and (if prescribed) what amounts to higher than average doses of antipsychotic, compared to bodyweight, can confer an increased risk of arrhythmias in anorexia nervosa patients. Potassium losing diuretics are another important cause of hypokalaemia.

7.5. Cardiac disease

Several cardiac diseases cause increased susceptibility to drug-induced arrhythmias. These include ischaemic heart disease, myocardial infarction, myocarditis, cardiac failure and left ventricular hypertrophy and dysfunction. In addition any disorder resulting in bradycardia will by its very nature result in QT prolongation due to the extended repolarisation phase (e.g. heart block). Individuals with ECG abnormalities such as large U-waves and T-wave abnormalities are also at increased risk of drug-induced QT prolongation.

7.6. Impaired hepatic or renal function

These conditions may increase the plasma concentrations of a QT prolonging drug and therefore increase the pro-arrhythmic risk.

7.7. Other diseases

A number of other diseases/ disease states are associated with an increased risk of arrhythmia. These include hypothyroidism, diabetic autonomic neuropathy and infection with human immunodeficiency virus.

7.8. Restraint and stress

Sudden death does occur in the general population at times of great physical or emotional stress (Hillis et al 1994; Engel et al 1971; Malik et al 1973). It is thought that increased sympathetic activity may cause myocardial instability and lead to fatal arrhythmias. Consequently the psychiatric patient requiring restraint, whilst prescribed antipsychotics, associated with QT prolongation, may be particularly vulnerable to such an event. Case reports have described sudden deaths in such circumstances (Jusic and Lader 1994; Lareya 1995) though

epidemiological studies have not identified such cases suggesting that the mechanism is rare (Reilly et al 2002).

7.9. Substance misuse

Some patients with alcohol induced liver disease have QT prolongation and this has been shown to be associated with sudden death (Day et al 1993). Cocaine (Perera et al 1997) and ecstasy (Drake and Broadhurst 1996) use are associated with QT prolongation and sudden death is recognised in individuals using cocaine (Karch et al 1996). Both alcohol and cocaine misuse are often co-morbid with psychotic illness and so may further increase any risk of sudden death associated with antipsythotic treatment.

8. SUDDEN UNEXPLAINED DEATH AND ANTIPSYCHOTICS

The most serious manifestation of TdP is sudden death. From as far back as the 1960's conventional antipsychotics have been linked to sudden unexplained and unexpected death (Hollister and Koesk, 1965). This can be defined as 'death within the hour of symptoms (excluding suicide, homicide and accident) which is both unexpected in relation to the degree of disability before death and unexplained because clinical investigations and autopsy failed to identify any plausible cause' (Jusic and Lader 1994). Sudden unexplained death occurs in the general population and so the key question is whether such deaths are causally related to the antipsychotic or represent a spurious association. Some case reports show a close temporal link between commencing an antipsychotic drug and sudden unexplained death suggesting a causal, or at least contributory, link between the two. Further evidence comes from epidemiological studies.

Perhaps the best epidemiological study is by Ray et al (2001) who conducted a retrospective cohort study of approximately 0.5 million Tennessee Medicaid enrolees for an average of 2.5 years from January 1988 to December 1993. They found 1,487 sudden unexpected cardiac deaths (equivalent to 11.6 per 10,000 person-years of observation) and from these calculated multivariate rate ratios adjusted for potential confounding factors including age, sex, race, non-cardiac and cardiac illness. The risk of sudden death for individuals receiving a moderate dose of antipsychotic drugs (>100mg thioridazine equivalent per day) was 2.39 times greater than for non-users (95% CI = 1.77-3.22). It was also greater than the risk of sudden death seen in current low-dose antipsychotic users ($p = 0.003$) and in former use ($p < 0.001$). Absolute rates of sudden cardiac death per 10,000 person-years of follow-up were calculated (standardised for age and sex distribution). Within the entire cohort the rate of sudden unexpected cardiac death per 10,000 person-years was 26.9 for use of moderate-dose antipsychotics compared with 11.3 for non-users, that is, for every 10,000 person-years of follow-up, antipsychotic use was associated with 15 additional deaths.

