PHARMACOGENOMICS

CHALLENGES AND OPPORTUNITIES IN THERAPEUTIC IMPLEMENTATION

SECOND EDITION

Edited by

Y. W. FRANCIS LAM

STUART A. SCOTT

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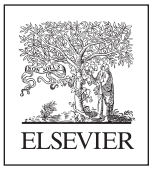

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525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-812626-4

For information on all Academic Press publications visit our website at https://www.elsevier.com/books-and-journals

Publisher: Andre Gerhard-Wolff
Acquisition Editor: Erin Hill-Parks
Editorial Project Manager: Jennifer Horigan
Production Project Manager: Poulouse Joseph
Cover Designer: Matthew Limbert

Typeset by TNQ Technologies

Dr. Lam dedicates this book to Jennifer, Jessica, and Derek; Aunt Chee-Ming and Uncle Po-Hon; Mom and Dad.

Dr. Scott dedicates this book to Gillian, Camille, and Harvey.

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List of Contributors

Rector Arya South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Edinburg, TX, United States

Mariana R. Botton Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Karla Claudio Campos Department of Pharmacotherapy and Translational Research, University of Florida College of Pharmacy, Gainesville, FL, United States

Kelly E. Caudle Pharmaceutical Sciences Department, St. Jude Children's Research Hospital, Memphis, TN, United States

Larisa H. Cavallari Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics, University of Florida, Gainesville, FL, United States

Katarzyna Drozda Office of Clinical Pharmacology, Food and Drug Administration, Silver Spring, MD, United States

Jorge Duconge Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico Medical Sciences Campus, San Juan, PR, United States

Ravindranath Duggirala South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Edinburg, TX, United States

Henry M. Dunnenberger Pharmacogenomics, Center for Molecular Medicine, NorthShore University Health System, Evanston, IL, United States

Roseann S. Gammal Department of Pharmacy Practice, MCPHS University, Boston, MA, United States; Pharmaceutical Sciences Department, St. Jude Children's Research Hospital, Memphis, TN, United States

Christopher P. Jenkinson South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Edinburg, TX, United States

Y. W. Francis Lam Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; College of Pharmacy, University of Texas at Austin, Austin, TX, United States

Edmund Jon Deoon Lee Pharmacogenetics Laboratory, Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Michael Limenta Health Products Regulation Group, Health Sciences Authority, Singapore

Surulivelrajan Mallayasamy Post-Doctoral Research Associate, Department of Pharmacotherapy, University of North Texas System College of Pharmacy, Fort Worth, TX, United States; Department of Pharmacy Practice, MCOPS, Manipal University, Manipal, India

Elsa Haniffah Mejia Mohamed Pharmacogenomics Laboratory, Department of Pharmacology, University Malaya, Kuala Lumpur, Malaysia; Pharmacogenetics Laboratory, Department of Pharmacology, National University of Singapore, Singapore

Kathryn M. Momary Department of Pharmacy Practice, Mercer University, College of Pharmacy, Atlanta, GA, United States

Aniwaa Owusu Obeng The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, Department of Pharmacy, The Mount Sinai Hospital, New York, NY, United States

Scott R. Penzak Department of Pharmacotherapy, University of North Texas System College of Pharmacy, Fort Worth, TX, United States

Matthias Samwald Center for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Vienna, Austria

Erick R. Scott Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; Clinical Data Science, Sema4, Stamford, CT, United States

Stuart A. Scott Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; Sema4, Stamford, CT, United States

Toshiyuki Someya Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Jesse J. Swen Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands

Wei Chuen Tan-Koi Health Products Regulation Group, Health Sciences Authority, Singapore

Alexander Vandell Daiichi Sankyo Pharma Development, Basking Ridge, NJ, United States

Richard L. Wallsten Clinical Data Science, Sema4, Stamford, CT, United States

Ophelia Yin Daiichi Sankyo Pharma Development, Basking Ridge, NJ, United States

Preface

Pharmacogenomics and pharmacogenetics are overlapping sciences, and, although the two terminologies have been used interchangeably in the literature, pharmacogenomics reflects a progressive transition that has taken place over the years within the broad scope of personalized medicine. As a discipline, pharmacogenomics is envisioned as a major societal benefit from all the scientific and technical advances related to the Human Genome Project. To date, much work remains to address the challenges with translating pharmacogenomics into clinical practice and drug development to achieve the ultimate goal envisioned many years ago. Nevertheless, examples of clinical applications of pharmacogenomics knowledge have emerged at several major academic medical centers.

This book differs from available pharmacogenomics books in several aspects. It neither contains significant materials on molecular genetics nor lists all the theoretical pharmacogenomics applications organized by therapeutic specialties. Rather, the focus of the book is to provide a timely discussion and viewpoints on a broad range of topics; from the academic, regulatory, pharmaceutical, clinical, socioethical, and economic perspectives that are relevant to the complex processes in translating pharmacogenomics findings into therapeutic applications.

As with the first edition, our goal has been to provide information that is not readily available in other books covering the same topic. Although the processes and implementation barriers are presented in depth in one chapter, perspectives on challenges and limitations, as well as examples of successful direct therapeutic applications, are presented throughout the book. In addition, we have included two chapters that discuss the complexity of ethnicity in pharmacogenomics studies and global drug development, and several chapters that discuss practical aspects of pharmacogenomics testing.

The book chapters are organized into three sections. The first section (Chapters 1 to 4) provides an introductory chapter on pharmacogenomics, one on industry perspective and insights for the role of pharmacogenomics in drug development, another on global academic and governmental efforts to advance and apply the relevant genomic knowledge, and an overview chapter on the challenges of moving the discipline into real-world settings over the last decade. The second section (Chapters 5 to 9) primarily focuses on clinical areas in which the evidence supports direct pharmacogenetic applications to patient care. When appropriate, unsuccessful applications are used to illustrate the challenges for the discipline. The third section (Chapters 10 to 15) is unique and covers diverse topics including looking to the future for pharmacogenomics data technologies, pharmacogenomics issues in different ethnic populations, as well as different models and economic evaluations of pharmacogenomics testing. The final chapter provides a resource as to how this textbook can be useful for teaching pharmacogenomics to students in various healthcare disciplines and graduate-level students in health and pharmaceutical sciences, as well as how pharmacogenomics information can be integrated into clinical practice.

Because the book details viewpoints on the challenges of translating pharmacogenomics, we intentionally did not "limit" our contributors with "organized content" for each chapter.

In essence, each chapter simply follows a general approach of including an overview of the potentials or opportunities within the context of the respective chapter, but the emphasis is on discussion of barriers with perspectives on how to move pharmacogenomics forward. Realizing that overlap is inevitable in a book with multiple authors, we took measures to minimize unnecessary duplicated materials, and cross-reference chapters whenever appropriate.

This book is intended not only as a reference book for scientists in academia and the pharmaceutical industry involved in pharmacogenomics research, but also for healthcare clinicians working or interested in the field. In addition, this text is useful as a textbook for teaching clinicians and students in different healthcare disciplines, and specific materials covered in the book would be useful resources for teaching graduate students in academic disciplines such as pharmacology, neuroscience, structural and cellular biology, and molecular medicine. It is our sincere hope that after completing the textbook, the readers not only have a critical awareness of the value of pharmacogenomic implementation with actual versus potential applications, but also a broad knowledge of the pertinent issues and challenges for pharmacogenomics before advances in scientific findings can be broadly and practically applied to patient care.

Y. W. Francis Lam
Stuart A. Scott

CHAPTER

1

Principles of Pharmacogenomics: Pharmacokinetic, Pharmacodynamic, and Clinical Implications

Y. W. Francis Lam[1,2]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]College of Pharmacy, University of Texas at Austin, Austin, TX, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00001-2

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OBJECTIVES

1. Describe how a single-nucleotide polymorphism (SNP) can affect protein function or expression, and consequently, influence drug response.
2. Explain how genetic polymorphisms for drug metabolism or drug-transporter proteins may influence drug pharmacokinetics.
3. Contrast phenotypic responses to genetic variation for drug metabolism versus drug-target proteins.
4. Describe novel drug developed based on an understanding of genes involved in disease pathophysiology.
5. Explain how genetic polymorphisms at the drug-target site may influence drug pharmacodynamics.

INTRODUCTION

Significant interpatient variability in drug response is largely attributed to innate differences among individuals in their capacity to process and respond to medications. Pharmacogenomics involves incorporating information about a person's genotype into drug therapy decisions, with the goal of providing the most effective and safest therapy for that individual. Over the last decade, there have been significant advances in our understanding of the contribution of genetic differences in pharmacokinetics and pharmacodynamics toward interindividual variability in drug response. Not only may pharmacogenomics lead to improved use of existing therapies, but it may also lead to novel drugs developed based on an improved understanding of genetic control of cellular functions.

The human genome comprises approximately 20,000 protein-coding genes. By far the most common variation is the single-nucleotide polymorphism (SNP), which is defined as single-base differences that exist between individuals. Over 22 million SNPs have been reported in the human genome [1]. SNPs that result in amino acid substitution are termed nonsynonymous. Nonsynonymous SNPs occurring in coding regions of the gene (e.g., exons) can impact protein activity and have significant consequences on responses to medications that depend on the protein for metabolism, transport, or eliciting cellular effects. Synonymous polymorphisms do not result in amino acid substitution; however, those occurring in a gene regulatory region (e.g., promoter region, intron) may alter gene expression and the amount of protein that is produced. Two or more SNPs are often inherited together more frequently than would be expected based on chance alone. This is referred to as linkage disequilibrium (LD). A haplotype refers to a set of SNPs that are in LD. Other types of variation that can affect gene expression or protein conformation include insertion–deletion polymorphisms (indels), short tandem repeats, and copy number variants (CNVs). A CNV represents a DNA segment (≥1 kb) with a variable number of copies of that segment, because of duplications, deletions, or rearrangement, and constitutes a major source of interindividual variation in the human genome. A unique reference SNP identifier (rs number) is assigned for each genetic variant, and exists as an SNP data repository, the National Center for Biotechnology Information (NCBI) Single-Nucleotide Polymorphism Database (dbSNP).

Polymorphisms commonly occur for genes encoding drug metabolism, drug transporter,

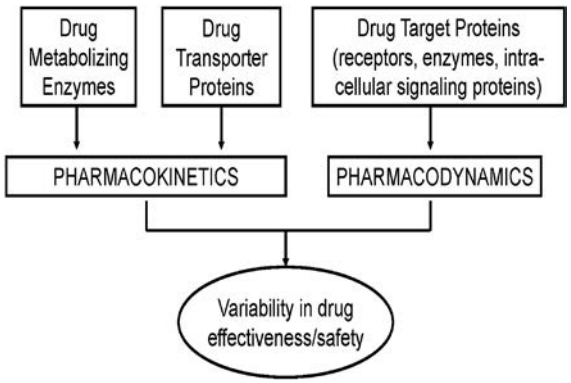

FIGURE 1.1 Location of genetic variations affecting drug response. Those occurring in genes for drug metabolism or transport can affect drug pharmacokinetics, whereas SNPs in genes encoding for drug-target proteins can impact drug pharmacodynamics.

and drug-target proteins (Fig. 1.1). Drug metabolism and transporter genotypes can affect drug availability at the target site, whereas drug-target genotype can affect a patient's sensitivity to a drug. In many instances, genes for proteins involved in drug disposition, together with genes for proteins at the drug-target site, jointly influence drug response. In addition, genetic polymorphisms in absorption, distribution, metabolism, and excretion (ADME) and target genes also contribute to ethnic heterogeneity in drug response [2]. Research advances have resulted in continued identification of association between genetic polymorphisms and response, with recent focus on genome-wide association studies (GWAS) in populations worldwide.

The terms pharmacogenetics and pharmacogenomics are often used interchangeably. Because drug responses are mostly determined by multiple, rather than single, proteins, recent trends of investigations on determinants of drug response have shifted from pharmacogenetics to pharmacogenomics. However, for simplicity, this chapter treats pharmacogenetics and pharmacogenomics as synonymous.

Despite the scientific advances made, personalized medicine envisioned many years ago has in many cases yet to become a reality. Exceptions to this largely exists in oncology and more recently in cardiology, in which genotyping to determine clopidogrel effectiveness is starting to become routine at some large academic medical centers [3,4]. Examples of genotype-guided therapies are beginning to emerge in other therapeutic areas, which are discussed in detail throughout this book. However, significant challenges still exist in ethical, socioeconomic, regulatory, legislative, drug development, and educational issues that need to be addressed and resolved before personalized medicine can be practically and satisfactorily implemented in clinical practice on a broader scale. The goal of this chapter is to review the pharmacokinetic and pharmacodynamics basis of individualized therapy, and briefly discuss the challenges of implementing pharmacogenomics in clinical practice. Further indepth discussion of specific therapeutic areas and/or disease states, as well as ethical, socioeconomic, regulatory, legislative, drug development, technological, and educational issues will be the focus of subsequent chapters.

POLYMORPHISMS IN CYTOCHROME P450 ENZYMES

The cytochrome P450 (CYP) superfamily of isoenzymes represents the most important and studied metabolic enzymes that exhibit clinically relevant genetic polymorphisms. Within this superfamily of isoenzymes, 57 different CYP genes and 58 pseudogenes have been identified, and, based on the similarity in their amino acid sequences, are grouped into 18 families and 44 subfamilies with increasing extent of sequence similarity. Of these genes and pseudogenes, 42 are involved in the metabolism of exogenous xenobiotics and endogenous substances, such as steroids and prostaglandins, and 15 are known to be

involved in the metabolism of drugs in humans [5]. Information regarding *CYP* allele nomenclature and specific genetic variations defining different metabolic phenotypes had been available at the Karolinska Institute website: www.cypalleles.ki.se, for more than a decade, and recently moved to the new Pharmacogene Variation (PharmVar) Consortium, which serves as a new hub for pharmacogene nomenclature [6].

The genes encoding CYPs are highly polymorphic, with SNPs in the *CYP* gene locus accounting for most of the variations in CYP activity, resulting in functional genetic polymorphism for several isoenzymes, including CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5. Additional types of *CYP* polymorphisms cause gene deletions, deleterious mutations resulting in premature stop codon or splicing defects, amino acid changes, gene duplications, and CNV. Different alleles or functional variants of these polymorphisms for individual drug metabolizing genes are defined with a "star" (*) designation. A combination of two *alleles, for example, *CYP2D6*1/*1*, is used to classify individuals into several genetically defined metabolic phenotypes with different expressions of enzyme activity. In general, the poor metabolizers (PMs) inherit two defective or deleted alleles and exhibit abolished-enzyme activity; the intermediate metabolizers (IMs) carry either one functional and one defective allele, or two partially defective alleles, and, in both cases, have reduced activity of the enzyme. The normal metabolizers are typically known as the extensive metabolizers (EMs) with two functional alleles and normal enzyme activity; and the ultrarapid metabolizers (UMs) carry a duplicated or amplified gene variant, resulting in two or multiple copies of the functional allele and very high enzyme activity.

In general, the clinical consequences of genetically altered-enzyme activity would depend on whether the pharmacological activity resides with the parent compound or the metabolite, and the relative contribution of the polymorphic isoenzyme to the overall metabolism of the drug. For the majority of the drugs, PMs would exhibit a higher risk of adverse drug reactions (ADRs), whereas UMs would experience lower efficacy when administered standard-dosage regimen of a drug that is mostly dependent on the polymorphic enzyme for elimination. In the case of a prodrug, the UMs exhibit higher incidence of ADRs, and the PMs experience lower efficacy, reflecting a difference in the extent of therapeutically active metabolite formed between the two metabolic genotypes.

Among the different *CYP* gene polymorphisms, those affecting CYP2D6, CYP2C19, and CYP2C9 are currently the most relevant with also the most abundant data, as well as representing most of the revised regulatory labeling information. Their potential role in translating the expanding pharmacogenomic knowledge into dose requirements and therapeutic decisions will be discussed first. An overview of the other major CYP isoenzymes will also be presented.

CYP2D6

CYP2D6 is the only drug-metabolizing CYP enzyme that is not inducible, and the significant interindividual differences in enzyme activity are largely attributed to genetic variations. *CYP2D6* is located on chromosome 22 and consists of 4382 nucleotides. The *CYP2D6* gene, which codes for the CYP2D6 enzyme, is composed of 497 amino acids. In addition, the *CYP2D6* gene polymorphisms are also the best characterized among all of the *CYP* variants, with at least 100 alleles identified. Nevertheless, Sistonen et al. [7] demonstrated that, even with the extensive number of alleles, determining 20 different haplotypes by genotyping 12 SNPs could predict the real phenotype with 90%–95% accuracy.

Among the multiple *CYP2D6* alleles, *CYP2D6*1*, *CYP2D6*2*, *CYP2D6*33*, and *CYP2D6*35* are active alleles with normal enzyme activity, whereas the two most

important null variants are *CYP2D6*4* (c.1846G>A, rs3892097) and *CYP2D6*5* (gene deletion), resulting in an inactive enzyme and absence of enzyme, respectively. Significant reduction in enzyme activity is commonly associated with *CYP2D6*10* (c.100C>T, rs1065852), *CYP2D6*17* (c.1023C>T, rs28371706, c.2850C>T, rs16947), and *CYP2D6*41* (c.2988G>A, rs28371725), and phenotypically expressed as IM. In addition, to these reduced function alleles, the IM phenotype has also been associated with the *CYP2D6*9*, **29*, and **36* variants [5]. Additional loss-of-function alleles include *CYP2D6*3*, **6–*8*, **11–*16*, **19–*21*, **38*, **40*, and **42*. CYP2D6 is also the first CYP isoenzyme for which CNVs were reported [8]. Individuals carrying up to 13 functional copies of the *CYP2D6*2* allele [9] have been reported to exhibit variation in response to different drugs [10,11]. After these initial reports, gene duplication has also been documented for the *CYP2D6*1*, **4*, **6*, **10*, **17*, **29*, **35*, **41*, **43*, and **45* variants [12]. Therefore, although UMs can result from duplication or multiduplication of the active CYP2D6 gene, duplication of partially functional and nonfunctional genes can also occur, resulting in different levels of gene expression and phenotypes of metabolic importance (Table 1.1). A CYP activity score has also been recommended for use in classifying the different 2D6 phenotypic groups [13]. More recently, a software tool (originally named "Constellation" and subsequently renamed as "Astrolabe") capable of allowing rapid, automated phenotype assignment has been made available for academic research at no cost [14].

Significant interethnic variations in *CYP2D6* allele and phenotype distributions have also been well documented. The normal function *CYP2D6*2* has been reported in approximately 25% of Caucasians, 31% of Africans, and 10%–12% of East Asians [15]. *CYP2D6*4* and *CYP2D6*5* (allelic frequency of about 20%–25% and 4%–6%, respectively) are predominantly found in Caucasian PMs, whereas the predominant variants in people of Asian and African heritage are *CYP2D6*10* (allelic frequency of about 50%) and *CYP2D6*17* (allelic frequency of about 20%–34%), respectively, both resulting in the IM phenotype. Therefore, even though the classic PM phenotypic frequencies determined

TABLE 1.1 Functional CYP2D6 Polymorphisms, Expected Enzyme Activity, and Predicted Metabolic Phenotypes for Selected Common Variants

Allelic Variants and Polymorphism	Functional Effect on Enzyme Activity	Predicted Metabolic Phenotypes
Active: **1, *2, *2A, *33, *35*	Normal activity	Extensive metabolizers: • Homozygous carriers of two active alleles • Heterozygous carriers of an active and a partially active allele
Partially active: **9, *10* (P34S), **17* (T107I, R296C), **29, *36, *41* (splicing defect)	Reduced activity	Intermediate metabolizers: • Heterozygous carriers of an active and a loss-of-function allele • Homozygous carriers of two reduced activity alleles • Heterozygous carriers of a partially active allele and a loss-of-function allele
Inactive: **3 (frame shift) *4 (splicing defect), *5 (gene deletion) *6 (frame shift), *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *38, *40, *42* Gene duplication have been reported for both **4* and **6*	Loss-of-function	Poor metabolizers: • Homozygous carriers of two loss-of-function alleles
Gene duplications/copy number variants **1, *2, *10, *17, *29, *35, *41, *43, *45*	Enhanced activity	Ultrarapid metabolizers: • Carriers of ≥3 active alleles

in Asians (about 0%–1% of population) and Africans (0%–5% of population) are lower than that reported for the Caucasians (5%–14% of population), the high prevalence of *CYP2D6*10* and *CYP2D6*17* in these two IM populations provides a biologic and molecular explanation for reported higher drug concentrations and/or the practice of prescribing lower dosage requirements in people of Asian and African heritage [16–19]. On the other hand, the UM phenotypic frequency is much higher in Northeast Africa and Oceania, including the Saudi Arabian (20%) and black Ethiopian (29%) populations when compared to Caucasians (1%–10%) and East Asians (0%–2%).

Even though accounting for a small percent of total CYP content in the liver, CYP2D6 mediates the metabolism of approximately 20%–30% of currently marketed drugs, and *CYP2D6* polymorphism affects significantly the elimination of 50% of these drugs [20], which include antidepressants, antipsychotics, analgesics, antiarrhythmics, antiemetics, and anticancer drugs. Although differences in pharmacokinetic parameters (elimination half-lives, clearances, and areas under the plasma concentration–time curves) for CYP2D6 substrates could be demonstrated among the different metabolic phenotypes, the significant overlap in CYP2D6 activities in EMs and IMs result in therapeutic implication mostly for the PM and UM phenotypes. In the past, the clinical relevance of *CYP2D6* polymorphism primarily concerned the increased prevalence of ADRs in PMs administered standard doses of drugs that rely significantly on CYP2D6 for elimination. These drugs include the antianginal agent perhexiline (neuropathy) [21], the antiarrhythmic agent propafenone (proarrhythmic events) [22], and neuroleptic agents such as perphenazine (sedation and parkinsonism) [23,24].

More recently, occurrences of ADRs have also been highlighted in UMs, primarily a result of a 10–30-fold increase in metabolite concentrations. The most cited example is that of codeine, which is converted by CYP2D6 to the pharmacologically more active metabolite morphine. UMs administered the usual therapeutic dose of codeine have been reported to exhibit symptoms of narcotic overdose associated with significantly elevated morphine concentrations. This toxicity potential had been highlighted in several case reports [25–29], including a fatal case of a breast-fed infant that was attributed to extensive formation of morphine from codeine taken by the mother who is a UM [26]. (Table 1.2) Prior to this unfortunate case, codeine has been considered safe for managing pain associated with childbirth, as literature reported low amounts of codeine are usually found in breast milk. Therefore, this fatal case underscores the importance of understanding how genes can affect pharmacological and therapeutic outcome associated with exposure to drug and/or active metabolite.

Given the high incidence of codeine use in postgestational women, Madadi et al. subsequently performed a case-control study in breast-fed infants with or without central nervous system depression signs and symptoms after exposed to codeine during breast feedings. They reported that breast-fed infants from mothers who are CYP2D6 UMs and homozygous carriers of *UGT2B7*2* (rs7439366; UGT2B7 is a phase 2 enzyme involved in codeine glucuronidation) have an increased risk of potentially life-threatening central nervous system depression [30]. Since 2007, the Food and Drug Administration (FDA) had issued several warning in revised prescribing information for codeine label. Citing the risk of morphine overdose in children and breast-fed infants and warnings from the FDA, the World Health organization, Health Canada, and the European Medicine Agency, the Academy of Pediatrics had recently cautioned the use of codeine in children, regardless of age [31].

Samer et al. reported higher incidence of oxycodone toxicity in UMs that could be partially related to CYP2D6-mediated metabolism to oxymorphone. The toxicity incidence is especially higher in those with concurrent

TABLE 1.2 Summary of Selected Literature on Impact of CYP2D6 Genotype and/or Drug Interaction on Opioid Safety

	Allelic Variants Reported	Adverse Events	References
Codeine	*CYP2D6*1* ×3	Life-threatening opioid intoxication exacerbated by drug interaction (with erythromycin and voriconazole) and renal insufficiency	[25]
	*CYP2D6*2A/*2*×2	Fatality in a breast-fed baby whose mother is a UM	[26]
	*CYP2D6*1* ×N	Fatality in a two-yr-old child due to respiratory arrest	[27]
	CYP2D6 ×2	Occurrence of apnea and brain injury in a 29-mo old child	[28]
	CYP2D6 gene duplication *CYP2D6*1* ×N	Fatality in two children who are UMs. Respiratory depression in an CYP2D6 EM who survived	[29]
Hydrocodone	*CYP2D6*2A/*41*	Fatality in a child who also received concurrent clarithromycin	[33]
Oxycodone	CYP2D6 UMs	Greater toxicity, especially in those administered ketoconazole	[32]
Tramadol	Heterozygous carrier of a wild-type allele duplication	22-yr with a near-fatal case of cardiac arrest and high concentration of tramadol metabolite	[34]
	CYP2D6 gene duplication	Tramadol-related respiratory depression	[35]

ketoconazole administration [32]. Similarly, drug interaction with clarithromycin might have played a role in the fatal case after hydrocodone exposure experienced by a 5-yr-old developmentally delayed child with a *CYP2D6*2A/*41* genotype [33]. In addition, tramadol cardiotoxicity and respiratory depression have been reported in UMs [34,35] with high level of the active O-desmethyltramadol [34], which has been reported to exhibit a high correlation with increased plasma epinephrine level [36]. The FDA also recently updated its safety warning for tramadol.

In addition to implications for ADR, the efficacy of prodrugs (such as codeine and hydrocodone) would also be reduced in PMs because less parent drug is converted by CYP2D6 to its respective active metabolite: morphine or hydromorphone, resulting in little analgesic relief [37]. However, despite strong evidence of a genotype effect on the pharmacokinetics of codeine and hydrocodone, the impact on dosage requirement is much less obvious. In this regard, the value of CYP2D6 genotype lies more with guiding the choice of the appropriate analgesic rather than genotype-based dosage recommendation [13,38]. In particular, avoidance of codeine, the only opioid analgesic with a Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline, is recommended for PMs and UMs. In addition, hydrocodone may not be a good alternative analgesic agent to codeine in these patient populations [13].

There are similar reports of lower efficacy in PMs with venlafaxine [39]. Another example is tamoxifen, in which CYP2D6 plays a major role in the formation of the abundant and pharmacologically more active metabolite, endoxifen [40]. Because endoxifen possesses greater affinity for the estrogen receptor than tamoxifen, PMs with the *CYP2D6*4/*4* genotype have been shown to have an increased risk of breast cancer recurrence and worse relapse-free survival, as well as a much lower incidence of moderate or severe hot flashes [40]. The results of Kiyotani et al. [41] showed that the association between tamoxifen

response and CYP2D6 genotype in Japanese breast cancer patients was only evident for those patients receiving tamoxifen monotherapy, and underscores the importance of considering concomitant drug therapy in pharmacogenomics association study with tamoxifen and possibly other drugs. Conflicting data and continued debate complicate the adoption of CYP2D6 genotyping in the therapeutic use of tamoxifen currently for patients with estrogen-receptor positive breast cancer. Nevertheless, available evidence strongly supports a role for CYP2D6 in pharmacological activation of tamoxifen [42] and possibly a likelihood of lesser therapeutic benefit in PMs [43], with the ultimate impact on patient outcome to be tested in prospective clinical studies.

Inadequate therapeutic response with implications for dosage adjustment had also been demonstrated for UMs administered CYP2D6 substrates. The best evidence described two patients with multiple copies of *CYP2D6*2* requiring the tricyclic antidepressant nortriptyline 500 mg daily (vs. usual recommended daily dose of 100–150 mg) in one patient [44] and clomipramine 300 mg/day (vs. 25–150 mg) in another patient [9] to achieve adequate therapeutic response. Similarly, lower efficacy in UMs has been reported with other antidepressants [45,46] and antiemetics such as ondansetron [47].

Nevertheless, the therapeutic significance of CYP2D6 is not only impacted by genetic polymorphism but also by the potential of CYP2D6-mediated drug–drug interaction, with clinical implications in patients with different metabolic phenotypes resulting from competitive inhibition of CYP2D6. As shown by Hamelin and colleagues [48], the pharmacological consequences of drug–drug interaction via CYP2D6 inhibition are of greater magnitude in EMs, with pronounced and prolonged hemodynamic responses to metoprolol, than in PM.

Potent CYP2D6 inhibitors had been shown to reduce the metabolic capacity of EMs significantly so that individual EM could appear *metabolically* as PM during concurrent administration [49] which could have therapeutic significance in patients taking multiple drugs. For example, it is not uncommon that tamoxifen-treated patients are also taking antidepressants such as selective serotonin reuptake inhibitors (SSRIs), for both their antidepressant effect as well as their offlabel use to manage hot flashes. In view of the abundance and greater antiestrogenic activity of endoxifen, concurrent administration of SSRIs that are potent inhibitors of CYP2D6 (such as fluoxetine and paroxetine) should best be avoided, and SSRIs with a lesser extent of CYP2D6 inhibition (such as citalopram and venlafaxine) would be better alternate antidepressants if there is a need for concurrent antidepressant therapy with tamoxifen.

Interestingly, Gryn et al. described significant reduction in endoxifen concentration in a patient with *CYP2D6*1/*41* genotype. The reported endoxifen concentration was described as "well below levels seen in most CYP2D6 poor metabolizers." Although the case report did not investigate the mechanism for the altered level, the authors suggested it could be secondary to the patient's concurrent treatment with phenytoin. Phenytoin is a potent inducer of multiple drug-metabolizing enzymes as well as the efflux drug-transporter ABCB1 (also known as multidrug resistance transporter, and described in more details in later sections) [50], which mediates the efflux transport of endoxifen [51]. Although the clinical outcome was not described, this case underscores the importance of evaluating the modulating effect of drug interaction when utilizing genotyping in individualized therapy [52]. Similar modulating effects on other genes encoding different metabolizing enzymes are described in later sections.

In addition, it is important to realize that the potential for drug interaction via CYP2D6 inhibition could also be affected by the basal metabolic activity of the individual patient. We have shown that the UM phenotype could affect the potential for drug interaction with paroxetine, a

CYP2D6 substrate as well as a potent CYP2D6 inhibitor, whence a UM with three functional *CYP2D6* copies had undetectable paroxetine concentration with standard dosing and showed no inhibitory effect at CYP2D6 [53].

CYP2C19

CYP2C19 is located on chromosome 10q23.33 and is a large gene consisting of 90,209 nucleotides and yet coding for CYP2C19 that contains only 490 amino acids. Compared to the *CYP2D6* polymorphism, polymorphisms in the *CYP2C19* gene do not affect as many drugs, and their clinical implication has not been extensively evaluated. However, studies involving the proton pump inhibitors (PPIs) provide extensive pharmacokinetic and clinical evidence, as well as the economic impact of the importance of taking into consideration of *CYP2C19* polymorphism in the management of gastroesophageal diseases.

Over the years, as many as 30 *CYP2C19* alleles, including those with no functional activity (**2, *3, *4, *6, *7)* and those associated with reduced catalytic activity (**5* and **8*), have been identified (www.cypalleles.ki.se/cyp2c19.htm). The principal null alleles are **2* (c.681G>A, rs4244285) and **3* (c.636 G>A, rs4986893), resulting in an inactive CYP2C19 enzyme, and accounting for the vast majority of the PM phenotype in Caucasians (1%–6%), black Africans (1%–7.5%), and Asians (10%–25%). Genotyping these two defective alleles has been shown to detect about 84%, greater than 90%, and about 100%, of PMs in Caucasians, Africans, and Asians, respectively. The detection rate for Caucasians PMs could be increased to about 92% by including the less common *CYP2C19*4* (rs28399504) and *CYP2C19*6* (rs72552267) in the genotyping assay. Similar to other *CYP2C19* rare variants, **5* (rs56337013), **7* (rs72558186), and **8* (rs41291556) have <1% allele frequency. Individuals carrying at least one functional allele are referred as EMs, whereas those with one functional and one loss-of-function allele are IMs. Of interest is that, similar to *CYP2D6* polymorphism, a "gain of function" *CYP2C19*17* allele (c.-806C>T rs12248560) was identified in the 5′-flanking region of *CYP2C19*, with increased gene transcription associated with high enzyme activity and an EM phenotype [54].

*CYP2C19*2* and **3* are commonly found in Asians, with allele frequencies of about 30% and approximately 10%, respectively. In contrast, the allele frequency of **3* is <1% in Caucasians and African Americans, even though the **2* occurs at a frequency of about 13% and approximately 18%, respectively, in these two ethnic groups. About 50% of the Chinese population possess either the **1/*2* and **1/*3* genotypes, and 24% have the **2/*2*, **2/*3*, or **3/*3* genotypes [55]. In contrast, only about 2%–5% and 30%–40% of the Caucasian population, respectively, have the **2/*2* and **1/*2* genotypes. Similar frequencies of the heterozygous and homozygous variant genotypes are reported in persons of African descent. The higher prevalence of PMs and heterozygote EMs carrying defective *CYP2C19* alleles in Asians likely account for reports of slower rates of metabolism of CYP2C19 substrates and the practice of prescribing lower diazepam dosages for patients of Chinese heritage [56,57]. An opposite direction in ethnic variation was observed in the prevalence of *CYP2C19*17* (18% in Swedes and Ethiopians vs. 4% in Chinese), with the **1/*17* and **17/*17* genotypes occurring in more Caucasians and Ethiopians (up to 36%) than Asians (8% of Chinese and 1% of Japanese) [54].

CYP2C19 accounts for about 3% of total hepatic CYP content, and *CYP2C19* polymorphism affects the metabolism of PPIs (omeprazole, lansoprazole, pantoprazole, rabeprazole), antidepressants (citalopram, sertraline, moclobemide, amitriptyline, clomipramine), the antiplatelet agent clopidogrel, the antifungal drug voriconazole, the benzodiazepine diazepam, and the anticancer drug cyclophosphamide. Similar to CYP2D6, CYP2C19 is also susceptible to inhibition by drugs such as cimetidine, fluoxetine, and diazepam. The inhibition occurs in a gene

dose-dependent manner in which carriers of two *CYP2C19*17* alleles exhibit the greatest extent of inhibition compared to little to no inhibition for patients with CYP2C19 PM phenotype.

The PPIs and clopidogrel provide the best examples of clinical relevance of *CYP2C19* polymorphism. When compared to EMs, PMs showed 5- to 12-fold increases in the area under the curve (AUC) of omeprazole, lanzoprazole, and pantoprazole [58,59], whereas homozygous carriers of the *CYP2C19*17* were shown to have a modest 2.1-fold lower AUC than EMs [60]. In addition, the *CYP2C19* genotype significantly affects the achievable intragastric pH with PPI therapy. In subjects who took a single 20-mg dose of omeprazole, Furuta et al. showed a good relationship not only between *CYP2C19* genotype and AUC, but also between the genotype and achievable intragastric pH: 4.5 in PMs, 3.3 in heterozygous EMs, and 2.1 in homozygous EMs [61]. Given the smaller dependency of esomeprazole and rabeprazole on CYP2C19 for metabolism, the pharmacological action of these two PPIs is less affected by the *CYP2C19* polymorphism [62,63].

An important treatment strategy in the management of patients with peptic ulcer disease is eradication of *Helicobacter pylori* with a regimen of PPI and antibiotics. *CYP2C19* genotype-related pharmacological effects have also been associated with improved eradication rate of *H. pylori* after dual [64] or triple therapy including omeprazole [65], lansoprazole [66], or pantoprazole [67]. The cure rate achieved with dual- and triple-therapy regimens was 100% in PMs compared with 29%–84% in EMs [64–67]. Furata et al. also reported a much higher eradication rate of 97% in EMs who failed initial triple therapy (lansoprazole, clarithromycin, and amoxicillin) and subsequently were retreated with high-dose lansoprazole (30 mg four times daily) and amoxicillin [68]. In addition, to showing a gene-dose effect in achieving desirable ranges of intragastric pH and *H. pylori* cure rates for lansoprazole, Furuta et al. also demonstrated the cost effectiveness of pharmacogenomics-guided dosing when compared to conventional dosing [69]. On the other hand, despite increased metabolism of PPI in carriers of *CYP2C19*17* and the potential of therapeutic failure [54,70], eradication rates of *H. pylori* have so far not to be shown to be associated with the *CYP2C19*17* allele, at least for patients with peptic ulcer disease and receiving the triple regimen of pantoprazole, amoxicillin, and metronidazole [67,71].

In healthy volunteers given a single 200-mg dose of voriconazole, Wang et al. demonstrated a 48% lower AUC in heterozygous carriers of the *CYP2C19*17* allele as compared to homozygous carriers of *CYP2C19*1* [72]. This finding is consistent with data that is more recent showing correlation between *CYP2C19* polymorphism and target voriconazole concentrations, with an increased risk of subtherapeutic trough concentration in patients with the CYP2C19 UM phenotype [73–75]. Investigators have also shown 42% lower escitalopram concentrations and 21% lower AUC in patients who are homozygous carriers of *CYP2C19*17* when compared to *CYP2C19*1* homozygotes [76]. Clearly, *CYP2C19*17* homozygotes might require higher doses of most CYP2C19 substrates, including PPIs [60,70], antidepressants, and voriconazole [72]. However, despite the presence of pharmacokinetic differences, the impact of *CYP2C19*17* on therapeutic outcomes with these CYP2C19 substrates have not been evaluated extensively or confirmed.

Clopidogrel is an antiplatelet prodrug that requires CYP2C19-mediated conversion to its active metabolite for therapeutic effect [77], with most of pharmacokinetic and pharmacodynamic evidence related to the *CYP2C19*2* allele [77–82]. Shuldiner et al. conducted a GWAS in which *ex vivo* adenosine diphosphate (ADP)-induced platelet aggregation at baseline and after 7 days of clopidogrel were measured in a genetically homogenous cohort of 429 healthy Amish subjects. In addition, 400,230 SNPs were evaluated in each subject for association with

platelet activity. They reported that the SNP rs12777823 on chromosome 10q24 with the greatest association signal is in strong LD with *CYP2C19*2*, accounting for 12% of the interindividual variation in platelet aggregation during clopidogrel treatment. As importantly, there was no association between the *CYP2C19* polymorphism and baseline platelet aggregation [83]. The results from this GWAS confirmed results from previous candidate gene studies regarding the role of *CYP2C19* as a major genetic determinant of clopidogrel response [78–82]. In a follow-up study of 227 patients undergoing percutaneous coronary intervention (PCI), the investigators also reported a higher incidence of cardiovascular death in carriers of the *2 allele (20.9% vs. 10%) at 1-yr follow-up. No association with response was found for other *CYP2C19* alleles, including *3, *5* (rs56337013), and *17*, that were also genotyped in the study [83]. A recent meta-analysis confirms the association of the *CYP2C19* nonfunctional allele and high-risk of adverse cardiovascular events in patients who underwent PCI [84].

Although the increased production of the active clopidogrel metabolite in carriers of the *17* allele has been associated with greater inhibition of platelet aggregation [85,86] and better clinical outcomes [87], there is also the potential of increased bleeding risk [88]. In addition, the increased response of the *17* allele has been suggested not as a direct effect, but rather attributed to that of the *1* allele [89]. Given this consideration, there is no specific therapeutic recommendation for this gain-of-function allele in the most recent practice guideline for *CYP2C19* genotyping [90].

Even with involvement of other non-genetic factors [91], the increased risks of major adverse cardiovascular events and stent thrombosis in carriers of at least one *CYP2C19*2* allele were confirmed in two meta-analyses that included almost 22,000 patients [88,92]. Differences in patient selection for analysis likely account for the lack of association reported in two other recent meta-analyses, which included a significant number of low-risk patients, such as those with acute coronary syndrome managed medically or patients with atrial fibrillation [93,94]. The meta-analysis of Hulot et al. [92] also evaluated the drug interaction potential of PPIs because of their inhibitory effect toward CYP2C19, resulting in a metabolic phenotype of CYP2C19 PM similar to that of carriers of the *2 allele. Both Hulot et al. and another study [92,95] suggest that the detrimental effects of PPIs on cardiovascular outcomes with clopidogrel likely occur at a higher frequency in high-risk patients receiving both drugs. Current data do not provide sufficient information to determine whether the observed adverse effects of PPI usage in high-risk patients (e.g., patients undergoing PCI) are related to CYP2C19 inhibition or yet-to-be-discovered mechanisms.

Based on the increasing amount of literature data supporting an association between *CYP2C19*2* and poor clopidogrel response, the FDA has made several revisions to the approved product label of clopidogrel. Although the March 2010 version specifically addresses the implication for homozygotes, there is no guidance on the implication for heterozygotes. In addition, as with other revised labels with additional genetic information, there is little guidance on clinical management of carriers of *CYP2C19*2*. The September 2016 label warns of diminished effectiveness in CYP2C19 poor metabolizers and suggests the use of different platelet P2Y12 inhibitors in those patients. In light of the scientific and clinical evidences as well as the regulatory decision, several recent clinical studies addressing alternative antiplatelet agents have been initiated and are discussed in Chapter 6.

CYP2C9

In addition to CYP2C19, another important member of the CYP2C subfamily of enzymes is CYP2C9 containing 490 amino acids. It is encoded by *CYP2C9* consisting of 50,708 nucleotides and located on chromosome 10q24.1 in close

proximity to *CYP2C19*. To date, approximately 60 *CYP2C9* alleles (www.cypalleles.ki.se/cyp2c9.htm) have been identified in the regulatory and coding regions of *CYP2C9*, with *CYP2C9*2* (c.430C>T, rs1799853) and *CYP2C9*3* (c.1075A>C, rs1057910) being the most common in persons of European descent and the most extensively studied. Both reduced-function alleles exhibit single amino-acid substitutions (p.R144C and p.I359L, respectively) in the coding region, accounting for lower enzyme activity by approximately 30% for *2 and 80% for *3 [96]. Other reduced-function alleles of potential importance included *5 (rs28371686), *6 (rs9332131), *8 (rs7900194), and *11 (rs28371685). [97–100] In addition, a "gain-of-function" *CYP2C9* (rs7089580) variant in intron 3 has been identified [97].

Significant variations in *CYP2C9* alleles and genotype frequencies exist among different ancestry groups. Both *CYP2C9*2* and *CYP2C9*3* are more common in Caucasians (11% and 7%, respectively) than in Asians and Africans. In fact, *CYP2C9*2* has not been detected in Asians, in whom *CYP2C9*3* is the most common allele. On the other hand, *CYP2C9*8*, as well as *5, *6, and *11 (albeit all at a lower frequency than *8*), are present almost exclusively in African Americans. The novel *CYP2C9* c.18786A>T variant (rs7089580) was reported to occur in about 40% of the African American population, and *CYP2C9*8* (c.449G>A, rs7900194) appears to be a major contributor to CYP2C9 expression in this ethnic group [97]. Approximately 1% and 0.4% of Caucasians have the *2/*2 and *3/*3 genotypes, respectively. The *1/*3 genotype occurs at a frequency of 4% in the Chinese and Japanese populations, with almost complete absence of the other genotypes (*2/*2, *2/*3, *1/*2, and *3/*3).

CYP2C9 accounts for about 20% of total hepatic CYP content and is involved in the metabolism of about 10% of currently marketed drugs. These CYP2C9 substrates include the nonsteroidal antiinflammatory drugs such as celecoxib, ibuprofen, and flurbiprofen; oral anticoagulants such as acenocoumarol, and phenprocoumon, and the S-isomer of warfarin; oral antidiabetic agents such as glibenclamide, glimepiride, glipizide, glyburide and tolbutamide; antiepileptic agents such as phenytoin, and antihypertensive agents such as candesartan, irbesartan, and losartan. The enzyme reduction associated with the *3 allele is greater than that with the *2 allele, with a 5- to 10-fold reduction in homozygous *3 carriers and two-fold reduction in heterozygous *3 carriers, when compared to homozygous *1 carriers. For example, clearance of warfarin is reduced by 90%, 75%, and 40% in subjects with the corresponding *CYP2C9* genotypes of *3/*3, *1/*3, and *1/*2 [101]. respectively. Interestingly, the effects of several reduced-function alleles appear to be substrate dependent. For the *2 allele, a significant effect was shown for clearances of acenocoumarol, tolbutamide, and warfarin but not for other substrates. On the other hand, nonsteroidal anti-inflammatory drug (NSAID)-associated gastrointestinal bleeding was shown to be related to the *3 but not the *2 variant [102]. Similarly, although the *8 allele has no effect on clearance of losartan, it decreases enzyme activity of warfarin and phenytoin, and exhibits an increased activity toward tolbutamide [103].

Of all of the CYP2C9 substrates, warfarin is the most extensively studied with dosing implications for different metabolic phenotypes. *CYP2C9* polymorphism, together with the literature information regarding the gene that encodes the warfarin target, vitamin K epoxide reductase complex (VKORC1) [104], provide promising translational use of the pharmacogenomic data [105,106], with revised language regarding their impact incorporated into the drug label [107]. CYP2C9 mediates the conversion of the active *S*-enantiomer of warfarin to an inactive metabolite. Most of the data document that the *2 and *3 alleles are associated with greater difficulty with warfarin induction therapy, increased time to achieve stable dosing, lower mean-dose requirement (e.g., as low as ≤1.5 mg/day with *3/*3), as well as increased risks of elevated , international normalized ratios (INRs) and bleeding [105,108,109]. Giving the 30%

and 80% difference in enzyme activity reduction between the *2 and *3 alleles, the warfarin-dose requirements differ between carriers of these two alleles. Compared to homozygous carriers of the *1 allele, data suggest a dose reduction of 30% and 47% for patients with the heterozygous genotypes of *CYP2C9*1/*2* and *CYP2C9*1/*3*, respectively, and up to 80% for patients with the homozygous *CYP2C9*3/*3* genotype [106,108,110,111].

In addition, with the difference in allele prevalence among different ancestral groups, the strength of association between the *2 and *3 alleles and genotypes is stronger in Caucasians [112,113]. Other recently identified alleles (*5, *6, *8, and *11) have been reported to better predict dose requirement (20% lower for *8 carrier) and adverse outcomes in African Americans [97–99,103,112,114]. On the other hand, the "gain-of-function" *CYP2C9* c.18786A>T allele was reported to contribute a higher-dose requirement (3.7 mg/week/allele) [97]. Finally, concurrent drugs with significant modulating effect on CYP2C9 activity would also have an impact on the association between *CYP2C9* genotypes and warfarin-dose requirement [115]. The effect of *CYP4F2* and *VKORC1* genotypes on warfarin pharmacokinetics and pharmacodynamics will be discussed in later sections of this chapter.

CYP2C8

In addition to *CYP2C9* and *CYP2C19*, the other clinically relevant members of the highly homologous genes (*CYP2C18–CYP2C19–CYP2C9–CYP2C8*) that cluster on chromosome 10q24 [83] is *CYP2C8*. To date, several SNPs within the coding region of the *CYP2C8* gene have been identified (www.cypalleles.ki.se/cyp2c8.htm). The more common variants are *2 (c.805A> T, rs11572103, resulting in p.I269F), *3 with two amino acid substitutions (c.416G >A, rs11572080 with p.R139K, and c.1196A >G, rs10509681 with p.K399R) reportedly to be in total LD, and *4 (c.792C >G, rs1058930, p.I264M). Both *3 and *4 alleles are more common in Caucasians (with the *4 variant reportedly only found in Caucasians). On the other hand, *2 and a rare allele, *5 (rs72558196, frame-shift deletion) are exclusively found in Africans and Japanese, respectively [116,117].

Accounting for about 7% of total hepatic content, the hepatic expression level of CYP2C8 lies between that of CYP2C19 and CYP2C9 [118], and it plays an important role in the metabolism of different drugs, primarily the antidiabetic agents (pioglitazone, repaglinide, rosiglitazone, and troglitazone), the anticancer agents (paclitaxel), the antiarrhythmic drug amiodarone, and the antimalarial agents amodiaquine and chloroquine. The smaller number of substrates as compared to CYP2C9 and CYP2C19 presumably leads to the lesser interest in studying *CYP2C8* polymorphism. As a result, the molecular mechanisms underlying interindividual variations in CYP2C8 activity remain unclear. Decreased elimination of R-ibuprofen has been reported in carriers of *CYP2C8*3* [119,120]. However, with the presence of a strong LD between *CYP2C8*3* and *CYP2C9*2* [119,121], the individual contribution of *CYP2C8*3* remains to be elucidated. In contrast, increased metabolism of repaglinide was reported in heterozygous carriers of *CYP2C8*3* when compared to carriers of either *1 or *4 [122]. Although this finding is interesting, other reports showed that genetic polymorphism of the hepatic uptake transporter plays a more important role in determining repaglinide pharmacokinetics [123]. The identification of two *CYP2C8* haplotypes: a high-activity allele associated with *CYP2C8*1B* and a low activity associated with *CYP2C8*4* [124], further highlights the need to characterize the different *CYP2C8* variants, including their functional relevance.

CYP3A4/5/7

A total of four CYP3A genes have been described in humans: *CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP3A43*; with *CYP3A7* primarily important in fetal CYP3A metabolism and

CYP3A43 exhibiting little functional or clinical relevance. More than 20 variants in the coding region of *CYP3A4*, most of them associated with reduced catalytic activity of the enzyme, have been identified to date. [125] The significance of the reduced-function allele *CYP3A4*22* C>T SNP (rs35599367) in intron 6, which results in 20% decrease in enzyme activity, has been extensively evaluated recently, especially in conjunction with *CYP3A5* SNP [126–129]. *CYP3A5* expression is highly polymorphic with the loss-of-function **3* allele (c.6986A>G, rs776746) in intron 3 as the most common variant, which results in a splicing defect and absence of enzyme activity. Other loss-of-function and reduced-function *CYP3A5* variants include the **2* (rs28365083; g.27289C>A; T398N), **6* (14690G>A; rs10264272), and **7* (rs41303343; 27131_27132ins T) alleles [130,131].

In general, *CYP3A4* polymorphism is more common in Caucasians, with **2* and **7* being the more prevalent alleles, whereas Asians have higher frequencies of **16* and **18* variants. Of note, is that *CYP3A4*22* is absent in both Asian and African populations. Carriers of the wild-type *CYP3A5*1* allele (also known as CYP3A5 "expressors") are more common in Asians (up to 50%) and Blacks (up to 90%) than in Caucasians (about 15%). The allele frequency of *CYP3A5*3* is much higher in Caucasians and Asians, occurring in 90% and 75% of the populations, respectively, *versus* a relatively low frequency of 20% in Africans. On the other hand, both *CYP3A5*6* and **7* are absent in Caucasians and Asians but present in Africans with frequencies up to 17% [130,132,133]. The *CYP3A5*2* allele has a frequency of less than 1% in Caucasians and is mostly absent in other ethnic populations.

CYP3A4 accounts for about 40% of the total hepatic CYP content and mediates the metabolism of more than 50% of currently used drugs with many examples from the pharmacological classes of macrolide antibiotics, antidepressants, antipsychotics, anxiolytics, calcium channel blockers, immunosuppressants, opiates, and the statins. The current consensus is that *CYP3A4* polymorphisms are mostly of minor clinical relevance, and unlikely responsible for the 10- to 40-fold interindividual variations in CYP3A4 activities. This is likely a result of low variant allele frequencies, only small changes in enzyme activity in the presence of a variant allele, as well as the overlapping substrate specificity between CYP3A4 and CYP3A5. The significant variability in CYP3A4 activity is more likely related to a large number of drugs capable of altering the enzyme through induction or inhibition in the liver and the gastrointestinal tract. Therefore, there is currently no uniform agreement on metabolizer subgroups for CYP3A4.

On the other hand, the clinical relevance of CYP3A genetic polymorphism is primarily associated with *CYP3A5*. The pharmacokinetics of the immunosuppressive agent tacrolimus is dependent on the *CYP3A5* genotype, with a higher-dosage requirement in homozygous or heterozygous carriers of *CYP3A5*1* [134,135]. In addition, results from a randomized controlled trial showed that pharmacogenetic-guided dosing based on *CYP3A5* genotype was associated with greater achievement of target tacrolimus concentrations when compared to standard dosing based on body weight [136]. Nevertheless, the overall clinical relevance of *CYP3A5* polymorphism is limited by its small contribution (2%–3%) to the total CYP3A metabolism. [137,138], and reportedly impacted by timing of tacrolimus therapy. In a meta-analysis of tacrolimus-dose requirement and rejection rate, Tang et al. indicated that the effect of *CYP3A5* polymorphism (*CYP3A5*3*) is most prominent during the first month of tacrolimus therapy, suggesting that *CYP3A5* genotyping might be useful to guide initial dosing of tacrolimus for prevention of early graft rejection [139]. Inclusion of both *CYP3A4*22* and *CYP3A5*3* status have been shown in many recent studies to significantly improve tacrolimus dose prediction [126–129,140]. Therapeutic and pharmacogenomic recommendations for tactolimus were included in the recent CPIC guideline [141].

On the other hand, despite significant effect of *CYP3A4*1G* (g.20230G>A, rs2242480) and *CYP3A5*3* on ticagrelor pharmacokinetics in a recent study of healthy Chinese subjects, there was no association on the extent of inhibition of platelet aggregation. Therefore, the investigators concluded that no dosage adjustment based on *CYP3A4* and *CYP3A5* genotypes is necessary [142].

CYP4F2

There are six members within the *CYP4F* gene subfamily residing on chromosome 19p13.1-2: *CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12*, and *CYP4F22*. The importance of CYP4F2, a vitamin-K oxidase, is related to the recent report of its role in mediating the conversion of vitamin K_1 to hydroxyvitamin K_1. Increased CYP4F2 activity causes decreased activation of vitamin K-dependent clotting factors, reflecting the consequence of reduced availability and reduction of vitamin K_1 to vitamin KH_2 necessary for carboxylation and activation of the clotting factors. On the other hand, the g.7253233C>T (rs2108622, p.V433M) SNP in exon 2 of the *CYP4F2* gene results in lower protein expression and enzyme activity, and consequently greater vitamin K_1 availability [143,144]. The T allele at rs2108622 confers the *CYP4F2*3* designation. Some ethnic differences in the V433M SNP has been reported, with the M433 allele occurring at a much lower frequency in African Americans [114], which contrast with its high occurrence in Indonesians and Egyptians [145,146].

Although genome-wide association studies enable detection of weaker genetic signals [144], *CYP4F2* genotype nevertheless only accounts for 1%–3% of the overall variability of warfarin-dose requirement [144,147], in contrast to *CYP2C9* genotype that accounts for approximately 10%–12% of the variability. Homozygous carriers of the M allele of the p.V433M SNP had been shown to require an approximate 1 mg/day higher dose of warfarin than homozygous carriers of the V allele [148]. However, additional studies demonstrated the association between the *CYP4F2* genotypes and dose requirements in Caucasians and Asians [144,147,149,150] but not in African Americans, Egyptians, or Indonesians [114,145,146]. This could reflect ethnic differences in *CYP4F2* allele and genotype frequencies distribution, the minor contribution of *CYP4F2* [151], as well as the modulating effects of other more important dose-requirement variables such as *CYP2C9* and *VKORC1*.

CYP2B6

Although several variant alleles with low enzyme expression, including *CYP2B6*6* and **18*, have been identified, to date there have not been any reports of the presence of an important loss-of-function allele. Among the variant alleles, the *CYP2B6*6* haplotype carrying two nonsynonymous SNPs (c.516G>T, rs3745274 and c.785A>G, rs2279343 causing two amino acid changes: p.Q172H and p.K262R, respectively) in exon 4 is the most common and occurs commonly in Caucasians and Asians (16%–26% allele frequency), whereas**18* (c.983T>C, rs28399499, I328T) is more common in Black subjects with allele frequencies of 7%–9% [152]. Interestingly, the 785A>G SNP resulting in the K262R amino acid change also occurs as a separate allele, *CYP2B6*4* (rs2279343 without rs3745274), and results in increased expression and enzyme activity [153,154]. Whether the 516G>T and 785A>G mutations are linked to additional mutations creating specific haplotypes causing either high or low CYP2B6 activities is not known. Gatanaga et al. also reported a new **26* allele containing 499G for the c.499C>G SNP (rs3826711), and 499G always coexists with 516G>T and 785A>G, thus representing a novel haplotype containing the 499C>G, 516G>T and 785A>G SNPs [155].

CYP2B6 accounts for up to 6%–10% of total CYP content in the liver [156,157], and known substrates include anticancer drugs such as

cyclophosphamide and ifosfamide, the smoking cessation agent bupropion, the antiretroviral agents efavirenz and nevirapine, as well as methadone. In addition to reduced activation of cyclophosphamide leading to lower antitumor efficacy, *CYP2B6* gene variants play a significant role in determining bupropion and methadone pharmacokinetic variabilities, in particular with *CYP2B6*6* (decreased clearance) [158–160] and *CYP2B6*4* (increased clearance) [159]. Levran et al. reported that the mean daily methadone dose in heroin addicts was 88 and 96 mg, respectively, for homozygous carriers of variant alleles 785A>G and 516G>T; as compared to 133 and 129 mg, respectively, for heterozygous carriers of the two variant alleles; and 150 and 151 mg, respectively, for wild-type homozygotes [161]. In individuals whose death was attributed to methadone poisoning, *CYP2B6*4*, **6*, and **9* alleles were associated with higher postmortem methadone blood concentrations ($P \leq .05$) [162]. However, despite report of longer corrected QT interval (QTc) interval in CYP2B6 slow metabolizers [163], a clear relationship between *CYP2B6* genotype and risk of cardiac arrhythmia and sudden death remains to be determined.

The potential clinical relevance of CYP2B6 has been evaluated primarily with the nonnucleoside reverse transcriptase inhibitors efavirenz and nevirapine. Increased central nervous system side effects associated with variable systemic exposure of efavirenz could be the result of patients being carriers of the **6* or **18* alleles [155,164]. Incorporating determination of additional less-frequent alleles such as **26* and **29* could further improve the prediction of elevated plasma efavirenz concentrations [155,164,165]. Altered concentrations of, and clinical outcome associated with, nevirapine have also been associated with *CYP2B6* rs3745274 SNP.