Several other studies support a causal link between antipsychotic use and sudden death. For example Reilly et al (2002) found an association between sudden unexplained death and thioridazine and Mehtohnen et al (1991) found that phenothiazines, particularly thioridazine, were over-represented in a series of autopsy-negative deaths in Finland in 1991. Although these studies are not without their weaknesses, in particular they cannot prove causality, a review of the whole evidence base provides a strong case that certain antipsychotic drugs can increase the risk of sudden unexplained death.

The most likely mechanism by which antipsychotics cause sudden unexplained death is blockade of Ikr channels leading to TdP. However other mechanisms have been proposed. These include peripheral vasodilatation leading to hypotension and collapse, (Cancro and Wilder 1970), respiratory dyskinesias that impair coordination of respiratory muscles leading to asphyxia (Weiner et al 1978), and oral laryngeal-pharyngeal dyskinesias causing acute airway obstruction (Modestin et al 1981). Certain other mechanisms have been implicated but these can be diagnosed at post-mortem (e.g. acute myocarditis and cardiomyopathy both observed in individuals on clozapine). It should also be noted that arrhythmias may develop through other pathways, for example suppression/acceleration of AV node conduction.

9. SUMMARY & CONCLUSIONS

A wide range of drugs can cause QTc prolongation and TdP. Among psychiatric drugs this includes certain conventional and atypical antipsychotics, lithium and tricyclic antidepressants. In recent years several drugs have been withdrawn from use because of these problems while other drugs have had their licences restricted. It is unclear how many drugs have had their development abandoned due to such concerns. TdP can manifest with syncope, dizziness, and palpitations but may also present with sudden death. Risk factors for TdP can be divided into genetic and non-genetic, the former being irreversible and fixed for any patient, the latter being potentially modifiable. Examples of genetic risk factors include gender (women are at higher risk of developing TdP than men), presence of congenital LQTS, mutations in genes coding for cardiac ion channels, and polymorphisms of CYP enzymes that make patients more susceptible to pharmacokinetic interactions. Non-genetic factors include pharmacodynamic and kinetic drug interactions, electrolyte disturbance, alcohol and substance misuse, hepatic and renal impairment and the presence of certain cardiac diseases.

In practice about 50% of cases of drug induced QT interval prolongation and TdP result from drug-drug interactions with metabolic inhibitors, about 10% result from electrolyte imbalance and 10% reflect pharmacodynamic interactions between two or more QTc prolonging drugs. In about 10-20% of cases there is no obvious risk factor and it is in this group that genetic factors appear to play the biggest cause. Mutations in genes coding for cardiac ion channels have been identified in only a minority of patients with drug-induced LQTC syndrome. However more systematic studies are needed to investigate this further and at

present routine genotyping of patients prior to prescribing drugs associated with QTc prolongation is not feasible (the genetic substrate remains uncertain) or practical.

Current strategies for preventing QTc prolongation and TdP centre on careful prescribing and patient monitoring. In particular one should avoid prescribing QTc prolonging drugs where safer and equally effective alternatives exist. If there are good clinical grounds for prescribing a QTc prolonging drug the prescriber should ensure that the patient does not have other risk factors for TdP e.g. cardiac failure. The minimum effective dose should be used. Drug combinations with the potential for pharmacokinetic and dynamic interactions that increase the risk of TdP should be avoided. Electrolyte levels should be regularly checked and ECGs should be obtained prior to starting the drug, after each dose increase and at regular intervals during long-term treatment. Patients prescribed QTc prolonging drugs need to be warned about which medications to avoid. Of course these are general guidelines and the exact strategies that are adopted will depend on the degree of risk associated with individual QTc prolonging drugs and the presence of other risk factors, which will differ on an individual patient basis.

10. REFERENCES

Algra, A., Tijsien, J., Roelandt, J. et al., 1991, QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest., *Circulation.* **83**: 1888-94.

Benton, R.E., Sale, M., Flockhart, D.A., Woosley, R.L., 2000, Greater quinidine-induced QTc interval prolongation in women, *Clin Pharmacol Ther*. **67**: 413-8.

Cancro, R. and Wilder, R.A., 1970, Mechanism of sudden death in chlorpromazine therapy, *Am J Psychiatry.* **127**: 368-371

Carella, M.J., Mantz, S.L., Rovner, D.R., Willis, P.W. 3rd., Gossain, V.V., Bouknight, R.R., Ferenchick, G.S., 1996, Obesity, adiposity, and lengthening of the QT interval: improvement after loss*, Int J Obes Relat Metab Disord.* **20**: 938-42.