In a prospective study of the effect of *CYP2B6* polymorphism on efavirenz concentrations and exposure, 456 patients infected with the human immunodeficiency virus type 1 (HIV-1) were genotyped for different SNPs, including the 499C>G, 15631G>T and 18053A>G polymorphisms [155]. All patients received the standard-dosage regimen of 600 mg/day, and extremely high concentrations (9,500 ± 2,580 ng/mL) were obtained in all 14 patients with the *CYP2B6*6/*6* genotype and in both patients with the *CYP2B6*6/*26* genotype. In contrast, only two patients with other *CYP2B6* genotypes had similarly high efavirenz concentrations, and both were heterozygous carrier of either the **6* allele (7,140 ng/mL) or **26* allele (9,710 ng/mL). Therefore, the **6* and **26* alleles were both associated with high efavirenz concentrations, and patients with the *CYP2B6*6/*6* or the *CYP2B6*6/*26* genotype had the highest concentrations with standard-dosage regimen of 600 mg/day.

To investigate the feasibility of dose reduction in patients with high efavirenz concentrations secondary to *CYP2B6* polymorphism, the investigators then reduced the efavirenz-dosage regimen to 400-mg/day in five patients and to 200 mg/day in another seven patients. The genotypes in these 12 patients included nine **6/*6* homozygotes, two **6/*26* heterozygotes, and one **1/*26* heterozygote. The plasma concentrations decreased proportionally with the dose reductions. Despite receiving the lower-dosage regimens for more than 6 months, the 12 patients were able to maintain therapeutically effective anti-HIV-1 activity with HIV-1 load continuously less than 50 copies/mL. Central nervous system side effects were reported to be much less frequent at the lower-dosage regimens. Similar therapeutic success with persistent suppressed HIV-1 load was also demonstrated in efavirenz-naïve patients (**6/*6* and **6/*26*), who were administered the lower-dosage regimen of 400-mg/day. The overall study results demonstrated the feasibility of genotype-based efavirenz-dose reduction in patients with *CYP2B6 *6/*6* and **6/*26* genotypes, with additional advantages of less central nervous system side effects and lower treatment cost.

CYP2A6

CYP2A6 only accounts for about 4% of total CYP450 content, and significant variations in CYP2A6 activity are primarily a result of genetic influence. The *CYP2A6* gene is located on chromosome 19 and codes for the protein CYP2A6 consisting of 494 amino acids. With more than 40 variants identified, the primary variants for *CYP2A6* polymorphism (www.cypalleles.ki.se) include *CYP2A6*2* (rs1801272, g.1799T >A), *CYP2A6*4* (gene deletion), *CYP2A6*5* (rs5031017 g.6582G > T), and *CYP2A6*20* (rs28399444, frame shift), all of which are associated with abolished enzyme activity. Additional alleles associated with reduced enzyme activity include *7 (rs5031016, g.6558 T>C), *10 (rs28399468, g.6600G>T) *11 (rs111033610. g.3391T>C), *17 (rs28399454, g.5065G>A), *18 (rs1809810 g.5668A>T), and *19 (rs5031016 g.6558T>C). As with other CYP polymorphisms, there are substantial interethnic differences in allele frequency. Deletion of the *CYP2A6* gene is very common in Asian patients [166], which likely accounts for the dramatic difference in the high occurrence of PMs in Asian (20%) versus Caucasian populations (≤1%).

Nicotine is metabolized by CYP2A6 to cotinine, and the clinical relevance of the *CYP2A6* polymorphism has been primarily investigated in managing patients with tobacco abuse. Nonsmokers were found to be more likely to carry defective *CYP2A6* alleles such as *7 and *9 than were smokers. In addition, smokers with defective *CYP2A6* alleles smoked fewer cigarettes and were more likely to quit. These results likely reflect higher nicotine concentrations, enhanced nicotine tolerance and increased adverse effects from nicotine in CYP2A6 poor metabolizers. Based on these observations, CYP2A6 inhibition may have a role in the management of tobacco dependency [166].

CYP1A2

Located on chromosome 15, *CYP1A2* consists of 7,758 nucleotides and encodes the enzyme CYP1A2 that contains 516 amino acids. Polymorphisms of the CYP1 family of genes have been studied for association with cancer susceptibility. Several *CYP1A2* SNPs have been identified, including *CYP1A2*1C* (rs2069514, –3860G>A) and the haplotype *CYP1A2*1K* containing three variants: –739T>G (rs2069526), –729C>T (rs12720461), and –163C>A (rs762551). However, to date there has been no consistent report of any functional *CYP1A2* alleles that result in important changes in gene expression and enzyme activity. Therefore, in contrast to other CYP isoenzymes such as CYP2C19 and CYP2C9, there is less agreement in the literature regarding acceptable method of defining CYP1A2 metabolic phenotype by *CYP1A2* genotype.

Nevertheless, a unique aspect of the *CYP1A2* gene is that a specific allele, *CYP1A2*1F* (rs762551) containing a c.-163C>A mutation in intron 1, has been shown to affect CYP1A2 inducibility [167] and the magnitude of increased caffeine metabolism in smokers [168,169]. However, conflicting reports have been reported for other CYP1A2 substrates [170–172]. This gene–environment interaction makes genotype–phenotype prediction of phenotype much more difficult. Finally, promoter variation is less likely to result in substrate-dependent effects, and the functional importance of increased CYP1A2 inducibility is currently unknown.

CYP1A2 contributes up to 10% of the total hepatic P450 content. However, unlike other CYP isoenzymes, it only mediates the metabolism of several commonly used drugs such as olanzapine, clozapine, duloxetine, and theophylline [173,174]. Although pharmacokinetic studies evaluating CYP1A2 inducibility by smoking or omeprazole have been performed, none of the studies have produced consensus information.

POLYMORPHISMS IN NON-CYP450 DRUG-METABOLIZING ENZYMES

Genetic polymorphisms in many non-P450 enzymes also play a role in influencing metabolism and elimination of many drugs. Among these enzymes, UDP-glucuronosyl transferase (UGT), thiopurine-*S*-methyltransferase (TPMT), dihydropyrimidine dehydrogenase (DPD), *N*-acetyltransferase (NAT), and glutathione-s-transferase (GST) have been characterized and their clinical relevance studied.

UDP-Glucuronosyl Transferase

The uridine diphosphate (UDP)-glucuronosyl transferase (UGT) enzymes are divided into two distinct subfamilies: UGT1 and UGT2. UGT1A1 has been the most extensively investigated among the UGT1A enzymes. A polymorphism with an extra thymine-adenine (TA) repeat (TA insertion) in the 5′-promoter region of the *UGT1A1* gene results in the *(TA)^{7}TAA* allele or *UGT1A1*28* (rs8175347), with a 35% decrease in transcriptional activity of *UGT1A1* and lower enzyme activity than the wild-type *(TA)^{6}TAA* allele (*UGT1A1*1*) [175]. Another UGT1A1 polymorphism, *UGT1A1*6* (rs4148323) carrying the c.211G>A SNP and p.G71R substitution in exon 1, has also been associated with lower enzymatic activity [176]. Although the **28* variant is more common in Caucasians (29%–40%) and Africans (36%–43%) than in Asians (13%–16%), the **6* is found only in Asians with a frequency of 16%–23%.

UGT contributes about 35% of phase II drug metabolism and is involved in glucuronidation of endogenous compounds and xenobiotics. For UGT1A1, the substrates include bilirubin, SN-38 (active and toxic metabolite of the anticancer drug irinotecan), raltegravir (inhibitor of the HIV integrase enzyme), clozapine, bazedoxifene (an investigational selective estrogen receptor modulator for prevention and treatment of postmenopausal osteoporosis), and eltrombopag (a thrombopoietin receptor agonist for the management of thrombocytopenia).

Irinotecan, also known as CPT-11, is a prodrug that requires metabolic activation via carboxylesterase to SN-38, a potent inhibitor of topoisomerase I. SN-38 is inactivated via glucuronidation by the polymorphic *UGT1A1* enzyme. Both *UGT1A1*28* and **6* had been associated with impaired SN-38 glucuronidation, especially in patients who are homozygous carriers (*UGT1A1*28 TA7/TA7*) [176,177]. The ensuing high SN-38 concentrations lead to increased SN-38 excretion into the gut lumen, predisposing patients to severe diarrhea even with standard irinotecan-dosage regimens. Abnormally high SN-38 concentrations have also been reported in patients with severe neutropenia [178]. These pharmacogenetic-related adverse reactions have also been demonstrated in prospective clinical trial [179] that led to FDA approval of the Invader UGT1A1 Molecular Assay (Third Wave Technologies) for genotyping *UGT1A1* alleles and revision of the irinotecan product label to include consideration of lower initial dose requirement for individuals who are homozygous for *UGT1A1*28*, although the genetic testing is not a requirement. The predictive value of *UGT1A1*28* polymorphism has recently been confirmed in a meta-analysis of 58 studies [180]. With the involvement of UGT1A1 in bilirubin glucuronidation confirmed by three meta-analyses [181–183] and the prevalence of *UGT1A1*6* among Asian populations, UGT1A1 may play a role in the high incidence of neonatal hyperbilirubinemia in those populations [184].

A different UGT enzyme, UGT2B7, plays an important role in mediating the conversion of morphine to the pharmacologically active metabolite, morphine-6-glucuronide. Darbari et al. reported that homozygous and heterozygous carriers of the G variant for the -840 G>A SNP (rs7438135) had significant higher parent to metabolite concentration ratio compared to individuals with the *A/A* genotype ($P = .03$) [185]. A second *UGT2B7* SNP (rs7439366, C802T) was

implicated for morphine toxicity in a patient with the *T/T* genotype that resulted in increased morphine-6-glucuronide formation [186].

Thiopurine-*S*-methyltransferase

Thiopurine-S-methyltransferase (TPMT) is encoded by the *TPMT* gene that has a nonsynonymous SNP and resulting in reduced TPMT enzymatic activity. Although more than 31 variants of the *TPMT* gene have been identified, the five most studied one are *TPMT**2 (rs1800462, G238>C, reduced activity), *TPMT*3A* (a haplotype consisting of the two nonsynonymous SNPs, G460>A and A719>G, abolished activity), *TPMT*3B* (rs1800460, G460>A, reduced activity) *TPMT*3C* (rs1142345, A719>G, reduced activity), and *TPMT*4* (rs1800584, G626>A, very low activity). About 95% of intermediate or low TPMT activity in affected patients are associated with *TPMT**2, *TPMT*3A*, or *TPMT*3C*. Approximately 10% and 0.3% of the Caucasian population is heterozygous and homozygous, respectively, of these mutant alleles.

TPMT mediates the inactivation of thiopurine drugs, including thioguanine, 6-mercaptopurine, and its precursor, azathioprine. Compared to patients who possess the wild-type alleles, homozygotes or heterozygotes for the *TPMT* mutant alleles have much higher levels of the cytotoxic thiopurine nucleotides and are at higher risk for developing serious hematological toxicities during treatment with standard-dosage regimens of the thiopurine drugs [187]. As a result, patients with absent and low TPMT activity can only tolerate 5 and 50% of the standard 6-mercaptopurine regimen.

The TPMT metabolism represents one of the most-investigated drug metabolic pathways that demonstrates the clinical relevance of genetic polymorphism. Currently, TPMT is the only drug-metabolizing enzyme with significant acceptance and widespread testing in clinical practice, with genotyping or phenotyping (determination of TPMT activity in red blood cells) recommended by the FDA, and the thiopurine drugs are one of several medications that have clinical guidelines available through the CPIC [188,189].

Dihydropyrimidine Dehydrogenase (DYPD)

In addition to being the initial and rate-limiting enzyme that catalyzes pyrimidines such as uracil and thymine, dihydropyrimidine dehydrogenase (DYPD), a minor phase I metabolizing enzyme, also mediates the metabolism of 5-fluorouracil (5-FU) and capecitabine. Genetic polymorphisms in the *DPYD* gene that encodes DYPD result in DYPD-deficiency phenotypes with an overall frequency in the general population of about 3%–5%, which varies significantly among many ethnic groups [190].

With more than 30 SNPs in *DYPD*, the most-relevant decreased functional variants associated with grade 3- and grade 4-toxicities in 5-FU-treated patients include c.1905+1G>A, also known in the literature as *DYPD*2A* or *DYPD*:IVS14+1G>A (rs3918290); c.1679 T>G, also known as *DYPD*13* (rs55886062, p.I560S); c.2846A>T (rs67376798 p.D949V); and c.1129-5923C>G (rs75017182) [191,192]. Other variants such as c.85T>C (*DYPD*9A*, rs1801265, p.C29R) do not result in altered DYPD activity [193]. Among the four functional SNPs, the G>A mutation within intron 14 results in a protein with no catalytic activity and is found in approximately 40%–50% of patients who have either a partial or complete DYPD deficiency. Homozygous and heterozygous carriers of the variant IVS14+1G>A allele have complete absence and 50%, respectively, of normal DYPD activity, and significant, sometimes life-threatening 5-FU-related toxicities [194]. However, the risk of severe toxicity is not necessarily related to *DYPD* genotypes [195]. This may be due to sensitivity of the *DYPD* genetic testing being dependent on which *DYPD* variants are included in specific test panels [193].

N-acetyltransferase

Genetic polymorphism in acetylation capacity was reported more than 50 yrs ago, when two distinct phenotypes of rapid acetylator (RA) and slow acetylator (SA) were noted in patients enrolled in a clinical trial of the antituberculosis drug isoniazid [196]. Subsequently, the phenotype differences were associated with enzyme activities of two cytosolic enzymes N-acetyltransferase-1 (NAT1) and N-acetyltransferase-2 (NAT2), which are encoded by the *NAT1* and *NAT2* genes, respectively. The NAT2 enzyme is primarily responsible for acetylation of aromatic amines and hydrazines. Polymorphism in *NAT2* results in more than 10 *NAT2* alleles, with *NAT2*4* reported as the wild-type allele, and *NAT2*5* (rs1801280) carrying the c.341T>C SNP that results in the p.I114T amino acid change, *NAT2*6* (rs1799930) with c.590G>A SNP and p.R197Q substitution, as well as NAT2*7 (rs1799931) with c.857G>A SNP and corresponding p.G286E substitution as the primary variant alleles [197]. These three variant alleles account for the majority of the SA phenotype. The prevalence of SAs varies significantly in different ethnic groups: 90% of Arab populations, 40%–60% of Caucasians, and 5%–25% of East Asians.

Substrates for NAT include numerous arylamine- and hydrazine-containing drugs such as sulfamethoxazole, hydralazine, isoniazid, and procainamide. High blood levels of these and similarly, acetylated drugs in SAs have been associated with lupus-like syndrome (hydralazine and procainamide), peripheral neuropathy (isoniazid), and liver damage (sulfapyridine). In addition, to drug therapy, *NAT2* polymorphism has also been implicated in susceptibility to developing different types of cancer, with SA having an increased risk after prolonged exposure to carcinogenic arylamines and other industrial chemicals [198].

Glutathione-S-transferase

The human glutathione-S-transferase (GST) family of cytosolic enzymes contains at least 17 genes divided into seven classes:α, μ, π, σ, θ, ζ, and ω. Of these, the most important genes are *GSTM1* of the μ class, *GSTT1* of the θ class, *GSTP1* of the π class, and *GSTA1* of the α class. Both deletion polymorphisms and SNPs exist for *GST* genes. Gene deletion results in the null variants, *GSTM1*0* and *GSTT1*0* and loss of GSTM1 and GSTT1 enzyme function, respectively. Two polymorphisms of the *GSTP1* gene have been described: rs947894 carrying the exon 5 c.A1404G SNP, and p.I105V substitution at codon 105, and rs1799811 carrying the exon 6 c.C2294T SNP and p.A114V substitution at codon 114. Four different haplotypes have been described for the population: *GSTP1*A* (^{105}Ile-^{114}Ala), *GSTP1*B* (^{105}Val-^{114}Ala), *GSTP1*C* (^{105}Val-^{114}Val), and *GSTP1*D* (^{105}ILe-^{114}Val) [199]. A point mutation in the promoter of the *GSTA1* gene results in lower promoter activity associated with the *GSTA1*B* allele. In contrast to deleted *GST* genotypes, the *GSTP1* and *GSTA1* polymorphisms result in genotypes with low-activity enzymes.

The frequency of occurrence of the two *GST* null alleles varies significantly among different populations. Between 42% and 58% of Caucasians and 27%–41% of Africans are reported to be lacking the *GSTM1* gene. For the *GSTT1* gene, the null-allele frequency ranges from 2% to 42% for Caucasians, 50%–60% in Asians, 15%–20% of African Americans, and less than 10% in Hispanics [200,201]. The *GSTP1* and *GSTA1* polymorphisms have been reported to occur in up to 40% of Caucasians and 54% of Africans, and 40% of Caucasians and 41% of Africans, respectively.

GSTs are detoxification enzymes that mediate the conjugation of reduced glutathione with different substrates that include carcinogens and chemotherapeutic agents, such as oxaliplatin-based chemotherapy and chlorambucil

[200,202–204], with poorer response and reduced overall survival in patients with *GSTM1*0* or *GSTT1*0* genotypes, and treated with oxaliplatin-based chemotherapy or anthracycline-based induction therapy [204–207]. Because GSTs are detoxification enzymes, the shortened survival in patients with reduced GST activity might be related to severe drug-related toxicity, as evidenced by a higher frequency of grade 4 neutropenia in homozygous carriers of *GSTM1*0* and treated with oxaliplatin-based chemotherapy for their metastatic colorectal cancer [208]. The important detoxification role of GST has also been reported in a recent study in which pediatric patients with *GSTMI* and *CYP2C9* variants have a higher risk of developing hemorrhagic cystitis when treated with the combined regimen of busulfan and cyclophosphamide [209].

POLYMORPHISMS IN DRUG-TRANSPORTER GENES

Membrane transporters are present at many endothelial and epithelial barriers, including the blood brain barrier (BBB), the intestinal epithelial cells, the hepatocytes, and the renal tubular cells. By facilitating drug excretion into the gastrointestinal tract and bile, from the liver and kidney, as well as limiting the amount of drug crossing the BBB, they provide an important physiological role of protecting humans against toxic xenobiotics, and have recently been recognized as important determinants of drug disposition and response [210]. These drug transporters can be broadly classified into two groups: the efflux adenosine triphosphate-binding cassette (ABC, and formerly known as multidrug resistance [MDR]) family of transporters, and the uptake solute carrier (SLC) family of transporters. In all, 49 members are present within the human ABC-transporter family. Based on homology of their amino acid sequences, they are further classified into seven subfamilies. Of all the ABC transporters, the better-known examples are ABCB1 (P-glycoprotein [Pgp] or MDR1), ABCC1 (multidrug resistance 1 [MRP1]), ABBC2 (multidrug resistance [MRP2]), and ABCG2 (breast cancer resistance protein [BCRP]). For the SLC family, there are 360 members that are subdivided into 46 subfamilies. The better-known SLC transporters are organic anion-transporting polypeptide (OATP), organic cation transporter (OCT), and organic anion transporter (OAT). Genetic variants of the genes encoding these drug-transport proteins (http://www.pharmGKB.org) have been discovered that affect their expression, substrate specificity, and/or intrinsic transport activity, and ultimately disposition, efficacy, and safety of many drug substrates.

The ABC-Efflux Transporters

ABCB1

ABCB1 was the first recognized and the most-studied ABC transporter. It is encoded by the highly polymorphic *ABCB1*, with more than 50 SNPs and three insertion/deletion polymorphisms reported. The most common studied SNPs are the c.C1236T (rs1128503) silent polymorphism in exon 12, the c.G2677A/T (rs2032582) polymorphism in exon 21, and the c.C3435T (rs1045642) silent polymorphism in exon 26. The c.G2677A/T polymorphism results in a change in amino acid sequence p.A893S (G2677T) SNP or p. A893T (G2677A) SNP. Ethnic variations in allelic variant distribution are well known [211,212]. In addition, strong LD among these SNPs had been reported to create haplotypes consisting of 1236C>T, 2677G>A/T, and 3435C>T. The three *ABCB1* SNPs and their haplotypes (Table 1.3) are important in expression and function of ABCB1.

The functional and clinical implication of the *ABCB1* polymorphism was first evaluated for the C3435T SNP with digoxin as the substrate, demonstrating a relationship between lower

TABLE 1.3 Selected ABC Transporters Polymorphisms Indicating Allele Variants and Frequency, and Drug Substrates

Genes	Allele Variants, Amino Acid Change	Frequency (%)	Drug Substrate Examples
ABCB1	C3435T	48%–59% in Caucasians 37%–66% in Asians 10%–27% in Africans	Protease inhibitors (ritonavir, saquinavir, nelfinavir) Anticancer drugs (anthracyclines, taxanes, vinca alkaloids, imatinib) Immunosuppressants (cyclosporine, tacrolimus) Antibiotics (erythromycin, levofloxacin) Calcium channel blockers (diltiazem, verapamil) Digoxin, pivastatin, simvastatin
	C1236T	34%–42% in Caucasians 60%–72% in Asians 15%–21% in Africans	
	G2677T, A893S	38%–47% in Caucasians 32%–62% in Asians ≤15% in Africans	
	G2677A, A893T	1%–10% in Caucasians 3%–22% in Asians	
	1236C>T/2677G>T/3435C>T haplotype	23%–42% in Caucasians 28%–56% in Asians 4.5%–8.7% in Africans	
ABCC2	1249G>A, V417I	22%–26% in Caucasians 13%–19% in Asians 14% in Africans	Reverse transcriptase inhibitors (tenofovir), Anticancer drugs (anthracyclines, vinca alkaloids, methotrexate, SN-38 glucuronide), pravastatin, rifampin
ABCG2	421C>A, Q141K	6%–14% in Caucasians 15%–36% in Asians 0%–5% in Africans	Anticancer drugs (methotrexate, imatinib, gefitinib, SN-38, SN-38 glucuronide, topotecan), Apixaban, atorvastatin, rosuvastatin, glyburide, dolutegravir

expression of ABCB1 and increased digoxin bioavailability and plasma concentration after oral administration in TT homozygotes with reduced ABCB1 activity [213]. In two separate studies, investigators showed that CC genotype of the C3435T SNP (increased Pgp expression) is associated with reduced efficacy and a higher risk of myalgia after treatment with atorvastatin [214] and increased statin-associated increase in serum creatine kinase [215], presumably due to lower intracellular concentration and higher plasma concentration of statin.

The polymorphism also affects plasma concentrations and clinical effects of protease inhibitors. After 6-mo therapy with efavirenz or nelfinavir, patients with the TT genotype had a greater rise in cluster of differentiation 4 (CD4) cell counts than patients with the CC genotype [216]. Therefore, *ABCB1* genotyping may have a role in predicting responses to protease inhibitors. The *ABCB1* haplotype TTT (rs1128503, rs2032582, rs1045642) was reported to be responsible for increased morphine exposure and the exhibition of morphine sensitivity in a patient [186]. In addition, Sadhasivam et al. reported an association between another *ABCB1* variant (rs9282564) and increased risk of morphine-induced respiratory depression in patients with the GG and GA genotypes of this SNP [217].

Nevertheless, conflicting results have been reported regarding the functional and clinical significance of the polymorphism for different substrates including psychotropics (see Chapter 7), antiretroviral protease inhibitors, immunosuppressants, and anticancer drugs. This may be due to the use of different assays

and study designs to identify ABCB1 substrates, the overlapping substrate specificity between ABCB1 and other enzymes and transporters; e.g., CYP3A4 for cyclosporine and OATP transporters for fexofenadine [218], the existence of strong LD necessitating a haplotype approach rather than individual SNPs in association studies or clinical evaluations. In addition, the 1236C>T/2677G>T/3435C>T haplotype was shown to affect the inhibition of substrate transport and not the transport process per se [219]. Thus, the functional effect of *ABCB1* polymorphism may be more modest than previously thought. Whether additional mutations resulting in loss of function, significant change in substrate specificity or functionality would have a bigger impact is not known, and awaits further studies for clarification [219,220].

ABCC1 and ABCC2

Both ABCC1 and ABCC2 are involved in the biliary excretion of conjugated drugs such as glucuronides or sulfates of tamoxifen and SN-38 glucuronide [221,222], organic anions, and some nonconjugated drugs such as methotrexate and pravastatin (Table 1.3), and exhibit overlapping substrate specificities for a variety of drugs. Genetic variation in *ABCC1* gene is rare, whereas polymorphisms of *ABCC2* gene are more common, including the c.1249G>A SNP (rs2273697) in exon 10 resulting in a p.V417I substitution and lower protein expression. Another identified polymorphism is the c.3972C>T SNP (rs3740066) in exon 28 with a p.I324I amino acid substitution [223].

Patients with the 1249G>A variant and receiving tenofovir were reported to have higher risk of drug-induced renal proximal tubulopathy, possibly a result of reduced renal drug excretion [224]. In an exploratory study of an association between *ABCC2* polymorphisms and haplotypes with irinotecan disposition in a cohort of 167 Caucasian patients with solid tumors, a total of 15 *ABCC2* haplotypes were constructed from six variants of *ABCC2* gene. The *ABCC2*2* haplotype (low activity) was found to be associated with lower irinotecan clearance of 28.3 L/h in 48 patients compared with 31.6 L/h in 75 patients not carrying the haplotype ($P = .02$). Interestingly, patients carrying the *ABCC2*2* haplotype but not the *UGT1A1*28* allele experienced lower incidence of severe grade 3–4 diarrhea (odds ratio of 0.15) compared to patients carrying at least one *UGT1A1*28* allele (odds ratio of 1.87), suggesting a protective effect of *ABCC2*2* haplotype against diarrhea occurrence [225]. Because ABCC2 mediates the secretion of SN-38 glucuronide into the bile, the protective effect might reflect a lower exposure of intestinal epithelial cells to SN-38 that is formed after cleavage of SN-38 glucuronide by β-glucuronidase within the intestine (Fig. 1.2).

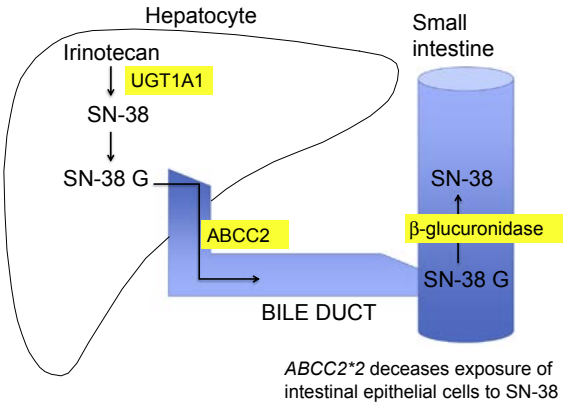

FIGURE 1.2 Schematic representation of potential protective effect of ABCC2 polymorphism against irinotecan-induced diarrhea.

ABCG2

The *ABCG2* gene encodes the BCRP, which is also known as mitoxantrone resistant protein (MXR), or placenta-specific ATP binding cassette transporter (ABCP). More than 80 polymorphisms in *ABCG2* have been reported, with the most studied being the c.421C>A SNP (rs2231142, p.Gln141Lys) in exon 5 that results in a p.Q141K substitution and lower protein expression (Table 1.3) [226]. The c.421C>A

variant with K141 is commonly present in different ethnic groups, being more common in Asians and Caucasians than in sub-Saharan Africans [211,212,227].

Patients carrying the c.421C>A SNP were reported to have increased concentrations of gefitinib and topotecan [228,229], resulting in higher incidence of gefitinib-induced diarrhea [230]. Increased risk of diarrhea was also associated with the *ABCG2* polymorphism in patients with cancer and receiving rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone (R–CHOP) therapy [231]. ABCG2 also plays a role in disposition of other drugs, with the c.421C>A variant reducing biliary excretion of apixaban [232], dolutegravir [233], and rosuvastatin [234]. In 305 Chinese patients with hypercholesterolemia treated with 10 mg of rosuvastatin per day, a gene dose-dependent reduction in low-density lipoprotein cholesterol levels was observed in a carrier of the C421A variant [235]. In both the JUPITER trial, with more than 4,000 patients, and another study with a cohort of 291 Chinese patients, a strong association at the genome-wide level significance has been reported between the C421A variant and altered statin efficacy [236,237].

The SLC-Uptake Transporters

Organic Anion Transporting Polypeptides

In contrast to ABCB1, OATPs are influx or uptake transporters. In addition, to facilitating hepatic uptake of drugs such as statins and antidiabetic agents from the blood into hepatocytes for further metabolism or biliary secretion, OATP also mediates the transport of several endogenous compounds, including bile salts, across the cell membrane. A total of 11 OATP transporters have been identified and classified into six families [238]. Of the human OATPs, OATP1A2, OATP1B1, OATP1B3, and OATP2B1 (Table 1.4) are the best characterized.

OAT1B1

The human *SLCO1B1* gene encodes OATP1B1, which is also known as OATP-C. Since the discovery of the first c.521T>C SNP [239], multiple SNPs have been reported for *SLCO1B1*, with 17 different *SLCO1B1* alleles identified [240]. The 521T>C SNP (rs4149056) with p.V174A substitution results in lower expression of the OATP1B1 protein and reduced transport activity. The 521T>C SNP is more common in Caucasians and Asians than in Africans (Table 1.4). Another very common mutation in all investigated ethnic groups is the c.388A>G SNP (rs2306283) resulting in p.N130D substitution, although conflicting results exist regarding associated changes in transport activity. More importantly, though, the 521T>C SNP and 388A>G SNP are in LD, resulting in several functionally distinct haplotypes, e.g., *OATB1B1*5* carrying the 388A/521C, *OATB1B1*15* carrying the 388G/521C, and *OATB1B1*17* carrying the 388G/521C with -11187A/-10499A of two additional SNPs in the promoter region of *SLCO1B1* [240,241]. Both **5* and **15* haplotypes contain the 521C allele and have been associated with reduced activity.

OATP1B1 plays an important role in hepatic uptake of the 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors such as pravastatin and rosuvastatin, as well as simvastatin acid, the active metabolite of simvastatin. The 521T>C variant has been associated with altered pharmacokinetics of simvastatin acid, with the CC homozygotes having more than two–three-fold increased systemic exposure compared to the other two genotypes [242], potentially resulting in increased toxicity [243], and with decreased intracellular concentration of simvastatin acid for inhibiting HMG-CoA reductase in hepatocytes, a lower efficacy for cholesterol reduction [244]. In a GWAS, 316,184 SNPs were compared between 96 patients treated with 80 mg/day of simvastatin and suffering from myopathy and 96 control subjects without the adverse drug effect. A noncoding rs4363657

TABLE 1.4 Selected SLC Transporters Polymorphisms Indicating Allele Variants and Frequency, and Drug Substrates

Genes	Allele Variants, Amino Acid Change	Frequency (%)	Drug Substrate Examples
SLCO1B1	521T>C, V174A	8%–22% in Caucasians 1%–19% in Asians 1%–5% in Africans	HMG-CoA reductase inhibitors (atorvastatin, simvastatin acid, pravastatin, rosuvasatatin), Anticancer drugs (SN-38, methotrexate), Antibacterials (rifampicin, cefazolin), repaglinide, valsartan
	388A>G, N130D	30%–46% in Caucasians 54%–84% in Asians 72%–81% in Africans	
SLCO2B1	1457C>T, S486F	1%–6% in Caucasians 25%–36% in Asians 10%–41% in Africans	HMG-CoA reductase inhibitors (atorvastatin, fluvastatin, pravastatin, rosuvasatatin), glibenclamide, fexofenadine, motelukast
	935G>A, R312Q	2%–14% in Caucasians 21%–40% in Asians 7%–15% in Africans	
SLCO1B3	334T>G, S112A	74%–89% in Caucasians 64%–83% in Asians 35%–41% in Africans	Anticancer drugs (docetaxel, paclitaxel), digoxin
	699G>A, M233I	71%–90% in Caucasians 64%–84% in Asians 34%–48% in Africans	
SLC22A1	1201G>A, G401S	1% in Caucasians 0% in Asians 1% in Africans	Metformin
	1393G>A, G465R	4% in Caucasians 0% in Asians, Africans	
	1256delATG, M420del	60% in Caucasians 74%–81% in Asians 74% in Africans	
SLC22A2	808G>T, A270S	16% in Caucasians 14%–17% in Asians 11% in Africans	Metformin

SNP within intron 11 of *SLCO1B1*, found to be in nearly complete LD with the rs4149056 polymorphism (521T>C, V174A) ($r^2 > 0.95$), was identified as the only strong SNP marker associated with simvastatin-induced myopathy. The odds ratio (OR) for myopathy was reported as 4.3 per copy of the C allele, and 17.4 in CC homozygotes compared with TT homozygotes [245].

In a more recent study, carriers of the T521C SNP were shown to have an OR of 8.86 ($P < .01$) for statin-induced serum creatine kinase elevation, whereas the impact of the A388G SNP was much smaller (OR of 0.24, $P < .05$) [215]. The magnitude of the clinical significance shown in these two studies [215,245] suggests potential value of genotyping to screen out patients with abnormal OATP1B1 activity to improve the therapeutic index of simvastatin, and may be for other HMG-CoA reductase inhibitors such as pravastatin that are also OATP1B1 substrates [246–248]. Indeed, both the 521T>C SNP and *SLCO1B1*17* haplotype had been shown to be associated with increased

pravastatin concentrations and decreased efficacy [249–251]. Based on the results of these and other studies, the CPIC has made recommendations for genotype-based dosing of statins in its 2014 guideline update [252]. In addition, both SLCO1B1 drug–drug interaction with concurrent drug therapy and drug–gene interaction with *SLCO1B1* variants can alter statin transport and subsequent metabolism and, hence, the risk for statin-related ADR such as rhabdomyolysis [253].

OATP2B1

OATP2B1, also known as OATP-B, possesses substrate selectivity similar to that of OATP1B1 [254]. OATP2B1 has also been found to be expressed in the luminal membrane of the small intestinal enterocytes [255], and hence would have a role in drug absorption. Since the first discovery of genetic polymorphism, several sequence mutations of *OATP2B1* have been described, including the c.1457C>T SNP (rs2306168), c.601G>A SNP (rs35199625), c.935G>A SNP (rs12422149), c.43C>T SNP (rs56837383), and a nine-nucleotide deletion of three amino acids 26–28 (26–28, p.QNT) of *OATP2B1* [256]. Although decreased transport activity had been shown mostly in vitro for most of these SNPs, the results are not consistent among all studies. In addition, significant ethnic variabilities exist in allele frequency of these SNPs; for example, the allelic frequency of c.1457C>T SNP is higher in Asians (31%) compared to Caucasians (3%)

A recent study evaluated the impact of the 1457C>T SNP on fexofenadine pharmacokinetics in Japanese subjects, and found similar pharmacokinetic parameters among the three genotype groups [257]. Although the same SNP did not affect the absorption of the leukotriene receptor antagonist motelukast, patients who carry the 935A variant allele of the c.935G>A SNP was reported to show lower plasma concentration and lesser pharmacological response [258]. Yet a separate study reported the lack of an association between motelukast and the c.935G>A SNP. Although this might suggest that the effect of *SLCO2B1* SNP on drug absorption could be substrate dependent, more importantly, additional studies with other substrates would need to be performed for clarification of the effect of *SLCO2B1* on drug disposition.

OATP1B3

In humans, the *SLCO1B3* gene encodes OATP1B3, which was previously also known as OATP8 and LST-2. Several sequence variations exist for the *SLCO1B3* gene. The c.334T>G SNP (rs4149117) and the c.699G>A SNP (rs7311358) occur at a high frequency in Caucasian populations. Although OATP1B3 mediates the hepatic uptake of several drugs, including taxanes [259], a study in 90 patients with cancer from six different ethnic groups reported that there were no associations between paclitaxel clearance and the two *OATP1B3* SNPs [260]. Similarly, no associations were found between docetaxel pharmacokinetics and OATP1B3 SNPs [261,262]. The role of *OATP1B3* polymorphisms in drug disposition and response await further clarification from future studies.

In summary, OATP polymorphisms can affect disposition and possibly response for a large number of drugs. Current evidence strongly suggests a vital role of specific SNPs of *SLCO1B1* gene (e.g., 521T>C) for statin efficacy and adverse effects. Similar data for other OATP1B1 substrates from future clinical studies would provide further evidence of the value of prospective genotyping for *SLCO1B1* variants in individualizing drug therapy. In contrast, the data for validating the functional role of *SLCO2B1* and *SLCO1B3* polymorphisms are not as clear. Much more work is needed for clarifying the clinical significance of these SNPs for predicting pharmacokinetic profile for, and clinical response to, OATP2B1 and OATP1B3 substrates.

Organic Cation Transporters

Three organic cation transporters (OCTs) have been identified in humans: OCT1, OCT2,

and OCT3, all of which are members of the SLC22A family, and are encoded by the corresponding *SLC22A1*, *SLC22A2*, and *SLC22A3* genes, respectively (Table 1.4). OCT1 is primarily expressed in the hepatocytes and mediates cellular uptake of drugs into the liver. OCT2 is primarily expressed in the proximal tubules of the kidney. In contrast, OCT3 is more broadly distributed in the body.

Located on chromosome 6, *SLC22A1* is highly polymorphic with reduced or loss of transporter functional activity secondary to four coding polymorphisms: c.181C>T (rs12208357, p.Arg61Cys), c.1393G>A (rs34059508, p.Gly465Arg), c.1201G>A (rs34130495, p.Gly401Ser), and OCT1 Met420 deletion of three bases ATG at codon 420 of exon 7 and collectively designated as rs72552763 [263]. The Met420 deletion variant commonly occurs in Caucasians and African Americans with a frequency of 18.5% and 5%, respectively. OCT1 genotypes have been shown to contribute to interindividual variability in disposition of several drugs, including ondansetron, metformin, morphine, and tramadol [264–267].

Of the different SNPs that have been identified for the *SLC22A2* gene, the most relevant one is the c.808G>T SNP (rs316019) that results in the p.A270S substitution. The antidiabetic drug metformin is primarily renally eliminated by active tubular secretion via OCT2. Homozygotes of the low-activity 270S variant had been shown to have lower renal clearance and higher plasma concentrations of metformin when compared to homozygous carriers of the wild-type 270A [268,269]. Interestingly, Tzvetkov et al. demonstrated that OCT1 is also expressed in the distal tubule and may play a role in tubular reabsorption of metformin. They reported that homozygous and heterozygous carriers of various haplotypes of low-activity alleles of several *SLC22A1* polymorphisms (c.1201G>A SNP with p.G401S substitution, c.1393G>A SNP with p.G465R substitution, and a deletion resulting in M420del) were associated with increases in metformin renal clearance by about 20%–30% [267]. Nevertheless, OCT1 is primarily expressed in the hepatocyte, the major site of action of metformin. The same low-activity OCT1 variant alleles of these polymorphisms have also been reported to decrease hepatic uptake of metformin with resultant lower blood glucose response [270], and more recently for fenoterol, resulting in increased systemic exposure and drug-related toxicities [271]. The effects of genetic polymorphisms in other transporters such as the multidrug and toxin extrusion transporters as well as pharmacological targets for metformin is further discussed in Chapter 9.

Organic Anion Transporters

In contrast to the OCT belonging to the same SLC22 family, the organic anion transporters (OATs) primarily mediates the transport of organic anions. Four OATs have been studied regarding their tissue location: OAT1, OAT2, and OAT3 are primarily expressed in the basolateral membrane of the renal proximal tubule, whereas OAT4 is located at the apical side. Therefore, OAT1, OAT2, and OAT3 are responsible for uptake of drug substrates into the tubular cells and OAT4 mediates their secretion into the renal tubule. Although several polymorphisms have been reported for *SLC22A6* encoding OAT1, SLC22A7 encoding OAT2, *SLC22A8* encoding OAT3, and *SLC22A11* encoding OAT4, the allele frequency of these SNPs are all ≤1% and their functional significance have not been clarified [272,273].

POLYMORPHISMS IN DRUG-TARGET GENES

The study of pharmacodynamics encompasses the biochemical and physiological effects of drugs on the body and the relationship between drug concentration and drug effect. Drugs exert their effects through interaction with numerous protein types, including cell

surface receptors (e.g., β-adrenergic, 5-hydroxytryptamine receptors, and μ–opioid receptors), enzymes (e.g., vitamin K epoxide reductase complex 1, adenosine monophosphate-activated protein kinase, and catecho-O-methyltransferase), and ion channel proteins (e.g., sodium and potassium channels, epithelial sodium channel). Additionally, numerous intracellular signaling proteins downstream from the target protein are involved in eliciting drug response. Genetic variation affecting either the activity or expression of a drug-target or intracellular signaling protein can have significant consequences for pharmacodynamic drug response.

Phenotypic response to genetic variation for drug-target proteins generally differs from that of drug-metabolizing enzymes and drug transporters (Table 1.5). As illustrated in Fig. 1.3, variation in drug-metabolizing enzymes results in distinct phenotypes (e.g., PMs, EMs, or UMs), as described in the earlier section. With precision oncology the expression of drug-target receptor gene for tumor cells predicts drug efficacy. In addition, there are a limited number of examples of genetic variants in drug-target proteins in germline cells that result in distinct pharmacodynamic effects. One of these examples involves mutations in the vitamin K epoxide complex subunit 1 *(VKORC1)* gene, in which rare nonsynonymous mutations result in warfarin resistance, and exceptional high doses (30 mg/day or higher) are required to achieve therapeutic anticoagulation. Most polymorphisms that impact drug pharmacodynamics tend to be much more subtle and help explain response variability across a single distribution curve. For example, commonly occurring variations in the *VKORC1* regulatory regions help explain the significant interpatient variability in the warfarin dose required to produce optimal anticoagulation, as described in detail in Chapter 6. The remainder of this section discusses examples of genes for various types of drug-target proteins that contribute to the interpatient variability in pharmacodynamic drug responses.

Drug Target Receptor Genes in Oncology

Several cancer chemotherapy agents have been developed based on findings that overexpression of certain tumor cell surface receptors drives tumor cell growth and which are further described in Chapters 3 and 5. Expression of the epidermal growth factor receptor type 2 (HER2), also known as Her2/neu and ErbB2, is one such example that influences disease prognosis and predicts drug. Overexpression of HER2 occurs in approximately 20% of metastatic breast cancers and is associated with more aggressive cancer and poor prognosis [274]. Trastuzumab is a recombinant monoclonal antibody that was developed to target HER2 and block growth and survival of HER2-dependent tumors. The addition of trastuzumab to chemotherapeutic regimens to treat breast cancer significantly slows the progression of breast cancer in women with HER2-positive tumors, with treatment effects positively correlated with the degree of HER2 overexpression [275]. Thus, testing to confirm HER2 overexpression is necessary before trastuzumab use. Although the data are conflicting and require further confirmation, a genetic association between HER2 655 A >G (Ile/Val) polymorphism (rs1136201) and trastuzumab cardiotoxicity has also been suggested [276].

The epidermal growth factor receptor (EGRF), also known as HER1 or ErbB1, is overexpressed in head and neck, colon, and rectal cancer. EGRF overexpression is associated with cancer growth and invasion and portends a poor clinical prognosis. The discovery of the *EGRF* gene and its role in cancer prognosis led to the development of EGRF antagonists, including cetuximab, panitumumab, erlotinib, and gefitinib. Cetuximab is a recombinant monoclonal antibody that binds to the extracellular domain of the EGFR, thus preventing epidermal growth factor and other ligands from activating the receptor. Cetuximab is indicated in the treatment of metastatic

TABLE 1.5 Consequences of Selected Genetic Variation in Drug Disposition and Drug Target Proteins

Gene	Drug Examples	Clinical Consequence
CYP2D6	Atomoxetine	PMs may have 10-fold greater atomoxetine exposure
	Codeine	UMs are at increased risk for morphine toxicity (details in Table 1.2)
CYP2C9	Warfarin	CYP2C9 deficiency increases bleeding risk
CYP2C19	Clopidogrel	CYP2C19 deficiency reduces drug effectiveness
G6PD	Rasburicase	G6PD deficiency increases risk for hemolytic anemia
TPMT	Azathioprine, 6-mercaptopurine, Thioguanine	Nonfunctional genotype increases the risk of serious, life-threatening myelosuppression with conventional drug doses
UGT1A1	Irinotecan	Reduced function genotype increases risk for drug-induced neutropenia
DPD	Capecitabine, 5-fluorouracil	DPD deficiency may lead to severe diarrhea, neutropenia, neurotoxicity)
SLCO1B1	Simvastatin	Increased risk for myopathy
DRUG-TARGET GENES		
EGFR	Cetuximab, Panitumumab	Determines drug effectiveness
HER2	Trastuzumab	Determines drug effectiveness
ADRB1	β-blockers	Influences variability in blood pressure response and possibly mortality reduction
VKORC1	Warfarin	Determines dose needed for optimal anticoagulation
KCNJ11 and *ABCC8*	Sulfonylureas	Drug effectiveness
KCNMB1	Verapamil	Possibly determines reduction in blood pressure
DRD3	Antipsychotics	Risk for tardive dyskinesia
GRK5	β-blockers	Drug effectives on clinical outcomes in heart failure
ATM	Metformin	Antidiabetic response
SLC6A4	SSRIs	Drug effectiveness
HTR2A	Clozapine	Drug effectiveness

colorectal cancers that overexpress EGRF, in which it has been shown to improve survival [277,278]. Similar to cetuximab, panitumumab is a monoclonal antibody that blocks activation of the EGRF and is indicated in metastatic colorectal cancer that progresses despite chemotherapy with fluoropyrimidine-, oxaliplatin-, and irinotecan-containing regimens. Erlotinib and gefitinib also target the EGRF and are indicated in nonsmall cell lung cancer.

Other examples of targeted chemotherapy developed based on genetic abnormalities include:

- rituximab, a monoclonal antibody used to treat CD20-positive, B-cell non-Hodgkin's lymphoma and chronic lymphocytic leukemia;

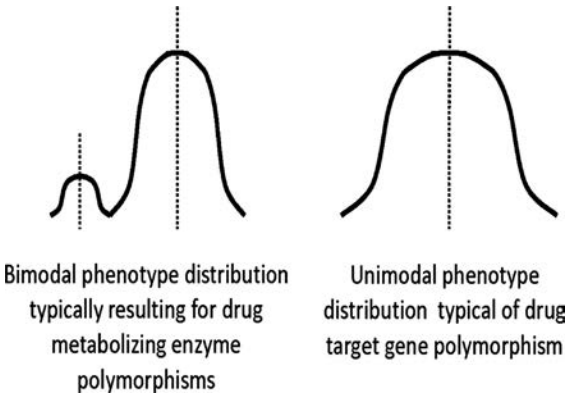

FIGURE 1.3 Many drug-metabolizing enzyme polymorphisms are inactivating resulting in distinct phenotypes, such as the poor metabolizer and extensive metabolizer phenotypes. In contrast, drug-receptor polymorphisms tend to be more subtle and help explain variability across single distribution curve.

- imatinib mesylate, a kinase inhibitor developed to block the product of a reciprocal translocation between chromosomes 9 and 22, occurring in 95% of patients with chronic myeloid leukemia; and
- crizotinib, an anaplastic lymphoma kinase (ALK) and c-ros oncogene1, receptor tyrosine kinase (ROS-1) inhibitor that targets the echinoderm microtubule associated protein like 4–anaplastic lymphoma kinase (EML4-ALK) gene fusion product in nonsmall-cell lung cancer.

Drug Target Receptor Genes in Cardiology

β_1-receptors are located in the heart and kidney, in which they are involved in the regulation of heart rate, cardiac contractility, and plasma renin release. β_1-receptor mediated effects contribute importantly to the pathophysiology of numerous cardiovascular diseases, including hypertension, coronary artery disease, and heart failure. In particular, plasma renin release and activation of the renin-angiotensin-aldosterone system lead to increased blood volume and vasoconstriction in hypertension. Increases in heart rate and cardiac contractility increases myocardial oxygen demand, thus contributing to myocardial ischemia in patients with coronary heart disease. Furthermore, increased sympathetic nervous system activity is one of the primary mechanisms contributing to cardiac remodeling and heart failure progression. Consequently, β-blockers exert beneficial effects across cardiovascular diseases, resulting in blood pressure reduction in hypertension, lowering of myocardial oxygen demand in ischemic heart disease, and attenuation of cardiac remodeling in heart failure. There is evidence that genetic variation for the adrenoceptor β-1 (ADRB1) may influence the effectiveness of β-blocker therapy.

The ADRB1 is encoded by an intronless gene, located on chromosome 10q24-26. Two common nonsynonymous SNPs in the *ADRB1*, p.S49G and p.R389G are in strong LD. The S49G SNP is located in the extracellular region of the receptor near the amino terminus, and the R389G variant is located in the cytoplasmic tail in the G-protein coupling domain of the ADRB1. In vitro studies show lesser receptor downregulation with the S49 form of the receptor and both greater receptor coupling to the G-protein and greater adenylyl cyclase activity with the R389 form [279,280]. There are ethnic differences in the S49G and R389G allele frequencies, with a G49 frequency of 12%–16% in Caucasians and 23%–28% in African Americans and a G389 frequency of 24%–34% in Caucasians and 39%–46% in African Americans [281].

The *ADRB1* gene has been the primary focus of research into genetic determinants of responses to *β*-blockers in hypertension, coronary heart disease, and heart failure [282–284]. In each case, the R389 allele or S49-R389 haplotype has been associated with greater response to β-blockade presumable because of greater adrenergic activity with this allele and haplotype. For example, treatment of hypertension with metoprolol produced greater blood pressure reduction in patients who were homozygous for the S49-R389

haplotype than in carriers of the G49 or G389 allele [285]. Among patients with coronary heart disease, the S49-R389 haplotype was associated with an increased risk for death compared to other haplotypes, an effect negated by treatment with atenolol [286]. In patients with heart failure, the homozygous R389 genotype was associated with greater improvements in left ventricular ejection fraction with carvedilol or metoprolol and greater survival benefits with bucindolol [287–289]. These clinical data are consistent with the in vitro data implying greater agonist-mediated effects (e.g., greater sympathetic nervous system-driven hemodynamic effects) with the S49 and R389 alleles and suggest that *ADRB1* genotype is an important determinant of blood pressure and cardiac responses to β-blockers.

The *ADRB1* genotype is also associated with β-blocker tolerability in heart failure. β-blockers are indicated for patients with heart failure because they attenuate the detrimental effects of the sympathetic nervous system on heart failure progression. However, because β-blockers have negative inotropic effects (i.e., reduced cardiac contractility), they can worsen heart failure when first started. For this reason, they must be started in very low doses with careful uptitration. Although most heart failure patients tolerate β-blocker initiation at low doses and slow uptitration, some experience significant heart failure exacerbation. The influence of *ADRB1* genotype on tolerability to β-blocker initiation and up-titration has been examined, and it was found that carriers of the Gly389 allele or the 49Ser/Ser genotype more frequently require increases in concomitant heart failure therapy (predominately diuretics) for management of symptoms of worsening heart failure during β-blocker titration than patients with other genotypes [290].

Stimulation of the presynaptic adrenergic alpha2C-receptor (ADRA2C) results in inhibition of norepinephrine release, and has been correlated with β-blocker response. The *ADRA2C* Del322-325 (α_{2Cdel}322-325) polymorphism causes an inframe deletion of 12 nucleic acids, resulting in the loss of four amino acids in the ADRA2C protein and loss of protein function. Loss of ADRA2C function would be expected to result in less inhibition of norepinephrine release, and consequently increased norepinephrine levels and sympathetic tone. The frequency of the Del322-325 variant exhibits marked variability by ancestry, with a frequency of approximately 0.40 in African Americans and <0.05 in those of European descent [291], and homozygosity for Del322-325 variant has been associated with higher risk of heart failure in African Americans compared to Caucasians.

In a large, multicenter, randomized, placebo-controlled heart failure trial, investigators found that individuals with the Del322-325 allele had greater reductions in sympathetic activity with bucindolol, a nonselective β-blocker with α1-receptor blocker properties. However, individuals with the wild-type (Ins322-325) *ADRA2C* genotype derived significant survival benefits from bucindolol, whereas Del322-325 allele carriers did not [292]. The mechanism underlying this association was not determined. However, it was hypothesized that the significant sympatholytic activity with bucindolol in Del322-325 allele carriers caused detrimental clinical effects. These findings might explain the negative association between bucindolol use and heart failure survival in the study population overall. Specifically, whereas carvedilol, metoprolol, and bisoprolol were all shown to improve survival in heart failure, bucindolol was not [293,294]. However, compared to other β-blocker trials, the trial with bucindolol enrolled a large number of African Americans, in whom the Del322-325 allele, associated with lack of benefit with bucindolol, is ten-times more common.

With its pharmacological targets at the ADRB1 on myocytes and on the adrenergic neurons, the interaction of both *ADRB1* and *ADRA2C* SNPs could further modulate the drug response. A recent clinical trial identified subsets of populations with different responses based

on evaluation of both genotypes. Enhanced bucindolol efficacy was associated with *ADRB1* homozygotes, whereas intermediate efficacy was observed in patients with Gly variant of the *ADRB1* SNP and homozygote carriers of the wild-type α_{2C}322-325. In contrast, a lack of efficacy was reported in carriers of the *ADRB1* Gly variant and homozygous carriers of the Del322-325 allele [295]. Similarly, Reddy et al. recently reported that in children with dilated cardiomyopathy, β-blockers produced better hemodynamics and preservation of cardiac function in those with high-risk genotypes, including α_{2Cdel}322-325 and β_1Arg389 [296]. Similar combinatorial pharmacogenomic approaches have also been investigated in psychopharmacology and is discussed in Chapter 7.

Drug Target Genes in Psychiatry

Antidepressants target 5-hydroxytryptamine (5-HT) receptors, and a number of studies have examined the association between antidepressant treatment response and 5-HT genotype, as described in more details in Chapter 7. However, results of these studies are largely inconsistent and even conflicting. For example, in a large-scale association study of 68 candidate genes, only the synonymous IVS2 A/G (rs7997012) SNP within intron 2 of the *HTR2A* gene, which codes for the postsynaptic 5-HT_{2A} receptor, was associated with response to citalopram [297]. Although a large study in European Caucasians confirmed the association between the rs7997012 SNP and antidepressant response, the findings were opposite of those in the initial study [298].

The majority of drug-target genetic associations discussed so far related to drug effectiveness. Variation in the *DRD3* gene, encoding for the dopamine D3 receptor, is an example of drug-target genotype linked to adverse drug effects. Specifically, the *DRD3* p.S9G variant has been implicated in risk for developing tardive dyskinesia, an irreversible movement disorder that develops after long-term antipsychotic treatment, particularly with typical antipsychotics. In a meta-analysis, the G9 allele was significantly overrepresented among 317 patients with tardive dyskinesia compared to 463 patients without this adverse drug effect [299]. Furthermore, G9 allele homozygotes had higher abnormal involuntary movement scores compared to both heterozygotes and S9 allele homozygotes. This association was confirmed in another meta-analysis [300].

Drug Target Genes in Pain Management

Over the years, several SNPs have been discovered for genes that encode different analgesic drug targets, and association studies had been carried out in various pain phenotypes. Not surprisingly, nonreplication of findings is common. The μ–opioid receptor (MOR) is the primary drug target for endogenous opioid peptides and the opioid analgesics. With more than 100 variants of the μ–opioid receptor gene *(OPRM1)* identified [301], the most studied polymorphism is the c.118A>G (rs1799971) SNP in exon 1 of *OPRM1* that results in p.N40D, and lower mRNA expression and protein amount associated with the G allele. This functional difference between the two alleles is reflected in stronger binding by the G allele to the endogenous opiate β-endorphins, thereby affecting opioid action at the receptor site, with decreased opioid potency by a factor of two to three [302]. This is evident by the report of Oertel et al., who showed that despite a stronger binding, the signal efficacy is weaker in regions of the brain that are important to pain perception and experience [303].

Decreased clinical response to opioids had been shown in carriers of the G allele. Klepstad et al. reported morphine-dosage requirements in 207 cancer patients differed among carriers of the wild type versus that of the variant allele. Four homozygous carriers of the G allele

required 225 ± 143 mg/day for effective pain control compared to 97 ± 89 mg/day in 78 wild-type homozygotes ($P = .006$). However, dosage requirement for heterozygote was 66 ± 50 mg/day, so there was no evidence of a gene-dose effect [304]. Chou et al. also reported similar findings of different dosage requirements for postoperative pain with patient-controlled analgesia, at 24 and 48 h after total knee arthroplasty respectively, of 22.3 ± 10 mg and 40.4 ± 21 mg in homozygous carriers of the G allele versus 16 ± 8 mg and 25.3 ± 15.5 mg in wild-type homozygotes [305]. Two patients identified as "low" responders of morphine requiring 1.8 and 2 gm/day were identified as a carrier of the G allele [306,307]. These and other clinical trial results [308–312]suggest that *OPRM1* genotype and haplotype analyses could have clinical implication for pain control in a variety of patients. Chidambaran et al. also reported a higher risk of morphine-induced respiratory depression associated with the A118G SNP [312].

In addition to morphine, a significant association has also been shown between the A118G SNP and decreased potency of morphine-6-glucuronide (M6G), the pharmacologically active metabolite of morphine. Using pharmacokinetic–pharmacodynamic modeling, the study showed that the effector site EC_{50} for M6G was 714 ± 197 nmol/L in six homozygous carriers of the wild-type, 1,475 ± 424 nmol/L in five heterozygotes, and 3,140 nmol/L in a homozygous carrier of the G allele [313]. Additional studies have reported decreased effect and higher-dosage requirement for other opioid agonists, including fentanyl and alfentanil, in carriers of the G allele [309,314–318]. However, negative association with A118G SNP has been reported for fentanyl, which was attributed by the investigators to the small sample size of the study [319]. In view of the low frequency of A118G SNP, the issue of small sample size with associated low statistical power to detect difference in analgesic doses and/or outcome is important.