Committee For Proprietary Medicinal Products (CPMP) Points to consider: The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products, London (December, 1997).

Day, C.P., James, O.F.W., Butler, T.J. et al., 1993, QT prolongation and sudden death in patients with alcoholic liver disease, *Lancet*. **341**: 1423-8.

De Ponti, F., Poluzzi, E., Montanaro., 2000, QT-interval prolongation by non-cardiac drugs: lessons to be learned from recent experience, *Eur J Clin Pharmacol*. **56**: 1-18.

Drake, W.M. and Broadhurst, P.A., 1996, QT-interval prolongation with ecstasy, *SAMJ.* **86**, 180-1.

Drici, M.D., Burklow, T.R., Haridasse, V. et al., 1996, Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart, *Circulation* **94**, 1471-4.

Engel, G.L., et al., 1971, Sudden and rapid death during psychological stress, *Annals of Internal Medicine.* **74**: 771-782.

FDA, Psychopharmacological Drugs Advisory Committee, 19 July, 2000. Briefing Document for Zeldox Capsules (zipriasone hydrochloride). http://www.fda.gov/ohrms/dockets/ac/oo/backgrd/3619b1a.pdf

Flockhart, D.A., Drici, M.D., Kerbusch, T. et al., 2000, Studies on the mechanism of a fatal clarithromycin-pimozide interaction in a patient with Tourette syndrome, *J Clin Psychopharmacol.* **20**: 317-24.

Fung, M.C., Hsiao-hui Wu, H., Kwong, K. et al., 2000, Evaluation of the profile of patients with QTc prolongation in spontaneous adverse event reporting over the past 3 decades; 1969-1998 (abstract), *Pharmacoepidemiol Drug Safety*. **9**, **suppl 1**:S24-5.

Goldberg, R.J., Bengtson, J., Chez, Z. et al., 1991, Duration of the QT interval and total cardiovascular mortality in healthy persons (The Framingham Study Experience), *Am J Cardiol* **67**: 55-8.

Haddad, P.M., Anderson, I.M., 2002, Antipsychotic-related QTc prolongation, Torsade de pointes and sudden death, *Drugs.* **62**: 1649-1671.

Haverkamp, W., Breithardt, G., Camm, J. et al., 2000, The potential for QT prolongation and proarrhythmia by non-antiarrhythmic drugs: clinical and regulatory implications. Report on a Policy Conference of the European Society of Cardiology*, European Heart Journal* **21**: 1216-31, published simultaneously in *Cardiovascular Research* 2000, **47**: 219-233.

Heiman, E.M., 1977, Cardiac toxicity with thioridazine-tricyclic antidepressant combination, *J Nerv Ment Dis.* **165**: 139-43.

Higham, P.D., Campbell, R.W.F., 1994, QT dispersion, *Br Heart J.* **71**:508-10.

Hii, J.T.Y., Wyse, D.G., Gillis, A.M. et al., 1992, Precordial QT interval dispersion as a marker of torsade de pointes. Disparate effects of class 1a antiarrhythmic drugs and amiodarone, *Circulation*. **86**:1376-82.

Hillis, W.S., et al., 1994, Sudden death in sport, *BMJ.* **309**: 657-660.

Hollister, L.E. and Koesk, J.C., 1965, Sudden death during treatment with phenothiazine derivatives, *JAMA.* **192**: 1035-1038.

Houltz, B., Darpo, B., Edvardsson, N. et al., 1998, Electrocardiographic and clinical predictors of torsades de pointes induced by almokalant infusion in patients with chronic atrial fibrillation or flutter: a prospective study, *Pacing Clin Electrophysiol*. **21**:1044-57.

Jusic, N. and Lader, M., 1994, Post-Mortem antipsychotic drug concentrations and unexplained deaths, *Br J Psychiatry.* **165**: 787-791.

Karch, S.B., 1996, Cardiac arrest in cocaine users, *Am J Emerg Med.***14**: 79-81.

Kroemer, H.K., Eichelbaum, M., 1995, "It's the genes, stupid". Molecular bases and clinical consequences of genetic cytochrome P450 2D6 polymorphism, *Life Sci.* **56**: 2285-98.