Signal Transduction Proteins

Signal transduction encompasses the cascade of events following drug binding to a receptor that ultimately leads to a change in cellular response. G-protein receptor kinase 5 (GRK5) is an example of a signal transduction protein linked to drug response. The ADBR1 and other adrenergic receptors are coupled to GTP-binding proteins also called G-proteins. Upon ligand binding, the receptor couples to the intracellular G-protein to elicit a cellular response. GRKs phosphorylate cardiac receptors, essentially inhibiting receptor-mediated signaling and, thus, serving in a manner analogous to natural β-blockade. The *GRK5* p.Q41L polymorphism occurs commonly in African Americans, with an allele frequency >30%. However, it rarely occurs in Caucasians. The L41 allele has been found to more effectively uncouple agonist-mediated receptor signaling and has been associated with increased transplant-free survival in African Americans with heart failure [320]. Patients with the L41 allele derived no benefit from β-blocker therapy, presumably, because they already have inherent downregulation of ADRB1 receptor signaling [321]. However, in patients with the *GRK5* 41QQ genotype, which is associated with a poor prognosis, treatment with β-blocker therapy significantly improved transplant-free survival [320].

The dopamine and serotonin receptors targeted by antipsychotics are also G-protein-coupled receptors (GPCRs) and signal to effector proteins through intracellular G-protein subunits. Regulators of G-protein signaling shorten the duration of neurotransmitter-mediated receptor signaling through the GPCRs. The regulator of G-protein signaling 4 (RGS4) is one such regulator, and it regulates the activity of the GPCRs. The gene that encodes RSG4 had been identified as a vulnerability gene for schizophrenia [322,323]. and variants of *RSG4* have been studied as predictors for antipsychotic treatment response.

Three SNPs of *RSG4* have been reported to confer differential treatment responses in three ethnic groups. In patients of African descent, those with the CC genotype of the rs951439 SNP had longer (391 days) and better (21% improvement based on the Positive and Negative Syndrome Scale [PANSS]) response to perphenazine than ziprasidone (124 days and 5% worsening, respectively). On the other hand, the same patient population with the TT genotype of the rs2842030 SNP responded better to perphenazine (24% improvement in the PANSS) than to quetiapine, risperidone, and ziprasidone. A sharp contrast in association was shown in patients with European descent, in which risperidone treatment resulted in better response with the TT genotype of the rs951349 SNP and GG genotype of the rs2842030 SNP [324]. In 120 schizophrenic patients of Chinese descent, rs2661319 of *RSG4* was found to predict response to risperidone treatment [325]. These data with *RSG4* polymorphisms underscore the importance of patient population stratification by ethnicity in pharmacogenomics investigations. It is also noteworthy that the investigators of the Chinese study also had reported in other studies that polymorphisms affecting the dopamine D_2 receptor (Ser311Cys), D_3 receptor (Ser9Gly), and 5-HT2A receptor (102-T/C) predict treatment response to risperidone [326–328]. Whether evaluating a combination of SNPs could result in better response prediction remains to be investigated.

The alpha adducin (ADD1) gene encodes for α-adducin, a cytoskeletal protein involved in signal transduction and renal sodium transport. The *ADD1* p.G460W variant is associated with greater renal sodium–potassium pump activity, renal sodium retention, and salt-sensitive hypertension [329,330]. Given its role in regulating sodium reabsorption and potentially mediating increased hypertension risk, the *ADD1* gene has been studied for its contribution to diuretic response. Although the 460W allele has been linked to greater blood pressure reduction with thiazide diuretics, the data are inconsistent [329,331]. The *ADD1* gene appears to interact with other genes involved in renal sodium reabsorption, including the neural precursor cell expressed, developmentally downregulated 4-like (NEDD4L) and lysine-deficient protein kinase 1 (WNK) genes [329]. This may explain the inconsistencies in the data when *ADD1* is analyzed alone rather than in the context of other genes involved in renal sodium handling, and illustrates the likely contribution of multiple genes to the efficacy of many drugs.

Enzyme Genes

VKORC1 is the target site for warfarin. Specifically, warfarin inhibits VKORC1 to prevent regeneration of a reduced form of vitamin K necessary for clotting factor activation. Two common variants, -1639G>A (rs9923231) and -1173C>T (rs9934438) in the *VKORC1* regulatory regions, are associated with reduced gene expression [332]. The frequency of 1639A allele of rs9923231 is highest in Asians (~90%) and lowest in persons of African descent (10%), with an intermittent frequency in populations of European descent (~40%) [333].

Numerous studies have documented the association between *VKORC1* genotype and warfarin-dose requirements. The -1639AA, AG, and GG genotypes are associated with average warfarin-dose requirements of approximately 3, 5, and 6 mg/day, respectively. The two SNPs are equally predictive for predicting warfarin-dose requirement. Recent data suggest that dosing based on one of the *VKORC1* SNP, in addition, to *CYP2C9* genotype, leads to more accurate dose prediction and may reduce the risk for adverse clinical outcomes early in the course of warfarin therapy [334,335]. The VKORC1 genotype is described in detail in Chapter 6.

The angiotensin-converting enzyme (ACE) gene has been widely studied for its effects on ACE inhibitor response. An insertion/deletion

(I/D) polymorphism in intron 16 of the *ACE* gene results in the presence or absence of a 287-base-pair fragment. The *ACE* D allele has been linked consistently to higher plasma concentrations of ACE, the enzyme responsible for the conversion of angiotensin I to the potent vasoconstrictor angiotensin II [336]. Given its association with ACE concentrations, a number of investigators have examined whether the I/D polymorphism contributes to the interpatient variability in ACE inhibitor response. However, much of the data with the I/D polymorphism and blood pressure response to ACE inhibitors are inconsistent and even conflicting, with some studies demonstrating greater response with the *D/D* genotype, whereas others have shown greater response with the *I/I* genotype, and further studies showing no association. In one of the largest pharmacogenetic studies to date, including nearly 38,000 patients, there was no association between the *ACE I/D* genotype and either blood pressure response or cardiovascular or renal outcomes with antihypertensive therapy [337].

Numerous polymorphic proteins are involved in the complex signaling pathway of the renin–angiotensin system, including renin, angiotensinogen, the angiotensin II type 1 receptor, bradykinin, and aldosterone synthase. Thus, a likely explanation for the inconsistent data with the *ACE* gene and ACE inhibitor response in hypertension is that a single polymorphism provides minimal contribution to ACE inhibitor response. Rather, ACE inhibitor response may be best determined by a combination of multiple polymorphisms occurring in multiple genes involved in the renin–angiotensin pathway.

The data with the *ACE I/D* genotype and ACE inhibitor response in patients with heart failure are more compelling. In this population, the *ACE* D allele has been associated with an increased risk for cardiac transplant or death [338–342]. As described in detail in Chapter 6, the detrimental effect of the *ACE* D allele on transplant-free survival appears greatest among patients who are taking lower than recommended doses of ACE inhibitors. These data suggest that maximizing the ACE inhibitor dose may be necessary in *ACE* D allele carriers to attenuate the harmful effects of this allele [342,343].

Metformin is an antidiabetic drug that works in part by activating adenosine monophosphate-activated protein kinase (AMPK), which is a master regulator of cell and body energy homeostasis and glucose uptake in skeletal muscle [344]. The ataxia-telangiectasia mutated (ATM) is a DNA repair gene that acts upstream of AMPK and appear necessary for metformin action [345]. A GWAS identified a significant association between metformin response in type 2 diabetic patients and a polymorphism in a locus containing the *ATM* gene [345]. The potential significance of ATM for metformin response is further described in Chapter 9.

The catecho-O-methyltransferase (COMT) enzyme is a key modulator of the adrenergic and dopaminergic systems, which play a role in pain modulation. A functional SNP in the *COMT* gene, c.472G>A (rs4680), results in a p.V158M substitution with three-to four-fold decrease in enzyme activity. The reduced enzyme activity leads to decreased dopamine degradation and subsequent increases in norepinephrine and epinephrine levels that may be associated with exaggerated levels of pain [346]. Down regulation of endorphins with compensatory upregulation of MOR has also been suggested to be a result of the SNP [347,348]. Cancer patients who are homozygous carriers of the M variant (high pain-sensitivity patients) reportedly required more morphine (155 ± 160 mg/day) than heterozygotes (117 ± 10 mg/day) and homozygous carriers of the wild-type V allele (95 ± 99 mg/day) [349]. These results were replicated in a later study [350]. Reyes–Gibby et al. also reported higher morphine-dosage requirement: 63% and 23%, respectively, for satisfactory pain control in patients with the *COMT Val/Val* and *Val/Met* genotypes compared to carriers of

Met/Met genotype ($P = .02$) [311]. The increased morphine toxicity reported in the patient by Madadi et al. had a *COMT* haplotype CCG (rs4633, rs4818, rs4680) and a *G/G* genotype for the *OPRM1* (rs1799971, A118G) SNP, in addition, to the *ABCB1* haplotype [186]. This again highlights the importance of multiple genes in mediating drug disposition and effect and the need of genotyping multiple functional polymorphisms in pharmacogenomic studies. Interestingly, in addition to reporting negative association for the *OPRMI* A118G (rs1799971) polymorphism that was discussed in the section on drug-target genes in patient management, Landau et al. also did not find a correlation between fentanyl therapeutic outcomes with *COMT* genotype [319].

Ion Channel Genotype

One of the most often cited examples of ion channel genes with consequences for drug response are genes for the pore-forming channel proteins that affect potassium and sodium transport across the cardiac cell membrane. Mutations in cardiac ion channel genes predispose individuals to congenital long-QT syndrome. Moreover, there is evidence that these mutations may increase the risk for drug-induced torsades de pointes [351,352]. This is discussed in detail in Chapter 6.

The large-conductance calcium and voltage-dependent potassium (BK) channel is another example of an ion channel with genetic contributions to drug response. The BK channel is found in vascular smooth muscle and consists of pore-forming-α and regulatory-β1 subunits. The β1 subunit enhances calcium sensitivity and decreases smooth muscle cell excitability, thus attenuating smooth muscle contraction. The *KCNMB1* gene encodes for the BK channel β1 subunit. A common SNP in the *KCNMB1* gene, Glu65Lys, results in a gain of function of the channel and increased calcium sensitivity compared to the wild type [353]. Given its role in mediating calcium sensitivity, the *KCNMB1* gene was examined for its effect on response to the calcium channel blocker, verapamil. Among patients with hypertension and coronary heart disease who were started on verapamil, 65Lys allele carriers achieved blood pressure control more rapidly than 65Glu homozygotes, suggesting that the Glu65Lys SNP enhances response to calcium channel blockers and contributes to the interpatient variability in blood pressure reduction during calcium channel blocker therapy [354].

The epithelial sodium channel (ENaC) is another example of an ion channel with genetic contributions to drug response. The ENaC is located in the distal renal tubule and collecting duct of the nephron and serves as the final site for sodium reabsorption. The channel is composed of α, β, and γ subunits, encoded by the *SCNN1A*, *SCNN1B*, and *SCNN1G* genes, respectively. In a healthy volunteer study, SNPs in the *SCNN1B* and *SCNN1G* genes were associated with natriuretic and diuretic responses to single oral doses of loop diuretics. Loop diuretics are commonly prescribed for managing symptoms of fluid overload in heart failure. Whether genes encoding for ENaC subunits influence response to loop diuretics in heart failure remains to be determined. But, given the significant consequences of under- or overdosing loop diuretics in this disease, such information could have significant clinical value.

The potassium inwardly rectifying channel, subfamily J, member 11 gene (KCNJ11) and the sulfonylurea receptor gene (ABCC8) encode the Kir6.2 and sulfonylurea receptor-1 (SUR1) subunits of pancreatic ATP-sensitive potassium (K_{ATP}) channels, respectively. Activating mutations in the *KCNJ11* and *ABCC8* cause K_{ATP} channels to remain open, which promotes hyperpolarization of the pancreatic β cell membrane and impaired insulin release [355,356]. Sulfonylurea drugs promote K_{ATP} channel closure, thereby attenuating the effects of activating mutations in *KCNJ11* and *ABCC8*. As such, sulfonylureas are especially

effective in patients with *KCNJ11* or *ABCC8* activating mutations [356,357]. Chapter 9 provides a more detailed discussion of these genetic variations and their effects on response to sulfonylurea agents.

CONCLUSION

Variations in genes influencing drug pharmacokinetics and pharmacodynamics often jointly influence drug response, as is the case with warfarin, the dose requirements for which are influenced by both the *CYP2C9* and *VKORC1* genotypes. Thus, when taking a candidate gene approach to discovery of variants impacting drug response, genes encoding proteins involved in determining drug bioavailability (transporter proteins, drug metabolizing enzymes) and response (receptor, enzyme, ion channel, and/or intracellular signaling proteins) should be considered. Genome-wide approaches to identifying determinants of drug response may reveal previously unknown proteins involved in eliciting drug response that represent potential biomarkers for predicting drug effectiveness or risk for toxicity. In addition, proteins involved in disease pathophysiology may represent attractive targets for drug development, as most often demonstrated in the area of oncology.

QUESTIONS FOR DISCUSSION

1. What are examples of drug metabolism and drug-transporter genotypes that affect drug response?
2. What are examples of drugs developed based on an understanding of genes involved in disease pathophysiology.
3. What are examples of drug-target genes with implications for drug response?
4. How might genes for drug metabolism, drug transport, and/or drug-target sites jointly influence drug response?

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CHAPTER

2

Governmental and Academic Efforts to Advance the Field of Pharmacogenomics

Kathryn M. Momary[1], *Katarzyna Drozda*[2]

[1]Department of Pharmacy Practice, Mercer University, College of Pharmacy, Atlanta, GA, United States; [2]Office of Clinical Pharmacology, Food and Drug Administration, Silver Spring, MD, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00002-4

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OBJECTIVES

1. Describe scientific and regulatory challenges associated with the clinical implementation of pharmacogenomics data.
2. Describe efforts by Academia and the National Institutes of Health to promote pharmacogenomic research discoveries.
3. Discuss pharmacogenomic initiatives by the Food and Drug Administration in regard to drug development and drug use.
4. Provide examples of regulatory activities by non-U.S. agencies in the area of personalized medicine.

INTRODUCTION

In 2010, leaders of the National Institutes of Health (NIH) and U.S. Food and Drug Administration (FDA) announced their shared vision of personalized medicine [1]. Together, they outlined the scientific and regulatory structure necessary to address the challenges in advancing personalized medicine. Examples of such challenges are listed in Table 2.1 and include identifying optimal genetic markers for drug response and encouraging the discovery of novel genetic targets for therapeutic intervention. In addition, personalized medicine is contingent on the accurate identification of patients who are likely to respond favorably to a particular drug. This identification may require the development of an in vitro companion diagnostic device (companion in vitro diagnostic [companion IVD]) product that is approved for use with a drug, similar to those available for predicting response to the anticancer drugs, e.g., trastuzumab, afatinib, and midostaurin. Thus, another challenge is to identify the optimal means for coordinated approval of drug therapy and companion IVD diagnostics.

As the field has grown, the term personalized medicine has been changed to precision medicine. The term precision medicine, as defined by the NIH, is "an emerging approach for disease treatment and prevention that takes into account individual variability in environment, lifestyle, and genes for each person" [2]. Precision medicine more accurately describes the research being done to predict drug response that extends beyond pharmacogenomics.

Academic institutions, the NIH, and the FDA are investing significant resources to address the challenges with advancing the field of pharmacogenomics and precision medicine from scientific discovery to clinical implementation. For example, the NIH is supporting pharmacogenomics discoveries through All of Us, which began as the Precision-Medicine Initiative, and promoting development of genotype-based therapies for rare inherited diseases through the Therapeutics for Rare and Neglected Diseases (TRND) Program [1,2]. The NIH has routinely offered several funding opportunities for pharmacogenomic analysis of biologic specimens from large NIH-sponsored epidemiologic studies and clinical trials. In addition, several large institutions are completing pharmacogenomics implementation projects to bring pharmacogenomics directly to the patient. Some examples include Vanderbilt University Medical Center,

TABLE 2.1 Challenges in Advancing Personalized Medicine and Efforts to Address These Challenges

Challenge	Effort to Address Challenge
Identifying genetic markers correlated with drug response	Pharmacogenomics Research Network (PGRN), NIH-supported analysis of tissue and sample banks from large epidemiologic studies
Identifying novel molecular targets for therapeutic intervention	Pharmacogenomics Research Network (PGRN)
Encouraging the development of novel therapies targeting gene-based disease pathways	NIH-funded Therapeutics for Rare and Neglected Diseases (TRND)
Delivering personalized medicine to patients	Clinical Pharmacogenetics Implementation Consortium (CPIC), IGNITE, eMERGE, and other consortia
Defining the process for coordinated approval of drug therapy and companion IVD diagnostics	FDA pharmacogenomics-related guidances, e.g., Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product
Ensuring high-quality diagnostic tests to predict drug response	FDA standards for the efficient review and oversight of genetic diagnostic tests and manufacturer claims are being established; NIH-funded voluntary genetic testing registry

University of Florida, Indiana University, St. Jude, and other consortia including nonacademic institutions. Pharmacogenomics-related activities of the FDA include active involvement in the Critical Path Initiative, development of guidance to industry related to the use of genomics in drug and diagnostic tests development, and engagement in the partnerships between scientists from academia, industry, and other federal agencies. Additionally, the NIH and the FDA are in the process of implementing legislation entitled the "21st Century Cures Act," which was signed by President Obama into law on December 13, 2016 [3]. Through these and other activities, Academia, the NIH, and the FDA hope to ease the transition from the identification of a genetic marker for drug response or a potential target for therapeutic intervention to the clinical implementation of novel therapies and strategies for improved disease management. This chapter will discuss specific initiatives by Academia, the NIH, and the FDA in coordination with external researchers to advance the field of pharmacogenomics. The chapter concludes with a brief discussion of pharmacogenomics-related activities of the European Medicines Agency (EMA) and the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan.

THE ROLE OF ACADEMIC INSTITUTIONS, THE NATIONAL INSTITUTES OF HEALTH (NIH), AND OTHER GOVERNMENT AGENCIES

Historical Efforts

Pharmacogenomics discoveries date back at least to the 1950s, when an inherited deficiency in the glucose-6 phosphate dehydrogenase enzyme was identified as the cause for primaquine-induced anemia [4]. There has been a resurgence of interest in pharmacogenomics since the completion of the Human Genome Project, which began in 1990 as a collaborative effort between the NIH National Human Genome Research Institute (NHGRI) and the U.S. Department of Energy Human Genome Project. The goal of the Human Genome Project was to sequence the entire human genome by 2005. The project was

completed in 13 years, 2 years ahead of schedule. The working draft of the human genome was published in companion papers in 2001 [5,6]. The final sequence includes 3 billion DNA base pairs and contains 99% of the gene-containing sequence, with 99.9% accuracy. Sequence data from the project were deposited into a freely accessible database run by the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov) to encourage genetic research and ultimately improve human health and well-being [7].

Medical advances from the Human Genome Project are many. For instance, pharmacogenomic discoveries have been made in the area of oncology, for which drugs have been developed to target specific cancer mutations. Afatinib, erlotinib, and gefitinib are inhibitors of the epidermal growth factor receptor (EGFR), indicated for treatment of patients with metastatic nonsmall-cell lung cancer (NSCLC), whose tumors have *EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test. Later discoveries were in the areas of cardiology, neurology, and infectious disease. As described in Chapter 8, genetic testing may influence the choice of antiplatelet therapy or dosing of the anticoagulant warfarin. In neurology, genetic testing for the major histocompatibility complex, class I, B (*HLA-B*15:02*) allele, is indicated in persons of southern Asian ancestry to determine risk for life-threatening skin reactions to the antiepileptic drugs carbamazepine, phenytoin, and to a lesser extent, lamotrigine. Similarly, screening for the *HLA-B*57:01* allele is recommended prior to initiation of the antiretroviral agent, abacavir, to predict risk for serious hypersensitivity reactions.

According to data from an independent analysis, the Human Genome Project has also had a considerable economic impact [8]. Specifically, the $3.8 billion ($5.6 billion in 2010 dollars) invested in the project over 13 years generated an economic output of $796 billion. In other words, every $1 invested in the Human Genome Project by the U.S. government generated a return of $141 to the U.S. economy. The cumulative economic impact of human genome sequencing includes 3.8 million job-years of employment (i.e., one person employed full time for 1 year) and $244 billion in personal income.

International HapMap Project

The International Haplotype Map (HapMap) Project followed the Human Genome Project. The purpose of the HapMap project was to create a publicly accessible database of common patterns of heritability in the human genome to facilitate genetic studies of common human diseases, including genome-wide association studies (GWASs). The HapMap Project was based on the occurrence of linkage disequilibrium (LD) among single-nucleotide polymorphisms (SNPs) in the genome, whereby SNPs are inherited together in sets or blocks more often than would be expected based on chance alone. A single SNP within the group is representative of SNPs within the haplotype block and thus may serve as a "tag SNP" for the haplotype. Based on patterns of LD in a given chromosomal region, only a few carefully chosen SNPs within the region need to be included to identify association at that locus with disease or drug response.

Patterns of LD may vary across ancestral groups, particularly for populations of recent African ancestry [9]. Thus, the HapMap Project was a collaborative effort among researchers from different countries (the United States, the United Kingdom, Canada, China, Japan, and Nigeria) to capture the LD patterns in various populations. For Phase I of the project, a total of 270 samples from populations with African, European, and Asian ancestry were genotyped.

The results of Phase I describing the LD patterns of approximately 1.1 million SNPs were published in 2005 [10]. The results of the Phase II second-generation human haplotype map, involving over 3.1 million SNPs, were published 2 years later [11]. To better define tag SNPs

across geographic regions, samples genotyped for Phase II included samples from the original HapMap populations plus samples from seven additional populations:

- Luhya in Webuye, Kenya (LWK)
- Maasai in Kinyawa, Kenya (MKK)
- Tuscans in Italy (TSI)
- Gujarati Indians in Houston, Texas (GIH)
- Chinese in metropolitan Denver, Colorado (CHD)
- Persons of Mexican ancestry in Los Angeles, California (MXL)
- Persons of African ancestry in the Southwestern United States (ASW)

Investigators have genotyped 1.6 million SNPs in an expanded set of HapMap I and II samples and sequenced ten 100-kilobase regions in 692 of these samples to create an integrated dataset of both common and rare alleles [12]. In 2016 the HapMap site was decommissioned due to security flaws. However, the NCBI had already planned to decommission the site as data from the 1000 Genomes Project has greater utility and HapMap site traffic had decreased significantly. Nevertheless, archived data from the HapMap Project are still freely available through the NCBI website (ftp://ftp.ncbi.nlm.nih.gov/hapmap/). According to the catalog of published GWASs maintained by the NHGRI, more than 170 studies of genetic associations with complex diseases and traits have been published based on HapMap data [13]. GWASs of drug response include studies of genetic associations with warfarin dose requirements [14], gemcitabine response in pancreatic cancer [15], amoxicillinclavulanate–induced liver injury [16], statin-induced myopathy [17], and metformin response in diabetes [18]. In many cases, GWASs have led to the discovery of variants in genes not previously suspected to have a role in disease pathology or drug response. For example, a GWAS identified the *SLCO1B1* gene that encodes the organic anion-transporting polypeptide (OATP1B1) as associated with risk of simvastatin-induced myopathy [17]. This association has since been confirmed by other investigators [19]. Per recommendations by the NIH, GWAS data from NIH-sponsored or conducted studies are made available to the scientific community through the NCBI Database of Genotype and Phenotype (DBGaP) [20].

1000 Genomes Project

The goal of a GWAS is to discover regions of the genome that are associated with an outcome of interest (e.g., drug response). The associated variant often serves as a marker (tag SNP) for the actual causal variant (the variant that underlies the observed association). Further studies of variants within the candidate region are required to identify the causal variant(s) and elucidate the mechanism underlying its effects. In many cases, rare variants within the candidate locus contribute to disease risk or phenotype. However, these rare variants are not included in the HapMap database or captured with available genotyping platforms and are thus missed in GWASs [13]. Resequencing of candidate-gene regions is historically necessary after GWASs to identify potentially causal, yet rare, variants. This is a time-consuming and costly process. Data provided by the 1000 Genomes Project are expected to limit the sequencing efforts necessary to identify rare variants underlying genetic associations discovered from GWASs.

The 1000 Genomes Project began in 2008 with the goal of developing a comprehensive catalog of common genetic variation through a DNA-sequencing approach. The project involved a pilot program and multiple research phases. Results from the initial pilot phase of the 1000 Genomes Project were reported in 2010 [21]. The first-phase analysis was published in 2012 and included the genomes of 1092 individuals from 14 populations constructed using a combination of low-coverage whole-genome and exome sequencing [22]. Phase I of the 1000 Genomes Project created a validated haplotype map of

38 million single-nucleotide polymorphisms, 1.4 million short insertions and deletions, and more than 14,000 larger deletions. They also demonstrated significant variation in the frequencies of rare and common variants in the different populations.

The main 1000 Genomes Project phase involved reconstructing the genome for 2504 individuals from 26 populations representing European, East Asian, South Asian, West African, and American populations [23]. The genetic analysis involved a combination of low-coverage whole-genome sequencing, deep exome sequencing, and dense microarray genotyping. In addition to the HapMap samples described previously, the 1000 Genomes Project included samples from the following groups, among others, to capture most of the genetic variation occurring in populations worldwide:

- British from England and Scotland (GBR)
- Finnish from Finland (FIN)
- Iberian populations in Spain (IBS)
- Han Chinese South (CHS)
- Chinese Dai in Xishuangbanna (CDX)
- Kinh in Ho Chi Minh City, Vietnam (KHV)
- Gambain in Western Division, The Gambia (GWD)
- African American in Southwest US (ASW)
- Puerto Rican (PUR)
- Colombian (CLM)
- Peruvian (PEL)
- Punjabi in Lahore, Pakistan (PJL)

The main 1000 Genomes Project phase characterized in total over 88 million variants (84.7 million SNPs), 3.6 million short insertions/deletions (indels), and 60,000 structural variants, all phased onto high-quality haplotypes [24]. The authors were able to determine greater than 99% of SNP variants with a frequency of greater than 1% for a variety of ancestries.

Similar to the HapMap Project, data from 1000 Genomes Project are made available through a publicly accessible database (http://www.internationalgenome.org). Investigators conducting GWASs may use computational approaches to impute variants from the 1000 Genomes Project into their dataset of genotyped SNPs, thus significantly expanding the number of variants interrogated for association with the phenotype of interest. This expansion increases the likelihood of capturing the causal variant underlying the observed association. The samples from the 1000 Genomes Project are available to researchers from the nonprofit Coriell Institute for Medical Research.

The ENCyclopedia of DNA Elements (ENCODE) Project

The ENCyclopedia Of DNA Elements (ENCODE) Project began in 2003 as an initiative of the NHGRI to develop a catalog of functional elements in the human genome. Functional elements refer to discrete genomic regions that encode for a particular product, e.g., a protein; or for a biochemical signature, e.g., a transcription or a chromatin structure [25]. The initial pilot phase of the project was a global, multidisciplinary effort of scientists from academia, industry, and governmental institutions to identify and analyze functional elements contained within a targeted 1% (about 30,000 kilobases, or kb) of the genome. Scientists involved included those from several American universities; the Municipal Institute of Medical Research, Barcelona, Spain; the Wellcome Trust Sanger Institute, Hinxton, UK; and Affymetrix, Inc. Additional investigators were funded to develop new technologies or to improve existing technologies to allow for efficient and cost-effective discovery and analysis of functional elements.

Results from the pilot project were published in 2007 [26]. Overall, the project showed that the organization and function of the human genome is much more complex than many had expected. Specific observations of the pilot project include the following [26,27]:

- The majority of the human genome is found in primary transcripts, which overlap and include nonprotein-coding regions;
- There are numerous unannotated transcription start sites;
- Many protein-coding genes have alternative transcription start sites that may be located greater than 100 kb upstream of the annotated start site;
- There is more alternative splicing than originally thought;
- Regulatory sites for a given gene may be located at a chromosomal position quite distal to the gene;
- Noncoding-RNA genes are involved in gene regulation (e.g., microRNAs [miRNAs]) and processing (e.g., small nucleolar RNAs [snoRNAs]), in addition, to protein synthesis;
- Noncoding "pseudogenes" can influence the structure and function of the human genome; and,
- The majority (approximately 60%) of bases under evolutionary constraint are related to functional sites rather than protein-coding exons or their associated untranslated regions.

In 2007, the ENCODE Project was expanded to cover the entire genome, with a focus on completing annotations for protein-coding genes, noncoding pseudogenes, noncoding transcripts, and their RNA transcripts and transcriptional regulatory regions [25]. Other areas of focus include DNA-binding proteins that interact with *cis*-regulatory regions, such as transcription factors and histones; DNA-methylation patterns; deoxyribonuclease (DNase) I footprints; long-range chromatin interactions; protein; RNA interactions; transcriptional silencer elements; and promoter-sequence architecture. Similar to other NIH initiatives, all data from the ENCODE Project are available through a freely accessible database (https://www.encodeproject.org).

Genotype-Tissue Expression (GTEx) Project

The Genotype-Tissue Expression (GTEx) Project was launched in 2010 by the NIH Common Fund with the goal of mapping genetic variation that affects gene expression. The project involves correlating genetic variation with tissue-specific gene expression levels through expression quantitative trait loci (eQTL) analysis. This information will be useful to researchers in selecting variants to interrogate for the effects on drug response. For example, variants found to affect expression of the ATP-binding cassette, subfamily B (*ABCB1*) gene, which codes for P-glycoprotein, are candidates for affecting disposition of drugs that are P-glycoprotein substrates. The database developed by NCBI (https://www.gtexportal.org/home) allows researchers to view and download GTEx data.

Therapeutics for Rare and Neglected Diseases (TRND) Program

Rare diseases are generally defined as diseases affecting fewer than 200,000 people in the United States. Examples of rare diseases are listed on the NIH Genetic and Rare Diseases website (https://rarediseases.info.nih.gov) and include Marfan syndrome, Gaucher disease, and severe combined immunodeficiency, all of which have a genetic cause. Because a rare disease affects a relatively small population, drug development is challenging, which is further compounded by a lack of financial incentive inherent in drug development for more common diseases.

The TRND Program is a congressionally mandated program for preclinical and early clinical development of new drug entities for rare diseases. TRND is overseen by the NIH National Center for Advancing Translational Science (NCATS) with laboratory operations administered by the NCATS Office of Strategic Alliances. Through TRND, the NIH supports development

of gene-based therapies targeting rare, inherited diseases. Examples of diseases targeted by the program include Duchenne muscular dystrophy, Niemann–Pick type C, and fibrodysplasia ossificans progressiva.

Million Veterans Program (MVP)

The Million Veterans Program (MVP) is a voluntary research program that is being termed a "mega-biobank" with a goal to partner with veterans and study how genes affect health. The overarching objective of MVP is to improve understanding of how health is affected by genetic characteristics, behaviors, and environmental factors [28]. The ultimate goal of MVP, by providing a framework for scientifically valid and clinically relevant genomic medicine, is to enhance the care of the veteran population. The MVP is a longitudinal study of veterans for future genomic (and nongenomic) research that combines data from survey instruments, the electronic health record, and biospecimens. A unique component of studying the veteran population is that data can be extracted from national Veterans Administration (VA) clinical and administrative databases, including the National Patient Care Database, VA-Medicare/Medicaid merge, and national Laboratory and Pharmacy extracts, to name a few. This study is funded entirely by the Department of Veterans Affairs Office of Research and Development.

MVP formal planning began in 2009 and enrollment began in 2011. As of August 2015, 397,104 veterans have been enrolled [28]. Subjects in the MVP complete two surveys that collect information regarding demographics, family pedigree, health status, lifestyle habits, military experience, medical history, family history of specific illnesses, and physical features to augment the data collected from the medical record. Subjects also provide a blood specimen for genotyping. The genotyping performed will be done via Affymetrix Axiom Biobank Array, the "MVP chip," with approximately 723K markers. This array is enriched for exome SNPs, has tag SNPs validated for diseases (including psychiatric traits), and has been augmented with biomarkers of specific interest to the VA population including enrichment for African American and Hispanic ancestry markers. Recruitment for this exciting study is still ongoing and will certainly contribute to our understanding of genetic contributions to disease and pharmacogenomics in this important patient population.

Pharmacogenomics Research Network (PGRN)

In contrast to the TRND Program that targets rare diseases, work by investigators within the NIH-sponsored Pharmacogenomics Research Network (PGRN) primarily targets common diseases, such as hypertension, asthma, cancers, and nicotine addiction. The PGRN consists of multiple research groups across the world with varied yet complementary expertise and the common broad objective of elucidating genetic contribution to drug response. The vision of the PGRN is "to lead discovery and advance translation in genomics in order to enable safer and more effective drug therapies" [29]. The ultimate goal of the PGRN is to catalyze and lead research in precision medicine for the discovery and translation of genomic variation influencing therapeutic and adverse drug effects.

The PGRN was initially funded in 2000, with funding renewed in 2005 and 2010. PGRN IV was established in 2015 and has a new model. The current PGRN invites participation of all investigators with an interest in pharmacogenomics research to be part of the new network, as opposed to only including those researchers funded through the PGRN. PGRN IV consists of three large center grant projects, two enabling resources for pharmacogenomics, a knowledge base, Pharmacogenomics Knowledge Base, and the PGRN Hub, established to coordinate

activities of the new PGRN to catalyze research in pharmacogenomics and precision medicine. The PGRN has evolved and expanded to catalyze research beyond the grants funded by the NIH.

Researchers within the network use both phenotype-to-genotype and genotype-to-phenotype strategies to identify and characterize genetic influences of drug response [30]. In the former, investigators search for variants predicting a phenotypic response in a well-characterized population (e.g., blood pressure response in a hypertensive patient cohort). In the latter, individuals with known genotypes may be exposed to a drug to determine response. Investigational approaches include in vitro mechanistic studies, sometimes using cell lines from the International HapMap Project, GWASs, next-generation sequencing (NGS), large-population studies, and clinical trials. In addition, to individual efforts, PGRN investigators work cooperatively through data and resource sharing and formation of cross-disciplinary teams to discover and disseminate new findings.

Pharmacogenomics Knowledge Base (PharmGKB)

The Pharmacogenomics Knowledge Base (PharmGKB) is a centralized resource providing the most comprehensive and up-to-date knowledge and tools in pharmacogenomics [31]. It is a resource that curates and disseminates knowledge about the impact of genetic variation on drug response for researchers and clinicians. The mission of PharmGKB is to advance research and facilitate clinical implementation of pharmacogenomics. Originated in 2000 as an NIH-sponsored pharmacogenomics knowledge base for the scientific community [32], the PharmGKB now encompasses clinical information including genotype-guided dosing guidelines and drug labels, potentially clinically actionable gene–drug associations, and genotype–phenotype relationships [31].

The PharmGKB is publicly available through the https://www.pharmgkb.org website. The website includes annotations of genetic variations and their relationship to disease and drug response, drug-centered pathways, and a clinical interpretation of the pharmacogenomics data. Very Important Pharmacogene (VIP) summaries are available for key genes that are of significant pharmacogenomics importance and include detailed description for individual variants and haplotypes that have been associated with drug response.

As outlined in Fig. 2.1, viewers may search the website by drug/small molecule, gene, gene variant (using the reference SNP ID number [rsID]) drug-centric pathways, or phenotype. The data are organized such that the same information can be retrieved from various starting points (e.g., searches by gene or by drug) [33]. Examples of information available when searching by drug include variants related to drug response, drug-labeling information, any pharmacogenomic-dosing

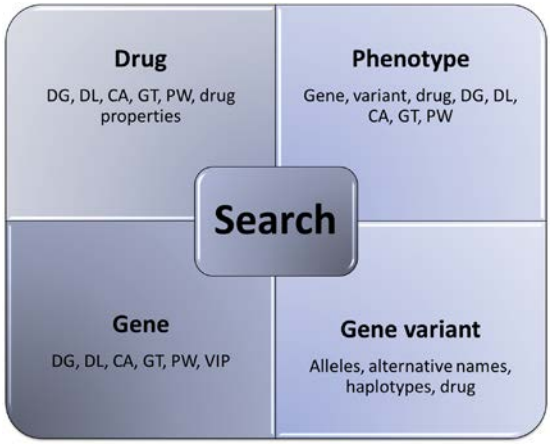

FIGURE 2.1 Information available through the Pharmacogenomics Knowledge Base website. As shown, the data are organized such that the same information can be retrieved from different starting points. *CA*, clinical annotation; *DG*, pharmacogenetic drug-dosing guideline; *DL*, drug label with pharmacogenomics information; *GT*, genetic test for pharmacogenomics; *PW*, pharmacokinetic and pharmacodynamic pathways; *VIP*, very important pharmacogenes.

guidelines, and any genetic tests available to predict drug response. A search by gene leads to information on gene location, alternative gene names, variants within the gene, related genes, and any PharmGKB-curated pathways for the gene. The viewer may also access available pharmacogenomics-dosing guidelines, labeling information, and genetic tests through the search by gene. The dosing guidelines currently available through PharmGKB are those published by the Clinical Pharmacogenetics Implementation Consortium (CPIC), described in detail in an upcoming section, the Royal Dutch Association for the Advancement of Pharmacy Pharmacogenetics Working Group (DPWG), and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS), manually curated by the PharmGKB staff. The CPNDS has a specific focus on drug safety and adverse drug reactions. The DPWG is focused on developing pharmacogenetics-based dose recommendations and assisting prescribers by integrating recommendations into computerized systems. Like the CPIC, the DPWG, and the CPNDS are composed of a small group of clinical pharmacology experts who provide consensus guidelines for integrating genetic information into therapeutic decisions [34]. However, due to the different focus of each group and medication utilization patterns by country, the medications reviewed differ, as do their recommendations (Table 2.2).

Clinical annotations related to specific gene variants are accessible to registered PharmGKB users. These annotations provide information about variants linked to drug response based on data from one or more research manuscripts, with links to access the original article and ratings for the strength of evidence. The drug-pathway view shows diagrams of proteins related to the pharmacokinetics or pharmacodynamics of a drug. The pathway for clopidogrel is shown in Fig. 2.2 [35]. Readers may click on individual proteins to view information about related genes and drugs. The VIPs and pathways on PharmGKB are peer-reviewed and published in *Clinical Pharmacology and Therapeutics* [36]. Although PharmGKB provides extensive summaries of pharmacogenetics-related information, the VIPs and guideline summaries provide a clinically relevant summary of actionable genetic variants for clinicians.

Another goal of the PharmGKB is to enable consortia examining important pharmacogenomics questions that are beyond the scope of individual research groups [37]. In this regard, the PharmGKB serves to curate (collect, format, and subject to quality control) data from disparate groups, facilitate communication among groups, actively participate in data analyses, and publish and disseminate the final data and research results to the community at large. Both data-centric and knowledge-centric consortia are ongoing, and these are described in the following subsections.

Clinical Pharmacogenetics Implementation Consortium (CPIC)

The Clinical Pharmacogenetics Implementation Consortium (CPIC) started as a joint project of the PharmGKB and PGRN to address barriers associated with transitioning data from the laboratory into clinical applications [38]. The CPIC is an international collaboration of PGRN members, PharmGKB staff, and other individuals from academic centers, clinical institutions, and pharmacy benefits management with expertise in pharmacogenomics or laboratory medicine. The consortium scores evidence linking drug-dosing decisions to genetic tests and provides consensus-based guidelines on how to use genetic test results to optimize pharmacotherapy [38,39]. Their goal is not to recommend whether genetic testing should be done, but rather, provide guidelines on how to use existing genetic information. A summary of guidelines published by CPIC can be found in Table 2.2.

TABLE 2.2 Examples of Pharmacogenomics Dosing Guidelines Curated by the CPIC and Other Organizations [34,38]

		Publishing Body		
Gene(s)	**Drug(s)**	**CPIC**	**DPWG**	**CPNDS**
HLA-B	Abacavir	X	X	
	Allopurinol	X		
	Carbamazepine	X		X
	Phenytoin	X		
	Ribavirin		X	
CYP2D6	Amitriptyline	X	X	
	Aripiprazole		X	
	Atomoxetine		X	
	Carvedilol		X	
	Clomipramine	X	X	
	Clozapine		X	
	Codeine	X	X	X
	Doxepin	X	X	
	Duloxetine		X	
	Flecainide		X	
	Fluvoxamine	X		
	Haloperidol		X	
	Imipramine	X	X	
	Metoprolol		X	
	Nortriptyline	X	X	
	Ondansetron	X		
	Paroxetine	X	X	
	Tamoxifen		X	
	Venlafaxine		X	
CYP2C9, VKORC1	Warfarin	X	X	
CYP2C9	Phenytoin	X	X	
	Glimepiride		X	

Continued

TABLE 2.2 Examples of Pharmacogenomics Dosing Guidelines Curated by the CPIC and Other Organizations—cont'd

		Publishing Body		
Gene(s)	**Drug(s)**	**CPIC**	**DPWG**	**CPNDS**
CYP2C19	Citalopram	X	X	
	Clopidogrel	X	X	
	Esomeprazole		X	
	Imipramine		X	
	Lansoprazole		X	
	Omeprazole		X	
	Pantoprazole		X	
	Sertraline	X	X	
	Voriconazole	X	X	
F5	Estrogen-containing oral contraceptives		X	
UGT1A1	Atazanavir	X		
	Irinotecan		X	
TPMT	Azathioprine	X	X	
	Fluorouracil	X	X	
	Mercaptopurine	X	X	
	Thioguanine	X	X	
DPYD	Capecitabine	X	X	
	Fluorouracil	X	X	
	Tegafur	X	X	

CPIC, Clinical Pharmacogenetics Implementation Consortium; *CPNDS*, Canadian Pharmacogenomics Network for Drug Safety; *CYP*, cytochrome P450; *DPWG*, Dutch Pharmacogenetics Working Group; *DPYD*, dihydropyrimidine dehydrogenase; *F5*, factor V Leiden; *HLA-B*, major histocompatibility complex, class I, B; *TPMP*, thiopurine-S-methyltransferase; *UGT1A1*, UDP glucuronosyltransferase 1 family polypeptide A1; *VKORC1*, vitamin K epoxide reductase complex 1.

Pharmacogene Variation (PharmVar) Consortium

Twenty years ago the star (*) nomenclature system for CYP450 variants was created by an international group of leading experts. This system was intended to provide the field with a systematic way to catalog allelic variants. As the number of known CYP450 polymorphisms continued to increase, *the Human Cytochrome P450 (CYP) Allele Nomenclature Database* was established in 2002 [40]. The PharmVar Consortium will be the new home for this gene nomenclature system and serve as a centralized "Next-Generation" Pharmacogene Variation data repository [41]. In September 2017, a new interactive database was launched https://www.pharmvar.org/. The inaugural version of PharmVar contained the

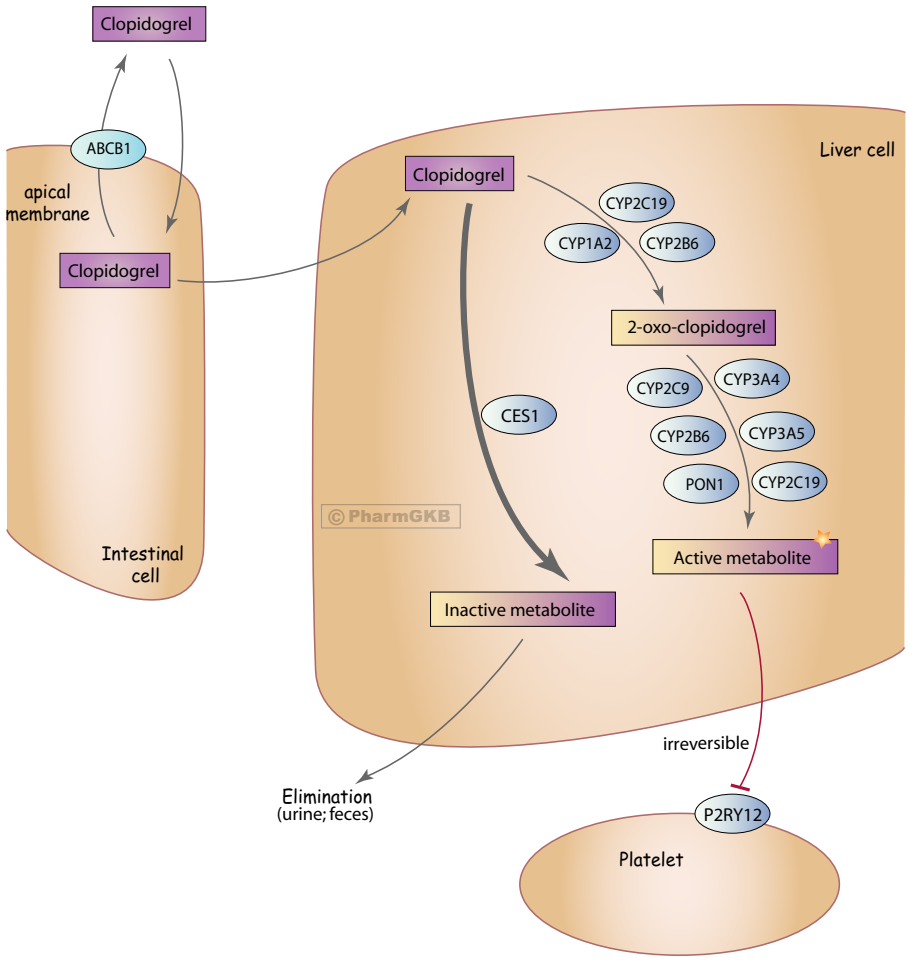

FIGURE 2.2 Clopidogrel pathway on the Pharmacogenomics Knowledge Base [35]. (See color plate 2.). *Copyright to PharmGKB with permission given by PharmGKB and Stanford University for reproduction. https://www.pharmgkb.org/pathway/PA154424674.*

CYP2C9, *CYP2C19*, and *CYP2D6* genes. Other pharmacogenomics (PGx) or pharmacogenetics genes including clinically actionable CPIC genes will be added in the future. Pharmacogene variation data in PharmVar will be directly accessible to all users, but will also be available through the PharmGKB.

ClinGen

Clinical Genome Resource (ClinGen) is an NIH-funded resource dedicated to building an authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research [42]. ClinGen is a partnership among public, academic, and private institutions [43]. The goals of ClinGen include:

- Share genomic and phenotypic data provided by clinicians, researchers, and patients through centralized databases for clinical and research use;
- Standardize the clinical annotation and interpretation of genomic variants;

- Implement evidence-based expert consensus for curating genes and variants;
- Improve understanding of variation in diverse populations to realize interpretation of genetic testing on a global scale;
- Develop machine-learning algorithms to improve the throughput of variant interpretation;
- Assess the "medical actionability" of genes and variants;
- Structure and provide access to genomic knowledge for use in electronic medical records (EMRs) ecosystems; and,
- Disseminate the collective knowledge and resources for unrestricted use in the community.

Variant information for ClinGen is housed in the Clinical Variations Resource (ClinVar), a similar database that houses genetic variant information.

ClinGen aims to help with problems with genetic studies published over the past several years [43]. Specifically, variants have been mischaracterized as being associated with disease due to insufficient standards for defining the evidence required to link a variant to disease causation and lack of information on common variation across many populations. The aggregation of data from many submitters in ClinGen permits the identification of some novel variants and better understanding of ones that have been previously studied. ClinGen-related working groups, with membership spanning more than 75 institutions, organizations, and commercial laboratories, have been assembled to tackle many of the key challenges to achieving the goals of ClinGen, including the establishment of standard procedures for evaluating genes, variants, genetic disorders, and phenotypes. Through these and other projects, ClinGen is working to improve interpretation of genetic information across the spectrum of patient care.

Curated genes currently include those effecting drug responses such as cytochrome P450, transporters, and others. However, of the currently curated 1356 genes, many are not related to drug response. In addition, clinical actionability is currently assessed by asking four questions. First, can variation in this gene cause disease? Second, does loss or gain of a copy of this gene or genomic region result in disease? Third, what changes in the gene cause disease? Fourth, are there actions that could be taken to improve outcomes for patients with this genetic risk? Thus, the current focus is not on pharmacogenomics. Although ClinGen may eventually become a go-to reference for pharmacogenomics information, this is not currently the case. Clinicians will find far more user-friendly information via PharmGKB.

Electronic Medical Records and Genomics (eMERGE) Network

The Electronic Medical Records and Genomics (eMERGE) Network was formed in 2007 with the goal of exploring the use of EMRs coupled to DNA repositories for large-scale genomic research [44]. An additional focus is on the social and ethical aspects (e.g., privacy and confidentiality) related to merging genomic information with the medical record. The eMERGE network is administered by the NHGRI's Division of Genomic Medicine, with additional funding from the National Institute of General Medical Sciences. The network is composed of experts in clinical medicine, genomics, health-information technology, statistics, and ethics. Vanderbilt University Medical Center serves as the coordinating center and supports and facilitates the work by network investigators.

The first phase of eMERGE included five institutions, each with a unique DNA biobank linked to the EMRs. Each site examined genome-wide associations in specific diseases, such as cataracts, type 2 diabetes, peripheral artery disease, Alzheimer's disease, and cardiac conduction defects [44]. A sixth GWAS was conducted using samples accrued across sites in the network. Results from eMERGE Phase I showed that linking data from the EMRs with

patient genotypes was feasible for identifying genetic contributors to disease phenotype and for large-scale GWASs across multiple clinical sites [45–48]. Phase II of eMERGE began in 2011 with the goal of exploring the incorporation of genetic information into the EMR for use in patient care. Additional sites, with focuses including abdominal aortic aneurysm, obesity, antipsychotic-induced weight gain, dementia, and infectious-disease susceptibility, among others, have been added to Phase II. Phase III of eMERGE began in 2015 with four additional sites. eMERGE-Pgx is a multicenter pilot of pharmacogenetic sequencing in clinical practice initiated through a collaboration between the eMERGE Network and the PGRN. Subjects enrolled in eMERGE will have 84 key pharmacogenes sequenced and the process for implementing preemptive genotyping for known pharmacogenetic drug–gene pairs at the 10 academic medical centers and health systems in the eMERGE-II Network will be evaluated. In addition, decision support for physicians surrounding these variants and tracking outcomes relating to implementation of these genetic test results will be evaluated, including physician actions and patient and physician attitudes and concerns. Although focus continues on genetic implementation, the network will also work to engage and educate Institutional Review Boards (IRBs), health system leaders, EMR vendors, and other stakeholders in implementing genomic medicine in clinical care.

Implementing Genomics in Practice (IGNITE)

eMERGE coordinates with IGNITE, which is an NIH-funded network dedicated to supporting the implementation of genomics in healthcare [49]. In 2013, six genomic medicine-research sites were tasked with finding ways to incorporate genomic information into EMRs and develop clinical decision support for providers across diverse healthcare settings. The IGNITE Network also disseminates the methods and best practices that its members develop to advance the implementation of genomics in healthcare. One outlet for public distribution is the Supporting Practice through Application, Resources, and Knowledge (SPARK) toolbox [50], which provides genomic medicine resources for clinicians and researchers. Within IGNITE the Pharmacogenetics Working Group was formed in January 2015 with the goal of broadly engaging institutions (funded IGNITE sites and affiliate members) implementing pharmacogenetics into practice.

Beyond eMERGE and IGNITE, other consortia and institutions are working on the implementation of pharmacogenomics in clinical practice. In addition, international consortia are working on the pharmacogenomics of clopidogrel, selective serotonin reuptake inhibitors, and tamoxifen, whose goals are to amass very large sample sizes and expertise from across the globe to dissect the genetic underpinnings of variable response to these medications.

In addition, many academic institutions and other healthcare providers are working individually on projects to expand pharmacogenomics implementation. These institutions include Vanderbilt, the University of Florida, Indiana University, St. Jude Children's Research Hospital, and many others. One such example is the 1200 Patients Project at the University of Chicago, which aims to develop a new model for personalized medical care through preemptive pharmacogenomics. Pharmacogenomic information is given to the provider via the Genomic Prescribing System (GPS). The GPS is a web-based portal used by physicians that displays interactive, patient-specific pharmacogenomics results in the form of succinct, electronic clinical consults. Patient-specific results are provided as a patient-tailored synopsis of the information translated into clinical meaning, and include prescribing recommendations and suggested alternative medications.

The 21st Century Cures Act

The 21st Century Cures Act is intended to help accelerate medical product development to address disease prevention, diagnosis, and treatment, and apply innovations into medical practice in a more efficient way [3]. One of the laws mandated by the 21st Century Cures Act is the Precision Medicine Initiative (PMI), which includes the following efforts:

- Developing a network of scientists to assist in carrying out the purposes of the Initiative;
- Developing new approaches for addressing scientific, medical, public health, and regulatory science issues;
- Applying genomic technologies, such as whole-genomic sequencing, to provide data on the molecular basis of disease;
- Collecting information voluntarily provided by a diverse cohort of individuals that can be used to better understand health and disease; and,
- Other activities to advance the goals of the Initiative, as deemed appropriate by the Secretary of Health and Human Services.

The law emphasizes that implementation of the PMI shall ensure collaboration of government agencies (e.g., the NIH, the FDA). As an example, in addition to the Precision Medicine Initiative, the 21st Century Cures Act includes Subtitle B "Advancing New Drug Therapies," which contains provisions related to the Qualification of Drug Development Tools (DDTs) and Targeted Drugs for Rare Diseases that pertain to matters related to biomarkers and rare genetic diseases.

All of Us Research Program

All of Us is part of the PMI launched in fiscal year 2016 when $130 million was allocated to NIH to build a national, large-scale research participant group, and $70 million was allocated to the National Cancer Institute (NCI) to lead efforts in cancer genomics as part of PMI for Oncology [51]. Much like MVP, All of US is a large-scale initiative to collect genetic and health information.

The All of Us Research Program aims to enroll one million or more participants from throughout the United Stated to provide insight into the substantial interindividual differences in physiology, risk of disease, and response to therapy [52]. Subjects will be recruited through social media and other tools as well as from participating healthcare organizations. Subjects can self-enroll via the internet and complete information via online forms. Participating subjects will provide some or all of the following during participation in the study: survey data, electronic health-record information, physical measurements, biospecimens (blood, urine, or saliva), and passive mobile digital health data.

The All of Us Research Program is expected to last at least 10 years, with active enrollment occurring in the first 5 years. Follow-up is expected to be continuous for the life of the project. Specimen analysis methods have yet to be published, but this study will create a vast amount of important data.

THE ROLE OF THE FOOD AND DRUG ADMINISTRATION AND OTHER INTERNATIONAL GOVERNMENT AGENCIES

Recognizing limitations of the trial-and-error approach to drug prescribing and the high attrition rates in drug development, the FDA has engaged in a number of activities to optimize drug use and enhance drug development through a better understanding of genetic determinants of drug response. Examples of FDA's initiatives toward personalized medicine include:

- Efforts of the Critical Path Initiative (C-Path), Critical Path Innovation Meeting (CPIM), and Biomarker Qualification Program (BQP) [53–55]; and,

- Enhancing regulatory science and expediting drug development by advancing the use of biomarkers and pharmacogenomics, as a part of the reauthorization of the Prescription Drug User Fee Act (PDUFA V) [56].

Critical Path Initiative (C-Path)

In light of rapidly evolving technologies and newly emerging areas of science, the FDA acknowledged the widening gap between scientific discoveries and its translation into innovative medical treatments. Based on this assessment, a list of potential opportunities for scientific improvements was propagated that included discoveries in genomics, development and utilization of biomarkers, modernization of clinical trial development, and intensive use of bioinformatics in disease modeling and trial simulation [57]. The FDA recognized that collaboration of all stakeholders is necessary to support 21st-Century medical product development [54]. Having a unique position, the FDA facilitated establishment of the Critical Path Initiative (C-Path). The C-Path alliance of international leaders from federal agencies, patient groups, academic researchers, industry, and healthcare providers works together to enhance and accelerate development of modern technologies and translation into successful medical product development. Some of the initiatives include establishment of the multiple consortia, e.g., the Polycystic Kidney Disease Outcomes Consortium (PKDOC), the Coalition Against Major Diseases (CAMD), the Duchenne Regulatory Science Consortium, and partnership with Parkinson's UK to launch the Critical Path for Parkinson's Consortium [58].

Critical Path Innovation Meeting (CPIM)

As a part of a broad C-Path initiative, the Critical Path Innovation Meeting (CPIM) was developed by the Center for Drug Evaluation and Research (CDER). The CPIM allows for nonbonding dialog between the meeting requestor (e.g., investigators from industry, academia, and patient advocacy groups) and FDA. It provides an opportunity for stakeholders from the public or private sectors to discuss with FDA issues related to early biomarker development, innovative clinical outcome assessments, emerging technologies (e.g., genomic technologies), and other novel approaches to develop medical products [59]. The goals of the CPIM are to provide general advice on how a novel methodology or technology might enhance drug development, or address existing knowledge gaps, as well as broaden regulatory perspectives. The CPIM may recommend pursuing work through existing consortia, or establishment of new consortia, and engagement of the wider scientific community by organizing public workshops [60].

Biomarker Qualification Program (BQP)

The Biomarker Qualification Program (BQP) was established to support the CDER's work with external stakeholders to develop biomarkers that can be applied in the drug-development process [61]. The BQP provides a framework for the development, regulatory acceptance, and integration of biomarkers for a particular context of use in drug development. In addition, the BQP encourages the identification of emerging biomarkers for evaluation and use in regulatory decision-making. Its supports outreach to stakeholders and facilitates communication and exchange of knowledge between involved participants. To streamline understanding and use of biomarkers, the FDA–NIH Joint Leadership Council worked together to develop the BEST (Biomarkers, EndpointS, and other Tools) Resource [62]. The first phase of BEST comprised a "living" glossary that clarifies important definitions and describes some of the hierarchical relationships, connections, and dependencies among the terms it contains. The NIH and the FDA intend to use the definitions included in the glossary when communicating on topics related to its contents (e.g., biomarkers) to

ensure a consistent use of the terms and, therefore, a common understanding of the issues.