Lareya, J., 1995, Sudden death, neuroleptics, and psychotic agitation, *Prog Neuropsychopharmacol Biol Psychiatry.* **19**: 229-41.

Llerena, A., Berecz, R., De la Rubia, A. et al., 2002, QTc interval lengthening is related to CYP2D6 hydroxylation capacity and plasma concentration of thioridazine in patients, *J Psychopharmacol.* **16**: 361-4.

Makkar, R.R., Fromm, B.S., Steinman, R.T. et al., 1993, Female gender as a risk factor for torsade de pointes associated with cardiovascular drugs, *JAMA.* **270**: 2590-7.

Malik, M.A.O. et al., 1973, Emotional distress as a precipitating factor in sudden deaths due to coronary insufficiency, *J Forensic Sci.* **18**: 47-52.

Mehtonen, O.P., Aranko, K., Malkonen, L., Vapaatalo, H., 1991, A survey of sudden death associated with the use of antipsychotics or antidepressant drugs: 49 cases in Finland, *Acta Psychiatrica Scand.* **84**: 58-64.

Meuleman, C., Jourdain, P., Bellorini, M. et al., 2001, [Citalopram and Torsades de Pointes. A case report] French., *Arch Mal Coeur Vaiss.* **94**: 1021-4.

Merri, M., Benhorin, J., Alberti, M. et al., 1989, Electrocardiographic quantitation of ventricular reporisation, *Circulation.* **80**, 1301-8.

Meyer, U.A., 1991, Genotype or phenotype: the definition of a pharacogentic polymorphism, *Pharmacogenetics.* **1**: 66-67.

Modestin J., Krapf R., Boker WA., 1981, Fatality during haloperidol treatment: mechanism of sudden death. *Am J Psychiatry* **138**: 1661-1617

Nagy, D., DeMeersman, R., Gallagher, D., Pietrobelli, A., Zion, A.S., Daly, D., Heymsfield, S.B., 1997, QTc interval (cardiac repolarisation): Lengthening after meals, *Obes Res,* **5**: 531-7.

Napolitano, C., Schwartz, P.J., Brown, A.M. et al., 2000, Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias, *J Cardiovasc Electrophysiol*. **11**: 691-6.

Perera, R., Kraebber, A., Schwartz, M., 1997, Prolonged QT interval and cocaine use, *J Electrocardiol.* **30**: 337-9.

Priori, S.G., Napolitano, C., Schwartz, P.J., 1991, Low penetrance in the long-QT syndrome: clinical impact, *Circulation.* **99**: 529-33.

Rautaharju, P., Zhou, S.H., Wong, S. et al., 1992, Sex differences in the evolution of the electrocardiographic QT interval with age, *Can J Cardiol.* **8**: 690-5.

Ray, W.A., Meredith, S., Thapa, P.B. et al., 2001, Antipsychotics and the risk of sudden cardiac death, *Arch Gen Psychiatry.* **58**: 1161-7.

Reilly, J.G., Ayes, S.A., Ferrier, I.N. et al., 2000, QTc interval abnormalities and psychotropic drug therapy in psychiatric patients, *Lancet.* **335**:1048-1052.

Salle, P., Rey, J.L., Bernasconi, P., et al., 1985, Torsades de pointe. Aapropos of 60 cases (in French), *Ann Cardiol Angerol* (Paris), **34**: 341-8.

Schoeten, E.G., Dekker, J.M., Meppelink, P. et al., 1991, Qt interval prolongation predicts cardiovascular mortality in an apparently health population, *Circulation,* **84**: 1516-23.

Stramba-Badiale, M., Locati, E.H., Martinelli, A., Courville, J., Schwartz, P.J., 1997, Gender and the relationship between ventricular repolarisation and cardiac cycle length during 24-hour Holter recordings, *Eur Heart J.* **18**: 1000-6.

Swanson, J.R., Jones, G.R., Krasselt, W., Denmark, L.N., Ratti, F., 1997, Death of two subjects due to imipramine and desipramine metabolite accumulation during chronic therapy: a review of the literature and possible mechanisms, *J Forenic Sci.* **42**: 3335-339.