Prescription Drug User Fee Act (PDUFA V)

On July 9, 2012, President Obama signed into law the Food and Drug Administration Safety and Innovation Act (FDASIA) of 2012 [63]. This new law included the reauthorization of the PDUFA that provided FDA with a source of stable and consistent funding that allows the agency to focus on supporting the development of innovative drug therapies and access to safe and effective new medicines. As a part of the PDUFA V, the FDA committed to provide emphasis on integration of biomarkers and pharmacogenomics in the process of drug development. To offer expertise, additional highly trained staff were integrated into the clinical review divisions, the clinical pharmacology, and statistical review disciplines. To maintain and expend FDA staff proficiency, additional training and public workshops were offered to provide scientific exchange of knowledge on the current status of biomarkers and pharmacogenomics between regulatory and nonregulatory stakeholders.

Guidance Development

The FDA routinely works with drug developers to provide advice on approaches to establish the safety and effectiveness of medical products before marketing applications are submitted. The reviewers at the FDA ensure that the appropriate biomarkers, including genetic markers, are evaluated at the Investigational New Drug (IND) stage, and the benefit–risk profile of the drug is assessed according to genetic and other biomarkers in the New Drug Application (NDA) and Biologic License Application (BLA) review stages [64,65]. In addition, the FDA also recommends pharmacogenomic-based postapproval studies, as appropriate, to better understand the drug-related benefits and risks in certain patient subsets.

FDA's positions may be more broadly clarified through guidance documents, and several guidance documents have been released in the past decade to provide specific advice on the development and use of pharmacogenomic biomarkers. In the final guidance, "Clinical Pharmacogenomics: Premarketing Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling," the FDA provides drug developers with advice on when to consider genetic information during the drug-development process and provides recommendations regarding appropriate collection and storage of genetic samples in clinical trials [66]. Specifically, the document outlines the application of genetic data for the assessment of:

- The basis for pharmacokinetics (PK) outliers and intersubject variability in clinical response;
- Ruling out the role of polymorphic pathways as clinically significant contributors to variable PK, pharmacodynamics (PD), efficacy, or safety;
- Estimating the magnitude of potential drug–drug interactions;
- Investigating the molecular or mechanistic basis for lack of efficacy or occurrence of adverse reactions; and,
- Designing clinical trials to test for greater effects in specific subgroups (i.e., use in clinical trial enrichment strategies) [64].

With regard to the latter, genetics can help to identify individuals most likely to respond to a particular therapy or most likely to experience a clinical event of interest, thus enabling enrichment strategies in Phase III trials (e.g., vemurafenib for the treatment of BRAF V600 mutation-positive unresectable or metastatic melanoma). These predictive and prognostic enrichment strategies are discussed in the draft guidance "Enrichment Strategies for Clinical Trials to Support Approval of Human Drugs and Biological Products" [67]. Utilization of an enrichment study design allows for detection of a real effect of a treatment more rapidly and with

a smaller sample size [68]. However, important concerns related to the generalizability, practical applicability of the study results, and potential lack of information for subjects excluded based on the enrichment strategy should be considered during the trial-design stage.

In the case of therapeutic products that clearly show differential effect in drug response or safety based on a genetic factor, it may be necessary to have tests that can adequately identify patients in the clinic (e.g., enasidenib, venetoclax, vemurafenib, dabrafenib, and trametinib). In those instances, the FDA recommends development and contemporaneous approval of a companion IVD. IVDs that are essential for the safe and effective use of a corresponding therapeutic product are referred to as companion IVDs, which was defined in the draft guidance entitled "In Vitro Companion Diagnostic Devices" [69]. FDA subsequently published a more practical guide to developing companion IVDs in the 2016 draft guidance entitled "Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product" [70]. The intent of this guidance is to assist therapeutic product and companion IVD diagnostic sponsors in the process of codevelopment. It provides considerations for planning and executing a therapeutic product clinical trial that also includes the investigation of a companion IVD. Furthermore, it provides examples of administrative issues and regulatory information for the submission of the therapeutic product and companion IVD.

As new genotyping technologies emerge (i.e., NGS), FDA focused on optimizing regulatory oversight for NGS in vitro diagnostic (IVD) tests to help accelerate research and the clinical adoption of precision medicine while assuring the safety and effectiveness of these tests. As part of the PMI effort, draft guidance entitled "Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases" were published [71]. In the process of guidance development, the FDA held public workshops to engage experts and community stakeholders in a discussion and to receive valuable feedback. This guidance provides recommendations for designing, developing, and validating NGS-based tests for germline diseases that FDA believes are appropriate for use in providing a reasonable assurance of the analytical validity of such tests. In addition, to the NGS-based in vitro diagnostics guidance, around the same time the FDA released draft guidance, "Use of Public Human Genetic Variant Databases to Support Clinical Validity for Next Generation Sequencing (NGS)-Based In Vitro Diagnostics" [72]. This draft guidance document describes how publicly accessible databases of human genetic variants can be utilized as sources of valid scientific evidence to support the clinical validity of genotype–phenotype relationships in FDA's regulatory review of NGS-based tests. By applying recommendations described in this guidance by the genetic-variant databases, FDA hopes to encourage the deposition of variant information in databases, decrease regulatory burden on test developers, and spur advancements in the interpretation and implementation of precision medicine.

Pharmacogenomics Biomarkers in Drug Labeling

The FDA routinely attends to emerging information relative to therapeutic risks and benefits. When a significant and clinically relevant genetic marker emerges in the pre- or postmarketing setting, a drug's labeling, which provides prescribers with a summary of the essential scientific information needed for the safe and effective use of the drug, will often reflect how that genetic factor pertains to its use. In the postmarketing setting, a request to update the labeling of an approved drug may be made by regulatory scientists at the FDA, the manufacturer of the drug in question, or an external researcher [64]. Based on the strength of evidence and potential impact of a gene–drug response association, FDA scientists, together with individuals from

the drug-manufacturing company, determine when a pharmacogenomics update to drug labeling is required.

Pharmacogenomics information may relate to alterations in gene structure (e.g., genetic polymorphism, mutations), gene-expression differences, functional deficiencies with a genetic etiology, or chromosomal abnormalities. Pharmacogenomics information may be included in various sections of the drug labeling (e.g., Dosage and Administration, Warnings and Precautions, Clinical Pharmacology). Pharmacogenomic associations with significant implications for drug safety may be included in a Boxed Warning (e.g., carbamazepine, clopidogrel). As of 2017, the labeling for approximately 200 FDA-approved drugs contain information related to genetic biomarkers with implications for drug exposure, clinical response, risk for adverse drug events, and/or dose optimization. About half have a prescribing recommendation tied to the genetic factor. Examples of drugs with pharmacogenomic labeling information are shown in Table 2.3. The FDA maintains a Table of Pharmacogenomic Biomarkers in Drug Labeling on its website [73].

Updates to the labeling of existing drugs in the postmarketing setting are usually based on data from the literature. In many cases, the data are based on retrospective assessment of genetic associations with drug response. For example, the data informing the decision to update the warfarin labeling were largely from pharmacogenomic studies using existing data generated 50 years after the drug was first approved for marketing [74]. In contrast, prospective data generated in properly designed drug clinical trials are available to FDA to guide pharmacogenomic labeling decisions for newly approved drugs. For instance, initial approval of trametinib for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations as detected by an FDA-approved test. Pharmacogenomics is only one of many factors to consider in individualization of drug therapy [75]. Therefore, to assess a drug response (beneficial or adverse) in the context of multiple patient factors (e.g., age, gender, genetics, organ impairment, concomitant medications), a systems-based approach [76], including physiologically based pharmacokinetic modeling, may be needed [77].

Diagnostic Approval/Clearance

An additional role of the FDA in advancing personalized medicine involves the approval of companion IVD diagnostics to predict drug response, or to select a population for whom the drug safety and effectiveness are known. The availability of an accurate diagnostic test is key for successful implementation of pharmacogenomic testing and precision medicine at large. One of the first successful examples of codevelopment of a drug and companion IVD was trastuzumab and an immunohistochemical test (HercepTest). The companion IVD test measures expression levels of HER2 in breast cancer tissue and identifies patients for whom trastuzumab therapy is indicated [78]. To date, the FDA has approved multiple companion IVDs for oncology medications including, e.g., afatinib, cetuximab, crizotinib, enasidinib, imatinib, olaparib, osimertinib, pertuzumab, panitumumab, rucaparib, trastuzumab, vemurafenib, and venetoclax (Table 2.3). However, clinical tests may also be manufactured and used in a single laboratory, commonly referred to as laboratory-developed tests (LDTs), that may not necessarily be FDA-cleared or -approved. To ensure the quality of diagnostic tests and protect those ultimately affected by test results (i.e., the patients), the FDA gathered feedback on the draft guidance "Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs)" and actively engaged in a public discussion with interested stakeholders [79]. Based on continued discussion, comments obtained on the draft guidance, and feedback from a broad range of stakeholders, the FDA issued a discussion paper on LDTs [80].

TABLE 2.3 Examples of Drugs With Pharmacogenomic-Related Information in the FDA-Approved Labeling

Drug(s)	Biomarker(s)	Context
Abacavir	*HLA-B*	Boxed Warning states that patients with the *HLA-B*57:01*allele are at increased risk for hypersensitivity to abacavir. Abacavir is contraindicated in HLA-B*57:01-positive patients. Genetic screening is recommended before starting abacavir.
Afatinib, Erlotinib, and Gefitinib	*EGFR*	The drug indicated for the first-line treatment of patients with metastatic nonsmall-cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test.
Azathioprine, 6-mercaptopurine	*TPMT*	Description of increased risk for myelotoxicity with conventional azathioprine or 6-mercaptopurine doses in patients with a nonfunctional *TPMT* allele in the Dosage and Administration section. Consideration of TPMT genetic testing is recommended.
Atomoxetine	*CYP2D6*	Dosage and Administration section provides dose adjustment in CYP2D6 poor metabolizers to avoid adverse drug effects.
Capecitabine	*DPYD*	Warnings and Precautions section provides information about an increased risk for severe toxicity (e.g., diarrhea, stomatitis, neutropenia, and neurotoxicity) in patients with dihydropyrimidine dehydrogenase deficiency.
Carbamazepine	*HLA-B*	Boxed Warning provides information of increased risk for serious dermatologic reactions (e.g., TEN, SJS) in patients with the *HLA-B*15:02* variant. Patients from genetically at risk regions (e.g., Southeast Asia) should be screened for the HLA-B*15:02 allele prior to starting carbamazepine.
Cetuximab	*EGFR, RAS*	The drug is indicated for EGFR-expressing, metastatic colorectal cancer. Cetuximab is not indicated for the treatment of RAS-mutated colorectal cancer or when the results of the RAS mutation tests are unknown.
Codeine	*CYP2D6*	Warnings and Precautions section informs about greater conversion to morphine and higher than expected morphine concentrations in patients who are ultrarapid metabolizers. These individuals are at increased risk for symptoms of overdose (e.g., extreme sleepiness, confusion, respiratory depression) with conventional doses of codeine.
Clopidogrel	*CYP2C19*	Boxed Warning of reduced drug effectiveness in CYP2C19 poor metabolizers with 2 loss-of-function alleles.
Crizotinib	*ALK*	Confirmation of the lymphoma kinase (*ALK*)-positive mutation is required using an FDA-approved test prior to drug use.
Irinotecan	*UGT1A1*	Dosage and Administration section provides recommendation to reduce irinotecan dosage by one level in homozygotes for the *UGT1A1*28* allele because of an increased risk for neutropenia.
Lenalidomide	Chromosome 5q deletion	Boxed Warning recommends monitoring complete blood counts weekly for the first 8 wks of therapy for patients with del 5q myelodysplastic syndrome.
Tetrabenazine	*CYP2D6*	Dosage and Administration section provides recommendation to test CYP2D6 genotype if the patient needs a higher than 50 mg/da dose.
Trastuzumab	*HER2*	The drug is indicated for *HER2* overexpressing cancers as detected by an FDA-approved companion diagnostic.

Continued

TABLE 2.3 Examples of Drugs With Pharmacogenomic-Related Information in the FDA-Approved Labeling—cont'd

Drug(s)	Biomarker(s)	Context
Vemurafenib	*BRAF*	The drug is indicated for the *BRAF* V600E mutation as detected by an FDA-approved companion diagnostic.
Venetoclax	Chromosome 17p deletion	The drug is indicated for the treatment of patients with chronic lymphocytic leukemia (CLL) with 17p deletion, as detected by an FDA approved test.
Warfarin	*CYP2C9, VKORC1*	Dosing recommendations are provided according to *CYP2C9* and *VKORC1* genotypes.

For FDA-cleared or FDA-approved tests, see the following website: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm; For information related to in vitro companion diagnostic devices, see the following website: https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm.

ACTIVITIES OF NON-U.S. AGENCIES

European Medicines Agency (EMA), European Union

The EMA is a decentralized body of the European Union (EU) responsible for the evaluation and supervision of drugs for the benefit of public and animal health in the EU. Similar to the FDA, the EMA has engaged in several activities related to pharmacogenomics. These include formation of a Pharmacogenomics Working Party (PgWP) and publication of scientific guidelines on pharmacogenomics.

The PgWP is composed of experts in the field of genetics or pharmacogenomics who are nominated by the Committee for Medicinal Products for Human Use (CHMP) and charged with providing recommendations to the CHMP on issues related directly and indirectly to pharmacogenomics. Activities of the PgWP include preparing guidelines for the evaluation of pharmacogenomic information in regulatory submissions and organizing pharmacogenomics workshops and training.

In 2012, the CHMP published a guideline for pharmaceutical companies on the use of pharmacogenomic methodologies in the evaluation of medicinal products [81]. The guideline focuses on genetic variation affecting drug pharmacokinetics and provides guidance on both required and recommended studies at different stages of drug development. The guideline specifically addresses:

- Circumstances in which pharmacogenomics studies are appropriate;
- Recommendations or requirements regarding study design, subject selection, and sampling; and,
- Evaluation of the clinical impact of genetic variability for treatment recommendations and labeling.

The PgWP plans to release an addendum to the guideline on the use of pharmacogenomics methodologies in the evaluation of medicinal products in early 2018. In addition, the PgWP is working on a guideline on a predictive biomarker-based assay in the context of drug development and life cycle.

Similar to the Critical Path Initiative, the European Union (represented by the European Commission) and the European pharmaceutical industry (represented by EFPIA, the European Federation of Pharmaceutical Industries and Associations) established a partnership named the Innovative Medicines Initiative (IMI) [82]. The IMI is Europe's largest public–private initiative aiming to speed up the development of better and safer therapies for patients. The IMI facilitates collaboration between industrial and academic experts to improve development and patients' access to innovative therapies, especially in areas of unmet medical or social need.

Pharmaceuticals and Medical Devices Agency (PMDA), Japan

The Pharmaceuticals and Medical Devices Agency (PMDA) is responsible for regulatory drug and medical devices review and approval in Japan. In 2009, the PMDA introduced a pilot scientific consultation program that focuses on pharmacogenomics and biomarker qualification [83]. This program involves consultation with drug sponsors to identify strategies for utilizing pharmacogenomics and biomarkers in drug development. The purpose of this program is to improve efficiency in drug development as well as enable development of personalized medicines.

Similar to FDA-approved drug labeling, pharmacogenomics information has been added to the package inserts for many drugs marketed in Japan. In a recent review, the authors found that the majority of drugs with FDA-approved pharmacogenomic labeling also contained pharmacogenomic information in their PMDA-approved package insert [84]. However, there are fewer instances of pharmacogenomics information included in the Warnings or Contraindications sections of labeling for drugs marketed in Japan versus the United States. In the case of carbamazepine, the pharmacogenomics information in U.S. labeling is specific for the patients of Chinese ancestry. Specifically, the *HLA-B*15:02* allele, associated with an increase in carbamazepine-induced Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in Han Chinese, is rare in persons from Japan (frequency <0.1%). In a recent GWAS conducted in Japanese patients, the *HLA-A*31:01*, but not the *HLA-B*15:02* allele, was associated with serious carbamazepine-induced cutaneous adverse reactions, including SJS and TEN [85]. The PMDA-approved package insert for carbamazepine has since been amended to include information about the HLA-A*31:01 allele [84].

CONCLUSION

The Human Genome Project and subsequent efforts in the field have led to an improved understanding of the structure and function of the human genome. This understanding has enabled numerous discoveries of genetic contributions to drug response in addition to discovery of novel therapeutic targets. Although there are examples of pharmacogenomics applications to patient care, broad application of personalized medicine has yet to be realized. However, the field of pharmacogenomics application is advancing rapidly with multiple consortia working in this area.

In 2011, the National Human Genome Research Institute published its updated vision for the future of genomic medicine. This vision focuses on prevention and treatment of disease based on an understanding of human biology and diagnosis [86]. Opportunities for genomic medicine are many. As outlined by the National Human Genome Research Institute [86], these include enabling routine use of genomic-based diagnostic panels; better characterizing genetic contributors to disease phenotype and drug response, thus allowing for improved therapeutic strategies, and revealing sites for novel drug development; and developing practical systems for applying genomic information to patient care. Ongoing efforts by the NIH and FDA are addressing each of these areas and their related challenges to further advance personalized medicine. Non-U.S. agencies, including the EMA and PMDA, are also engaged in regulatory activities with the goal of safer medication use through individualized therapy.

QUESTIONS FOR DISCUSSION

1. What are the scientific and regulatory challenges of advancing personalized medicine?
2. What are some examples of efforts by the NIH and the FDA to address the challenges associated with advancing personalized medicine?

3. What resources are available to assist clinicians with implementing pharmacogenomics into patient care? How do you gain access to these resources?
4. How is the FDA encouraging the incorporation of genetic information in drug development?
5. What efforts are non-U.S. agencies taking to advance pharmacogenomics?

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CHAPTER

3

Incorporating Pharmacogenomics in Drug Development: A Perspective From Industry

Ophelia Yin, Alexander Vandell

Daiichi Sankyo Pharma Development, Basking Ridge, NJ, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00003-6

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OBJECTIVES

1. Discuss the role of pharmacogenomics in various aspects of drug development.
2. Identify major opportunities and challenges of applying pharmacogenomics in drug development.
3. Propose potential strategies toward increased success of pharmacogenomic research in the pharmaceutical industry

INTRODUCTION

The pharmaceutical industry works to develop safe and effective therapeutics. Thousands of new molecular entities are examined each year, but only a few are able to progress through the drug-development pipeline, achieve successful regulatory approval, and enter the marketplace. In a research heavy industry, a high dropout rate is expected. However, the industry is facing significant headwinds that are steadily decreasing productivity in drug development.

The onerous time, cost, and risk associated with new drug development pose a major challenge to the pharmaceutical industry. Since 1950, the number of new drugs achieving United States Food and Drug Administration (FDA) approval and being successfully brought to market per billion US dollars spent is dropping by 50% every 9 years [1]. A recent analysis of the development procedures for new molecular and biological entities shows that the average time required for a product to progress from the start of clinical testing to acquiring regulatory approval is 7.6 years [2]. However, the likelihood that a compound that enters clinical testing will eventually reach the market continues to be low, averaging 16% across different therapeutic areas. The long development time and low success rate result in high costs of developing drugs [3]. In 2014, overall research and development (R&D) costs for the pharmaceutical sector exceeded $79 billion in the United States [4].

In response to these challenges, the industry is undergoing a paradigm change. For the past several decades, a blockbuster model was the standard and the focus has been put on developing drugs for large markets consisting of patients with chronic illnesses [1]. Recently, both the industry and regulatory agencies are transitioning to a new precision-medicine model which aims to target the right treatments to the right patients at the right time. Key to successful execution of precision medicine is the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or response to a specific treatment. Realizing the vision of precision medicine will require the successful integration of many different medical and scientific disciplines.

One well-publicized example of a pharmaceutical company shifting its R&D framework toward a precision medicine-based approach comes from AstraZeneca. From 2005 to 2010, AstraZeneca's R&D performance was well below industry averages. In 2011, the company implemented a new decision-making process focused on the right target, tissue, safety, patient, and commercial potential, what it referred to as the "5 Rs." As part of this initiative, substantial focus was placed on having a firm biological basis for target and patient selection, which included increased emphasis on pharmacogenomics research. In 2016, AstraZeneca reported that success rates for drug candidates had improved from 4% to 16%, suggesting that precision-medicine approach can improve industry performance [5].

Among these disciplines, disease genetics and pharmacogenomics are poised to play major roles. The primary processes in the development of new therapeutic modalities are identification and characterization of drug target, and evaluation and optimization of the pharmacokinetics, pharmacodynamics, efficacy, and safety of drugs. Pharmacogenomics can be utilized in

TABLE 3.1 Application of Pharmacogenomics in Different Aspects of Drug Development

Drug Development Phase	Applications
Target screening/identification	• Identification of potential drug targets • Characterization of the gene encoding the drug target • Assessment of drug target variability
Preclinical/animal toxicity	• Identification of safety markers • Provision of potential early safety indicators or warning signals
Pharmacokinetics and pharmacodynamics	• Explanation of outliers and interpatient variability in pharmacokinetics • Patient selection—inclusion/exclusion criteria • Bridge to other ethnic populations • Identification of drug responders and nonresponders • Dose-range selection and dose modification
Safety	• Patient selection—inclusion/exclusion criteria • Analysis of reported adverse effects with pharmacogenomic tests • Identification of patients at high risk of adverse drug effects

all these aspects (Table 3.1) to improve the efficiency of drug development through:

- Validating more genomically diverse and higher-quality drug targets;
- Eliminating unsuitable drug candidates and therapeutic targets at earlier stages of development;
- Accelerating clinical development by facilitating the design of trials that more clearly show improved efficacy and safety; and
- Optimizing risk–benefit profiles of drugs in targeted patient populations.

GENETIC RESEARCH AND NEW DRUG TARGET IDENTIFICATION

The field of genetic research has greatly advanced over the last two decades as a result of several critical initiatives, such as the Human Genome Project, HapMap, 1000 Genomes, and Encyclopedia of DNA Elements (ENCODE). The completion of the Human Genome Project not only provided a map of complete sequence of human genome (~20,000 protein coding genes), but also led to a huge improvement in sequencing technology. In just 6 years from 2008 to 2014, the cost of sequencing an average human genome dropped precipitously from about US$10 million to US$1000 [6]. It is projected that with some additional improvements, next-generation sequencing (NGS) technology will further reduce the cost to US$100.

The International HapMap Project began in 2002 with the aim of describing common patterns of human genetic variation across 11 global ancestry groups. Using the technology available at the time, the HapMap project was able to reliably catalog variants present in at least 10% of a population [7]. The 1000 Genomes Project, initiated in 2008, made use of NGS technology to catalog variants present in 0.5%–1% of a population [8].

Another critical project, the Encyclopedia of DNA Elements (ENCODE), also commenced in 2003 as a follow up to the Human Genome Project. The approximately 20,000 protein coding genes only account for about 1.5% of the human genome. The rest of the genome, often regarded as "junk DNA," contains a wide variety of regulatory elements such as promoters, transcriptional regulatory sequences, and regions of chromatin structure and histone modification. Changes in gene regulation either through inherited polymorphisms or spontaneous point mutations have the ability to disrupt protein

function and cell processes, resulting in disease. The ENCODE project aimed to determine the location and function of these regulatory elements to better understand how they influence gene transcription [9]. Results of ENCODE have provided numerous leads for potential new drug targets by revealing that over 80% of the human genome participates in at least one biochemical-, RNA-, or chromatin-associated process in at least one cell type. Many variants located within "junk DNA" regions previously associated with disease by genome-wide association studies are now known to be enriched within noncoding functional elements identified by ENCODE [10].

Understanding how genetic polymorphisms affect protein function and downstream physiologic or pathophysiologic processes can provide insight into potential drug targets. In recent years pharmacogenomics research has made high-profile contributions toward the identification of several novel drug targets. Recent well-known examples in oncology include vemurafenib for melanoma patients harboring a *BRAF* V600E mutation [11], and crizotinib and ceritinib, for nonsmall-cell lung cancer (NSCLC) patients with echinoderm microtubule-associated protein-like 4 (*EML4*)– anaplastic lymphoma kinase (*ALK*) translocation [12,13].

Many examples also exist in other therapeutic areas, such as evolocumab and alirocumab, two recently approved monoclonal antibodies that block anti-proprotein convertase subtilisin/kexin type 9 (PCSK9). In 2003, mutations in PCSK9 were identified in two French families with familial hypercholesterolemia (FH), a disorder characterized by severe elevations of Low-Density Lipoprotein (LDL) and increased cardiovascular risk. Subsequent in vitro studies revealed that PCSK9 plays a critical role in regulating LDL receptor trafficking. Overexpression of PCSK9 using in vivo models was found to be associated with increased LDL, whereas PCSK9 knockout mice displayed increased LDL receptor expression and reduced circulating LDL levels. Large-cohort studies identified further associations between polymorphisms in the PCSK9 gene, LDL levels, and cardiovascular risk in humans [14].

In 2010, the first Phase I clinical trial for evolocumab was initiated by researchers at Amgen, and a similar Phase I clinical trial for alirocumab was initiated as collaboration by researchers at Sanofi and Regeneron. Both novel PCSK9-inhibiting antibodies were shown to be safe in healthy volunteers and appeared to have LDL-lowering properties. Additional Phase II and Phase III clinical trials were conducted, and confirmed the efficacy of these drugs in reducing LDL levels in hypercholesterolemic subjects. Both drugs received FDA approval in 2015 [14–16].

A third PCSK9 inhibitor, bococizumab, was under development by Pfizer, but was discontinued in November 2016 due to high rates of injection-site reactions, the development of antidrug antibodies, and subsequent attenuation of LDL-lowering effects observed in two large cardiovascular outcome studies [17,18]. On the other hand, clinical study data suggest that immunogenicity is not an issue for alirocumab and evolocumab [19,20]. The reason for such a difference is probably because bococizumab is a partially murine monoclonal antibody, whereas alirocumab and evolocumab are both fully humanized. These examples illustrate that, although pharmacogenomic research can provide valuable insight into identifying novel drug targets, target identification alone is not a guarantee of successful development. A robust clinical development plan is still needed, and industry researchers must be prepared to address challenges presented by the pharmacokinetic profile, lack of efficacy, or safety findings.

Following the success of the Human Genome Project, HapMap, 1000 Genomes, and Encyclopedia of DNA Elements (ENCODE), the pharmaceutical industry is now investing substantially in genetic research. The industry hopes these investments will further their precision-medicine initiatives by identifying new drug targets for oncology indications, rare and

orphan diseases, and individuals with common diseases who are currently treatment refractory. The DiscovEHR Collaboration is an excellent example in this regard.

In 2007, Geisinger Health System began collecting blood, serum, and DNA samples from their patients. Their goal was to create a central biobank of samples that would be linked to information in electronic health records, allowing samples and data to be used to address broad research questions. Currently, there are approximately 100,000 participants in this program, and Geisinger's eventual goal is for participation to be offered to every active patient, which would create a database of over a half million individuals [21].

In 2014, the DiscovEHR Collaboration was formed from a partnership between Regeneron and Geisinger Health System. The goal of DiscovEHR Collaboration was to build a platform for discovering and validating genetic variants that may be linked to diseases with major unmet medical needs. By 2016, DiscovEHR had sequenced the exomes of more than 50,000 Geisinger patients, and identified more than four million rare single-nucleotide polymorphisms (SNPs) and insertion–deletion events. Approximately 176,000 of these may be linked to loss of gene function [22].

Angiopoietin-like 4 (ANGPTL4) is involved in inhibiting lipoprotein lipase, the primary enzyme responsible for releasing free fatty acids from lipoprotein triglycerides for utilization by tissues. Reduced function of ANGPTL4 by the missense E40K mutation has been shown to be associated with increased High-Density Lipoprotein (HDL) and decreased triglycerides. However, the effect of this polymorphism on risk of coronary artery disease remained to be determined. Researchers sequenced the exons of ANGPTL4 in over 42,000 individuals in the DiscovEHR cohort to identify E40K heterozygotes and homozygotes. Triglyceride levels per allele were 13% lower among carriers of the E40K variant than among E40 homozygotes ($P = 2.0 \times 10–23$), and HDL cholesterol levels per allele were 7% higher among E40K carriers than among E40 homozygotes ($P = 1.6 \times 10–17$). E40K variant carriers were significantly less likely than noncarriers to have coronary artery disease (odds ratio, 0.81; 95% CI, 0.70 to 0.92; $P = .002$) [23]. These findings stimulated the subsequent exploration of ANGPTL4 by researchers at Regeneron Pharmaceuticals, Inc. as a potential drug target in a dyslipidemic monkey model. In this model, blockade of ANGPTL4 by the monoclonal antibody REGN1001 decreased circulating triglyceride levels by approximately 60% [23]. Such a substantial decrease is encouraging and supports the further development of REGN1001 as a drug candidate.