Tateishi, T., Chida, M., Ariyoshi, N. et al., 1999, Analysis of the CYP2D6 gene in relation to dexytomethophan -demethylation capacity in a Japenese population, *Clin Pharmacol Ther.* **65**: 570-5.

Thanaccody, R., Daly, A.K., Thomas, S.H., 2003, Influence of CYP2D6 genotype on the QTc interval and plasma concentrations of thioridazine and its metabolites in psychiatric patients taking chronic therapy (abstract), *Clin Pharmacol Ther.* **73**: 77.

Viskin, S., 1999, Long QT syndromes and torsade de pointes, *Lancet.* **354**: 1625-1633.

Von Bahr, C., Movin, G., Nordin, C. et al., 1991, Plasma levels of thioridazine and metabolites are influenced by the debrisoquine hydroxylation phenotype*, Clin Pharmacol Ther.* **49**: 234-40.

Warner, J.P., Barnes, T.R. and Henry, J.A., 1996, Electrocardiographic changes in patients receiving neuroleptic medication, *Acta Psychiatr Scand.* **93**: 311-313.

Website. An overview of the inherited long QT syndrome and sample materials, online (accessed August 2004) URL: http://www.sads.org/LQTS.html.

Weiner, W.J., Goetz, C.G., Nausieda, P.A. et al., 1978, Respiratory dyskinesias: extrapyramidal dysfunction and dyspnea, *Annals of Internal Medicine.* **88**: 327-331.

Woosley, R.L., Chen, Y., Freiman, J.P., Gilles, R.A., 1993, Mechanism of the cardiotoxic actions of terfenadine, *JAMA.* **2569**: 1532-1536.

Wysowski, D.K., Corken, A., Gallo-Torres, H., Talarico, L., Rodriguez, E.M., 2001, Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and Food and Drug Administration regulatory actions, *Am J Gastroenterology.* **96**: 1698-1703.

20. GLOSSARY:

Genetics and Pharmacogenetics Related Terms

Philip Gorwood and Michel Hamon*

Allele
One of the different forms of a gene that can exist at a single locus.

Annealing
Pairing of complementary single stands of DNA to form a double helix.

Back mutation
Reversing the effect of a mutation that had inactivated a gene, thus it restores wild type.

Base pair (bp)
Partnership of A with T, or C with G, in a DNA double helix. Other pairs can be formed in RNA under certain circumstances.

CAAT box
Part of a conserved sequence located upstream of the startpoints of eukaryotic transcription units; this sequence is recognized by a large group of transcription factors.

cDNA
Single-stranded DNA complementary to an RNA, synthesized from it by reverse transcription *in vitro*.

Philip Gorwood, INSERM Unit 675 (IFR02) Faculty Xavier Bichat. 16 rue Henri Huchard, 75018 Paris-France and CHU Louis Mourier (AP-HP, Paris VII), 178 rue des Renouillers, Colombes, France.
Michel Hamon, INSERM Unit 677 Neuropsychopharmacology, Faculty Pitié-Salpêtrière, 75013 Paris, France.

Centromere
Constricted region of a chromosome that includes the site of attachment to the mitotic or meiotic spindle. (*see also* kinetochore.)

Chromosome
Discrete unit of the genome carrying genes. Each chromosome consists of a long molecule of duplex DNA and only visible as a morphological entity during cell division (mitosis).

Clone
Cells or molecules indentical with a single ancestral cell or molecule.

Cloning vector
Plasmid or phage that is used to « carry » inserted foreign DNA for the purposes of producing more material or a protein product.

Coding region
The exons part of genes.

Codon
Triplet of nucleotides representing an amino acid or a termination signal.

Conservative
Modifying the structure of the protein, but not its function.

Conservative recombination
Breakage and reunion of pre-existing strands of DNA without any synthesis of new stretches of DNA.

Crossing-over
Reciprocal exchange of material between chromosomes that occurs during meiosis and is responsible for genetic recombination.

Cyclic AMP (cAMP)
Molecule of AMP in which the phosphate group is joined to both the 3' and 5' positions of the ribose; its binding activates the CAP, a positive regulator of prokaryotic transcription.

Deletions
Generated by removal of a sequence of DNA, the regions on either side being joined together.

Denaturation
Describes DNA or RNA conversion from the double-stranded to the single stranded state; separation of the strands is most often accomplished by heating.

Denaturation
Conversion of protein from the physiological conformation to some other (inactive) conformation.

Diploid
Set of chromosomes containing two copies of each autosome and two sex chromosomes.

DNAase
Enzyme that attacks bonds in DNA.

DNA polymerase
Enzyme that synthesizes a daughter strand(s) of DNA (under direction from a DNA template). May be involved in repair or replication.

Domain
Discrete structural entity of a chromosome defined as a region within which supercoiling is independent of other domains; *or* an extensive region including an expressed gene that has heightened sensitivity to degradation by the enzyme DNAase I; *or* is a discrete continuous part of the amino acid sequence of a protein that can be equated with a particular function.

Dominant allele
An allele that determines the phenotype displayed in a heterozygote with another (recessive) allele.

Epigenetic
The influence the phenotype without altering the genotype. They consist of Changes in the properties of a cell that are inherited, but that do not represent a change in genetic information.

Epistasis
Situations in which expression of one gene modify the phenotypic effects of another gene.

Exon
Any segment of an interrupted gene that is represented in the mature RNA product.

Gamete
Either type of reproductive (germ) cell-sperm or egg-with haploid chromosome content.

Gene
The segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (TATA box) as well as intervening sequence (introns) between individual coding segments (exons).

Gene dosage
Number of copies of a particular gene in the genome.

Gene family
Set of genes whose exons are related; the members were derived by duplication and variation from some ancestral gene.

Gene cluster
Group of adjacent genes that are identical or related.

Genotype
Genetic constitution of an organism.

G proteins
Guanine nucleotide-binding trimeric proteins that reside in the plasma membrane. When bound by GDP the trimer remains intact and is inert. When the GDP bound to the α subunit is replaced by GTP, the α subunit is released from the βγ dimer. One of the separated units (either the α monomer or the βγ dimer) then activates or represses a target protein.

Haplotype
A particular combination of alleles in a defined region of some chromosome, in effect the genotype in miniature. Originally used to described combinations of MHC alleles, it now may be used to describe particular combinations of RFLPs.

Hemizygote
Diploid individual that has lost its copy of a particular gene (for example, because a chromosome has been lost) and which therefore has only a single copy.

Heritability
Ratio of additive genetic variance. The phenotypic variance is the result of the interaction of genetic and nongenetic factors in a population.

Homozygote
Individual with the same allele at corresponding loci on the homologous chromosome.

Housekeeping (constitutive) genes
Genes (theoretically) expressed in all cells because they provide basic functions needed for sustenance of all cell types.

Hybridization
Pairing of complementary RNA and DNA strands to give an RNA-DNA hybrid.

Immortalization
Acquisition by a eukaryotic cell line of the ability to grow through an indefinite number of divisions in culture.

Imprinting
Change in a gene that occurs during passage through the sperm or egg with the result that the paternal and maternal alleles have different properties in the very early embryo. May be caused by methylation of DNA.

***In situ* hybridization**
Denaturing the DNA of cells squashed on a microscope slide so that reaction is possible with an added single-stranded RNA or DNA; the added preparation is radioactively labeled and its hybridization is followed by autoradiography.

Insertions
Presence of an additional stretch of base pairs in DNA.

Intron
Segment of DNA that is transcribed, but removed from within the transcript by splicing together the sequences (exons) on either side of it.

Kb (kilobase)
Abbreviation for 1,000 base pairs of DNA or 1,000 bases of RNA.

Kinase
Enzyme that phosphorylates (adds a phosphate group) to a substrate; the substrates for **protein kinases** are amino acids in other proteins, and they are divided into those specific for tyrosine and those specific for threonine/serine.

Linkage
Tendency of genes to be inherited together as a result of their location on the same chromosome; measured by percent recombination between loci.

Linkage desequilibrium
Situation in which some combinations of genetic markers occur more or less frequently in the population than would be expected from their distance apart. It implies that a group of markers has been inherited coordinately. It can result from reduced recombination in the region or from founder effect, in which there has been insufficient time to reach equilibrium since one of the markers was introduced into the population.

Locus
Position on a chromosome at which the gene for a particular trait resides; A locus may be occupied by any one of the allele for the gene.

LOD score
Measure of genetic linkage, defined as the $\log_{10}$ ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

Map distance
Measured as cM (centiMorgans) = percent recombination (sometimes subjet to adjustments).

Mb (megabase)
Abbreviation for 10^6 bp of DNA.

Meiosis
Occurs by two successive divisions (meiosis I et II) that reduce the starting number of 4*n* chromosomes to 1*n* in each of four product cells. Products may mature to germ cells (sperm or eggs).

Microarray
Set of miniaturized chemical reaction areas that may also be used to test DNA fragments, antibodies, or proteins.

mtDNA
Mitochondrial DNA.

Mutation
Any change in the sequence of genomic DNA.

Mutation rate
Rate at which a particular mutation occurs, usually given as the number of events per gene per generation.

Noncoding regions
A region concerning introns of genes

Nonsense codon
Any one of three triplets (UAG, UAA, UGA) that cause termination of protein synthesis.

Oncogenes
Genes whose products have the ability to transform eukaryotic cells so that they grow in a manner analogous to tumor cells. Oncogenes carried by retroviruses have names of the form *v-onc.*

PCR (polymerase chain reaction)
Technique in which cycles of denaturation, annealing with primer, and extension with DNA polymerase, are used to amplify the number of copies of a target DNA sequence by > 10^6 times.

Pharmacodynamic
Genetically based differences in the proteins at which a drug acts

Pharmacogenetics
Study of genetically determined interindividual differences in pharmacoresponse, concerning drug metabolism (pharmacokinetic level), drug targets (pharmacodynamic level) and global response to pharmacological agents. May ultimately propose genome-based 'rational therapeutics'. Its final goal being to make a prediction of drug response at the level of the individual patient.

Pharmacogenomics
The application of genome-wide approaches to the study of interindividual differences in response to pharmacological agents. Drug discovery based on knowledge of genes, offers insight into aetiologic mechanisms, and possible prevention and treatment.

Pharmacokinetic
Bioavailability analyses of a drug.

Phenotype
Appearence or other characteristics of an organism, resulting from the interaction of its genetic constitution with the environment.

Pleiotropic gene
Gene affecting more than one (apparently unrelated) characteristic of the phenotype.

Polymorphism
Simultaneous occurrence in the population of genomes showing allelic variations (as seen either in alleles producing different phenotypes or - for example – in changes in DNA affecting the restriction pattern).

Primer
Short sequence (often or RNA) that is paired with one strand of DNA and provides a free 3'-OH end at which a DNA polymerase starts synthesis of a H deoxyribonucleotide chain.

Promoter
Region of DNA involved in binding of RNA polymerase to initiate transcription.

Pseudogenes
Inactive but stable components of the genome derived by mutation of an ancestral active gene.

Recessive allele
Obscured in the phenotype of a heterozygote by the dominant allele, often due to inactivity or absence of the product of the recessive allele.

Renaturation
Reassociation of denatured complementary single strands of a DNA double helix.

Repeating unit
Length of the sequence that is repeated.

Restriction enzymes
Enzymes that recognize specific short sequences of (usually) unmethylated DNA and cleave the duplex (sometimes at target site, sometimes elsewhere, depending on type).

Restriction fragment length polymorphism (RFLP)
Inherited differences in sites for restriction enzymes (for example, caused by base changes in the target site) that result in differences in the lengths of the fragments produced by cleavage with the relevant restriction enzyme. RFLPs are used for genetic mapping to link the genome directly to a conventional genetic marker.

RNA polymerase
Enzyme that synthesizes RNA using a DNA template (formally described as DNA-dependent RNA polymerase).

Satellite DNA
Many tandem repeats (identical or related) of a short basic repeating unit.

Sex chromosomes
Those whose contents are different in the two sexes; labeled X and Y, one sex has XX, the other sex has XY.

Silent mutations
No change in the product of a gene.

SNP
Single nucleotide polymorphisms (variation of one nucleic acid) which is the most common type of human genetic variation (currently, around 2 millions of SNPs are publicly available).

Stop codons
The three triplets (UAA, UAG, UGA) which terminate protein synthesis.

Synonymous
Nucleotide polymorphisms in coding regions that do not influence the structure of the protein.

Transcription
Synthesis of RNA on a DNA template.

Transfection
The acquisition of new genetic markers by incorporation of added DNA in eukaryotic cells.

INDEX

Page numbers followed by t and f indicates tables and figures, respectively