GENETICS AND PRECLINICAL ANIMAL TOXICOLOGY STUDIES

In preclinical animal toxicology studies, the use of validated predictive safety biomarkers can enhance the understanding of toxicity mechanisms, aid the selection of drug candidates that are more likely to be tolerated in humans, potentially reduce the cost and time required for preclinical evaluation, and ultimately reduce late-phase failures. Thus, toxicogenomic profiling via the use of DNA microarray-based approaches has provided the most striking advances in understanding both disease mechanisms and the effects of drug treatment. Several consortia, through the partnerships among industry, academic, and other nonprofit groups, were formed to build toxicogenomic profiling platforms for drug safety assessment (Table 3.2) [24]. For example, the Predictive Safety Testing Consortium (PSTC) was launched by Critical Path Institute (C-Path) in 2006, with goals to [1]: validate predictive animal model-based biomarkers aimed at reducing the cost and time involved in conducting nonclinical safety studies [2]; provide potential early indicators of clinical safety in drug development and postmarketing surveillance, and [3] provide new

TABLE 3.2 Examples of Consortia Using Pharmacogenomics for Drug Safety Biomarker Assessment

Consortium	Species and Study Design
CEBS programme of the NIEHS proteomics	Rat/mouse; primary focus on liver toxicity
Japanese toxicogenomics project	Rat plus in vitro; primary focus on liver toxicity
C-Path institute: Predictive Safety Testing Consortium	Rat and human markers being sought for liver and kidney toxicities, myopathy, vasculitis, and carcinogenicity
HESI Genomics Committee	Rat and in vitro markers being sought for kidney and heart toxicities, and genotoxicity
InnoMed PredTox	Rat (focus on liver and kidney toxicities)

CEBS, chemical effects in biological systems; *FDA*, Food and Drug Administration; *HESI*, Health and Environmental Sciences Institute; *NIEHS*, National Institute of Environmental Health Sciences.

tools to assist in regulatory decision-making [25]. This group currently includes a membership of 19 pharmaceutical companies and is actively engaged with a number of projects to identify, evaluate, and qualify biomarkers for cardiac hypertrophy, nephrotoxicity, hepatotoxicity, skeletal myopathy, testicular toxicity, and vascular injury. Some of the achievements to date include the development of a qualification package of seven nonclinical kidney safety biomarkers that was approved by the FDA, the European Medicines Agency (EMA), and the Pharmaceuticals and Medical Devices Agency (PMDA), Letters of Support from FDA and EMA for two new kidney safety biomarkers and four new skeletal muscle injury biomarkers [26]. Most recently, through the collaboration with another C-Path consortium, Duchenne Regulatory Science Consortium (D-RSC), PSTC received a Letter of Support from EMA for the measurement of glutamate dehydrogenase (GLDH) as a biomarker of hepatocellular liver injury [27]. As part of the InnoMed PredTox project, a panel of novel biomarkers for improved detection of liver injury and renal toxicity in preclinical toxicity studies was also reported [28,29]. The 10-year Japanese Toxicogenomics Project (TGP) produced one of the largest public toxicogenomics databases, Open Toxicogenomics Project-Genomics Assisted Toxicity Evaluation Systems (TG-GATEs). The database consists of approximately 24,000 microarray samples of about 200 different compounds, studied in rat tissues in vivo and rat- or human-derived primary cultured hepatocytes in vitro. Both single- and repeat-dose samples are available [30]. These new preclinical safety markers and toxicogenomics database are useful tools in accelerating decision-making (i.e., the interpretation of isolated histopathological findings in some drug safety–evaluation animal models) and risk assessment choices.

PHARMACOGENOMIC/ PHARMACOKINETIC RESEARCH

Phase 1 research is traditionally conducted to characterize the pharmacokinetic profile (i.e., absorption, distribution, metabolism, and elimination [ADME]) of an investigational drug, and to gain an understanding how extrinsic (i.e., drug–food and drug–drug interactions) and intrinsic (i.e., demographics, organ function) factors may affect the drug's pharmacokinetics. Genotype is one of the important intrinsic factors. Performing genotype and phenotype determinations to establish genotype–phenotype relationships in early pharmacokinetic studies will allow an assessment of the effects of specific polymorphisms on the pharmacokinetics of drugs, and provide explanations for outliers or intersubject

variability in response. The information thus derived, together with other properties of drug (i.e. safety margin, dose–response curves for both safety and efficacy) and types of disease, will then facilitate the evaluation of clinical consequences of the observed pharmacokinetic variability.

The effects of ADME-related polymorphisms on enzyme or transporter function and pharmacokinetics can be dramatic, with effects large enough to warrant the inclusion of genotype-based dosing guidelines in drug labels. Some well-known examples include cytochrome P450 2C9 (CYP2C9) and warfarin [31], CYP2C19 and clopidogrel [32], and CYP2D6 and atomoxetine [33].

Atomoxetine

Atomoxetine is a selective norepinephrine reuptake inhibitor for the treatment of attention-deficit hyperactive disorder. Atomoxetine is primarily metabolized in the liver via the CYP2D6 enzyme. The CYP2D6 gene is highly polymorphic, and a number of variants produce reduced or non-functional CYP2D6. To investigate the effects of genetic variation on atomoxetine pharmacokinetics, researchers at Lily administered 14C-labeled atomoxetine to healthy volunteers that were either genetically determined CYP2D6 extensive metabolizers (EMs) or poor metabolizers (PMs). Plasma clearance of atomoxetine was reported to be 0.35 L/h/kg in CYP2D6 extensive metabolizers (EMs) and 0.03 L/h/kg in CYP2D6 poor metabolizers (PMs), with the area under the concentration-versus-time curve (AUC) being approximately 10-fold and steady-state peak plasma concentration (Cmax) being 5-fold greater in PMs than in EMs. At the same dose level, atomoxetine AUC in PMs was similar to that observed in EMs with concomitant administration of strong CYP2D6 inhibitors [34]. Pharmacogenomic samples were collected in efficacy and safety trials of atomoxetine. Given the anticipated impact of CYP2D6 genotype on atomoxetine pharmacokinetics, these samples were analyzed and a database of clinical and pharmacogenomic data was constructed. Such a database allowed a retrospective analysis, which revealed that some adverse drug effects occurred twice as frequently or statistically significantly more frequently in PM patients when compared with EM patients. Such adverse effects included decreased appetite (23% of PMs, 16% of EMs); insomnia (13% of PMs, 7% of EMs); sedation (4% of PMs, 2% of EMs); depression (6% of PMs, 2% of EMs); tremor (4% of PMs, 1% of EMs); early morning awakening (3% of PMs, 1% of EMs); pruritus (2% of PMs, 1% of EMs); and mydriasis (2% of PMs, 1% of EMs). Although pharmacogenetic testing is not mandated before prescribing atomoxetine, the updated product label suggests that dose adjustment of the drug may be necessary when administered to patients known to be CYP2D6 PMs or when coadministered with potent CYP2D6 inhibitors [33].

Pharmacogenomic/Pharmacodynamic Research

Although the ability of genotype to affect the pharmacokinetics of drugs is reasonably well understood and accepted (as illustrated by the previous example), genetic variation can also independently affect the pharmacodynamics of drugs. Polymorphisms present in drug targets, such as g-protein-coupled receptors, ion channels, and signaling molecules, may contribute to variability in pharmacodynamic effects. A well-known example is the VKORC1—1639 G>A polymorphism and warfarin. The −1693 G>A polymorphism is located in the promoter region of warfarin, and is associated with increased copies of the variant allele resulting in decreased expression of VKORC1. Thus, individuals with reduced VKORC1 expression require lower warfarin doses to achieve the same anticoagulant effect.

Incorporating pharmacogenomic/pharmacodynamic research into drug development can optimize clinical trial design or patient stratification to match the right drug with the right

patients, so that the risk of failure is reduced, and the likelihood of success is increased. For example, if genotypes are predictive of drug effects in early trials, enrichment or stratification strategies can be implemented in late-phase patient trials to ensure appropriate representation of genotypes of interest. Continued assessment of pharmacogenomic/pharmacodynamic data during clinical trials or postmarket stage can also enhance the confidence in continuing the clinical development program or optimize therapy in individual patients. Pharmacogenomic research can also provide critical insights into reasons for clinical trial failure and help industry researchers rescue compounds by refining of the target patient population.

Gefitinib

Gefitinib inhibits the tyrosine kinase activity associated with the epidermal growth factor receptor (EGFR), and thus blocks intracellular signal transduction pathways emanating from this receptor implicated in the proliferation and survival of cancer cells. In mouse xenograft models, gefitinib shows a significant inhibition on tumor growth in a dose-dependent manner [35]. In phase-I trials in patients with nonsmall-cell lung cancer (NSCLC), gefitinib was found to be well tolerated. Inhibition of EGFR and its related downstream signaling is achieved at a dosage of 250 mg/day, whereas the maximal tolerated dose of gefitinib is 700 mg/day [36]. Accordingly, randomized phase-II trials (Incremental Decrease in Endpoints Through Aggressive Lipid Lowering [IDEAL]-1 and IDEAL-2) were conducted to evaluate the activity of gefitinib at two dose levels, 250 and 500 mg/day, in pretreated patients with advanced NSCLC [37,38]. Similar results were obtained from both studies. The response rate of gefitinib was moderate and similar for the 250 versus 500 mg doses (18.4% vs. 19% in IDEAL-1 and 12.0% vs. 9.0% in IDEAL-2). However, from these trials important evidence emerged showing major efficacy of gefitinib in some specific subgroups of patients, such as females, those with adenocarcinoma histological subtype, and those of Asian ethnicity. In IDEAL-1, the odds of responding (i.e., having complete or partial responses) was approximately 3.5-fold higher for patients with adenocarcinoma than for patients with other tumor histology ($P = .021$), 2.5-fold higher for females than males ($P = .017$), and 1.6-fold higher for Japanese than non-Japanese patients ($P = .25$) [37]. In IDEAL-2, the response rate was greater in adenocarcinoma than in other histologies (13% vs. 4%, $P = .046$), and greater in female than in male patients (19% vs. 3%, $P = .001$) [38].

On the basis of these data, several phase-III trials were launched. The Iressa Survival Evaluation in Lung Cancer (ISEL) study enrolled 1692 patients with advanced NSCLC, who had previously received chemotherapy [39]. Median survival did not differ significantly between gefitinib versus placebo groups, either in the overall population (5.6 vs. 5.1 months, $P = .087$) or among the patients with adenocarcinoma (6.3 vs. 5.4 months, $P = .089$). However, preplanned subgroup analyses showed significantly longer survival in the gefitinib group than the placebo group for nonsmokers (8.9 vs. 6.1 months, $P = .012$) and patients of Asian origin (9.5 vs. 5.5 months, $P = .01$) [20]. In addition, analysis of ISEL tumor biopsy samples suggested that high EGFR gene copy number was predictive of gefitinib-related effect on survival. Patients with EGFR mutations obtained higher response rates than those with wild-type EGFR genotype (37.5% vs. 2.6%) [40].

To further understand the role of clinicopathologic features versus molecular selection, the phase-III Iressa Pan-Asia Study (IPASS) used several clinicopathologic criteria to identify a group of patients who may derive further benefit from gefitinib therapy [41]. The study included Asian, chemotherapy-naïve patients who never smoked and had adenocarcinoma of the lung. In this carefully selected population, progression-free survival (PFS) was found to

be superior for gefitinib as compared to carboplatin/paclitaxel. In the subgroup of patients who were positive for EGFR mutation, PFS was significantly longer among those who received gefitinib than among those who received carboplatin–paclitaxel, whereas in the subgroup of patients who were negative for EGFR mutation, PFS was significantly longer among those who received carboplatin–paclitaxel. Thus, this was the first study that definitively identified EGFR mutation status as an important predictive marker for gefitinib therapy. The use of first-line gefitinib in a selected patient population was further supported by other phase-III trials, in which only patients with chemotherapy- naïve advanced NSCLC-harboring EGFR mutations were enrolled [42,43].

Based on additional phase II and III trials [44–53] conducted with gefitinib as a first-line treatment of NSCLC (patients were either unselected or selected based on clinical characteristics or EGFR mutation), it becomes obvious that gefitinib, when used in unselected patients, produces only a modest response rate of 10%–20%. A greater benefit was obtained in clinically selected subgroups of patients, such as nonsmokers, Asians, and patients with adenocarcinoma histology. Although clinical characteristics may identify potential candidates for gefitinib therapy, the most predictive marker is the presence of EGFR gene mutations, present in approximately 10%–20% of NSCLC patients and more frequently found in nonsmokers, Asians, and patients with adenocarcinoma. The two most common mutations are small inframe deletions in exon 19 and amino acid substitution in exon 21 (L858R), which collectively account for >90% of known activating EGFR mutations. In an in vitro experiment using NSCLC cell lines, it has been shown that gefitinib is approximately 70–200-fold more potent in inhibiting these mutant forms than the wild-type form [54].

It is worthwhile to note that the important association between EGFR mutation and treatment outcome with gefitinib also explains the failure of gefitinib in combination with chemotherapy (gemcitabine–cisplatin, or paclitaxel–carboplatin) in previous trials. For example, two randomized phase-III trials, Iressa NSCLC Trial Assessing Combination Treatment-1 and -2 (INTACT-1 and INTACT-2) evaluated the drug in combination with chemotherapy as first-line treatment. Both studies failed to demonstrate a survival advantage when gefitinib was administered with chemotherapy [55,56]. One of the most likely reasons could be that patients were not selected based on any of the criteria later found to be associated with a sensitivity to gefitinib. Thus, in both trials, the population who was most likely to receive a real benefit from the treatment (EGFR mutation) was not large enough to statistically change the overall results.

Imatinib

Imatinib was among the first targeted anticancer agents, developed based on the understanding of the genomic basis of chronic myeloid leukemia (CML) to specifically inhibit the tyrosine kinase activity of BCR–ABL. Although imatinib has been proven to be a remarkably successful treatment for CML, nonresponse or disease progression occurs in some patients. The emergence of new BCR–ABL tyrosine kinase domain mutations and clonal evolution are the known mechanisms for the acquired drug resistance [57,58]. However, increasing evidence also suggests that for a substantial number of patients, resistance may be apparent (pseudoresistance), and other factors such as drug transporters and imatinib plasma levels may play a contributing role to the therapeutic outcome in imatinib-treated CML patients. Thomas et al. were the first to show that inhibition of the organic cation transporter 1 (OCT1) in peripheral blood leukocytes from CML patients caused a decrease in intracellular imatinib uptake [59]. This finding was confirmed by others, showing that influx of imatinib into the CML cell is mediated by OCT1 [60]. Subsequent

studies demonstrated that low OCT1 activity is a major determinant of suboptimal response to imatinib. More patients who had high OCT1 activity achieved major molecular response (major molecular response was defined as a BCR–ABL transcript level ≤0.1%, measured by real-time quantitative reverse transcriptase polymerase chain reaction and expressed on the International Scale) by 5 years compared with patients who had low OCT1 activity (89% vs. 55%; $P = .007$). Moreover, a low OCT1 activity was significantly associated with lower event-free survival (48% vs. 74%, $P = .03$) and overall survival (87% vs. 96%, $P = .02$) following 5 years treatment with imatinib (Fig. 3.1) [61]. A recent analysis further suggested that the combination of low OCT-1 activity and low-trough imatinib levels defines a group of patients who achieve the lowest rates of major molecular response by 24 months when compared to all other patients. These patients are also at the highest risk for imatinib failure when compared to all other patients [62]. Hence, it has been proposed that for poor responses seen in patients with low OCT1 activity, increasing the initial dose of imatinib from 400 to 600–800 mg/day may provide a strategy to overcome low OCT1 activity. Alternatively, patients with low OCT1 activity who are unable to tolerate with higher imatinib doses may benefit from the second-generation tyrosine kinase inhibitors, dasatinib or nilotinib, because they are not transported into CML cells by OCT1 [57].

Pharmacogenomic/Safety Research

Pharmacogenomics can improve patient care by predicting adverse drug effects or identifying patients at high risk of adverse drug effects. In the case of irinotecan, polymorphisms in the metabolic pathway of irinotecan significantly affect the risk of neutropenia. Genetic polymorphisms can also impact drug safety through mechanisms unrelated to a drug's pharmacokinetics and pharmacodynamics. This is illustrated by abacavir, in which polymorphisms in an immune system-related gene drive severe-hypersensitivity reactions.

Irinotecan

Irinotecan is a topoisomerase I inhibitor approved as a single agent for second-line treatment and in combination with 5-fluorouracil (5-FU) and leucovorin for first-line treatment in metastatic colorectal cancer. In vivo, irinotecan is hydrolyzed by carboxylesterase to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which is 1000-fold more cytotoxic than the parent drug [63,64]. SN-38 is inactivated via glucuronidation catalyzed primarily by the uridine diphosphate glucuronosyltransferase (UGT)1A1 isoform. The formed conjugated product, SN-38G, is eliminated in the bile and can be deconjugated back to SN-38 via the action of intestinal β-glucuronidase enzyme [65]. The presence of a dinucleotide (Thymine Adenine [TA]) insertion in the TATA box of the UGT1A1 promoter results in a 70% reduction in enzyme expression, and in several studies, it was suggested that UGT1A1*28 polymorphism was significantly associated with decreased glucuronidation of SN-38 [66–69].

The use of irinotecan has been associated with severe grade 3 and 4 toxicities, primarily neutropenia and diarrhea, in a considerable number of patients [70]. It is generally considered that this toxicity is mediated by the active metabolite of irinotecan, SN-38. Based on a prospective study in 66 cancer patients who received irinotecan monotherapy, grade 4 neutropenia occurred in 50% and 12.5% of patients who were homozygous and heterozygous carriers of UGT1A1*28, respectively, whereas no grade 4 neutropenia occurred in patients who had the UGT1A1 wild-type genotype [71]. Data from a large clinical trial (North Central Cancer Treatment Group N9741) in 520 patients with metastatic colorectal cancer showed similar results. The overall risk of grade 4 neutropenia was higher in homozygous UGT1A1*28 patients than in those with the other genotypes: 36.2% in the homozygous UGT1A1*28 group versus 18.2% in the

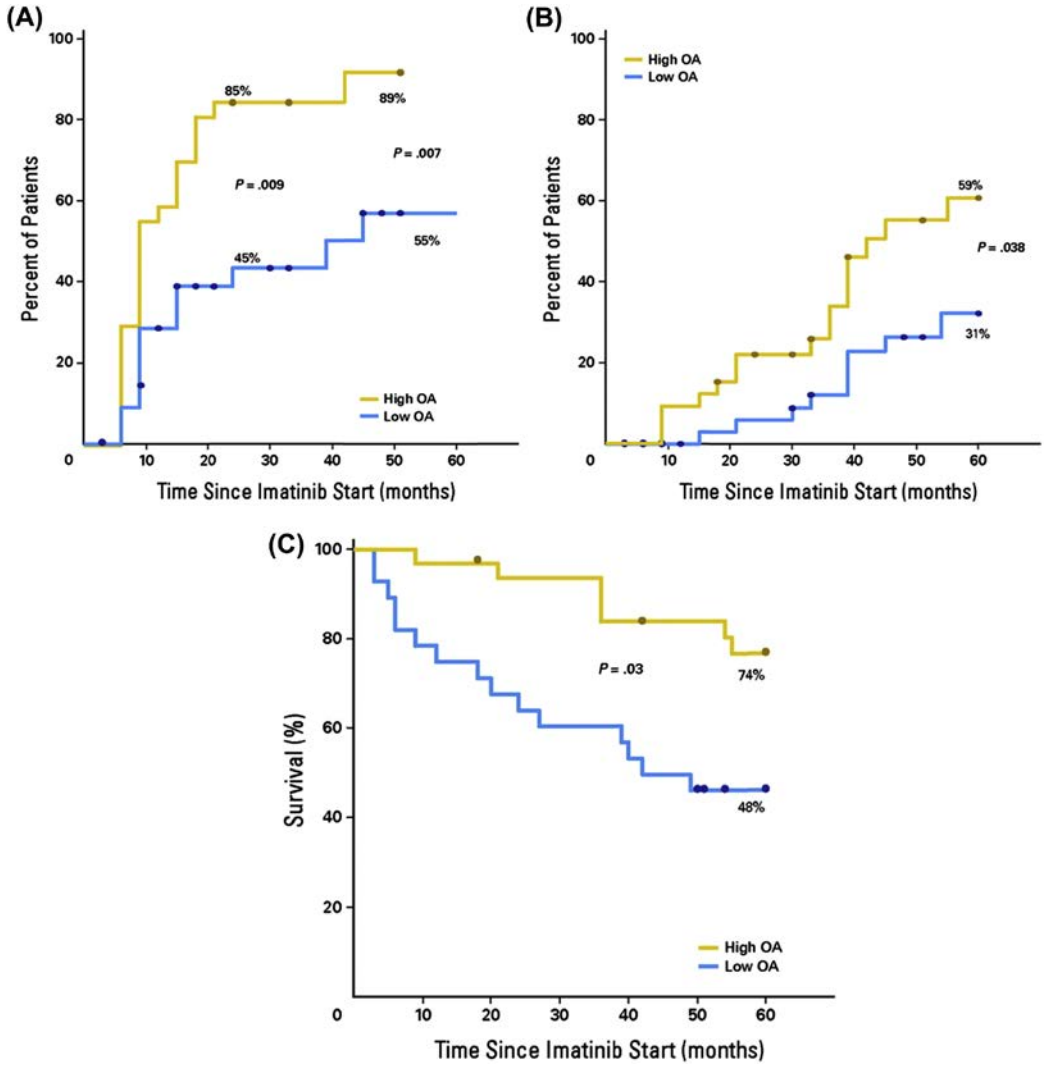

FIGURE 3.1 The percentage of patients achieving (A) major molecular response (MMR), (B) complete molecular response (CMR), or (C) event-free survival on the basis of low and high organic cation transporter-1 (OCT-1) activity groups. Kaplan–Meier curves demonstrate that a significantly greater proportion of patients who had high OCT-1 activity achieve MMR and CMR by 5 years when compared with patients who had low OCT-1 activity. There is also significant event-free survival advantage for patients with high OCT-1 activity. *Adopted from Yang CH, Yu CJ, Shih JY, Chang YC, Hu FC, Tsai MC, Chen KY, Lin ZZ, Huang CJ, Shun CT, Huang CL, Bean J, Cheng AL, Pao W, Yang PC. Specific EGFR mutations predict treatment outcome of stage IIIB/IV patients with chemotherapy-naive non-small-cell lung cancer receiving first-line gefitinib monotherapy. Journal of Clinical Oncology 2008;26:2745–53.*

heterozygous UGT1A1*28 group and 14.8% in the homozygous wild-type group [72].

Based on these findings and other reports, the irinotecan label was modified in 2005 to indicate the role of UGT1A1*28 polymorphism in the metabolism of irinotecan and the associated increased risk of severe neutropenia. The label modifications also include recommendations for lower starting doses of irinotecan in patients homozygous for the UGT1A1*28 polymorphism

[73]. Subsequent to the label update, a genetic test kit for UGT1A1*28 was approved by the FDA.

Abacavir

Abacavir is a nucleoside analog inhibitor of human immunodeficiency virus (HIV) type-1 (HIV-1) reverse transcriptase approved for the treatment of HIV-1 infection in both treatment-naïve and treatment-experienced patients. When administered in combination with other antiretroviral drugs, usually as a fixed-dose combination of abacavir/lamivudine given together with a nonnucleoside reverse transcriptase inhibitor or a ritonavir-boosted protease inhibitor, abacavir has good efficacy against susceptible HIV-1 isolates [74,75].

Early in the clinical development of abacavir, it was observed that the drug caused a hypersensitivity reaction in some patients. This reaction typically appears within 6 weeks of initiation of therapy [76]. Symptoms resolve within a few days after discontinuation of abacavir, but rechallenge results in the rapid onset of an overwhelming immediate-type hypersensitivity reaction that can lead to hypotension, respiratory failure, and death. The incidence of abacavir-induced hypersensitivity was shown to be higher in white patients than in black patients [77], indicating a potential genetic basis for susceptibility to this hypersensitivity reaction.

Subsequently, retrospective analyses identified a significant association between the presence of human leukocyte antigen (HLA)-B*5701 allele and risk for abacavir-induced hypersensitivity [78,79]. Prospective screening for HLA-B*5701 in patients who were candidates for abacavir-containing antiretroviral therapy showed a reduction in the incidence of abacavir hypersensitivity reaction. In a large, prospective, double-blind, randomized trial conducted to validate the use of genetic testing to prevent abacavir hypersensitivity (Prospective Randomized Evaluation of DNA Screening in a Clinical Trial [PREDICT]-1 study), 1956 patients were randomly assigned to undergo prospective HLA-B*5701 screening. This screening excluded HLA-B*5701-positive patients from abacavir treatment (prospective-screening group), or those who have undergone a standard-of-care approach of abacavir use without prospective HLA-B*5701 screening (control group). A hypersensitivity reaction was clinically diagnosed in 93 patients, with a significantly lower incidence in the prospective-screening group than in the control group (3.4% vs. 7.8%, $P < .001$). These results established the effectiveness of prospective HLA-B*5701 screening to prevent the hypersensitivity reaction to abacavir [80]. The abacavir package insert and antiretroviral treatment guidelines recommend screening for HLA B*5701 before prescribing abacavir [81].

Several other drugs display severe adverse reactions now known to be associated with genetic polymorphisms. For example, allopurinol hypersensitivity is associated with HLA B*5801 [82], and flucloxacillin hepatoxicity is associated with HLA B*5701 (the polymorphism responsible for abacavir hypersensitivity discussed earlier) [83]. However, unlike abacavir, the package inserts for these drugs do not include guidelines recommending genetic screening as prospective randomized trials have not yet been conducted to demonstrate the effectiveness of genetic screening in limiting adverse events. As both drugs are currently off patent, it is unlikely that funding will materialize for randomized controlled trials similar to the one that was conducted with abacavir and funded by GlaxoSmithKline.

CHALLENGES IN APPLYING PHARMACOGENOMICS TO DRUG DEVELOPMENT

Over the last decade, our ability to interrogate the genome has increased dramatically, whereas the cost of doing so has dropped considerably. As the pharmaceutical industry moves toward a full embrace of precision medicine, pharmacogenomics is being applied to all aspects of the drug

development process. Knowledge of potential relevant polymorphisms is critical to designing a rational clinical development plan, and aids the "go" or "no-go" decision process. As a common practice for the pharmaceutical industry, if a compound is metabolized extensively by an enzyme exhibiting genetic polymorphisms and consequently predicted to show extremely high interindividual variability, it may be abandoned for further development, or the development plan may be modified. Similarly, polymorphisms related to adverse drug safety or a lack of efficacy may trigger "no-go" decisions. If development is continued, genetic testing may be necessary. For example, dosing regimens may need to be based on the metabolizer group (e.g., poor, extensive, and rapid metabolizers) to which a patient belongs. In some cases, certain genotypes related to safety or efficacy may be contraindications for administration. Such genotype-guided approaches to dosing may be crucial to achieve optimal safety and efficacy results and maximize the probability of a successful late-phase trial.

Using genomic markers to prescreen patients for a preregistration clinical study is likely to restrict the indication, and consequently the market potential of the approved drug. This may be a disincentive for companies to develop therapies for less severe diseases. However, excluding potential nonresponders will increase the risk–benefit ratio for the drug. As illustrated with gefitinib clinical development, earlier trials without carefully selected patient populations have resulted in disappointing outcomes, whereas restriction of study participation to the likely responders (i.e., patients with a *EGFR* mutation) has made the drug viable. In these cases, incorporating pharmacogenomics into clinical trial design allowed a reduction in sample size and trial duration, and assisted in increasing the likelihood of therapy success. In addition, pharmacogenomics can help manage postapproval risks. This is well demonstrated in the examples of abacavir and irinotecan, in which genetic testing is used to identify patients at high risk for serious adverse effects and to assist with treatment decisions in those patients.

Although the benefits of applying pharmacogenomics in drug development have been increasingly recognized in recent years, and pharmacogenomics studies are becoming an integral part of drug development, several scientific and practical challenges remain, including a lack of existing hypotheses, sample-size requirements, and operational and logistic issues.

For pharmacogenomic research to have a meaningful impact on drug development, each study should test a clearly defined hypothesis based on what is known or suspected about the effects of a given polymorphism. However, because of the complex nature of disease, the role of genetics may not be sufficiently well understood to enable selection or prioritization of candidate genes, such as drug targets, disease, or pathway-related genes, for evaluation. Recent advances in genetic research has simultaneously begun filling our gaps in our knowledge base, and greatly expanded the list of potential targets. Creating and mining large datasets consisting of genetic data with associated clinical information, as in the DiscovEHR initiative, is an approach being taken to help generate testable hypotheses that can drive future drug-development efforts.

Ensuring adequate sample size is another challenge. In planning a pharmacogenomic study, the size of both the expected drug response and the relevant genetic effect are important considerations [84]. When there is no clear existing genetic hypothesis, pharmacogenomic study evaluation is planned as a supplemental component or "add-on" to clinical trials. In this setting, the sample size of the trial is determined by the primary objective, such as the expected drug response. As a result, depending on the frequency of genotype/phenotype in the study population, the statistical power needed to detect a gene effect may not be adequate, leading to uninterpretable or inconclusive results. Even when the initial clinical studies with limited

numbers of subjects generated positive correlation results, in many cases they are hypothesis-generating and still required confirmation in a larger population.

Industry-led pharmacogenomic research has been mostly retrospective in nature; only until recently has there been a shift in the oncology space. Conducting prospective pharmacogenomic studies requires a clear testable hypothesis. Without such a hypothesis, pharmacogenomic research is at risk of becoming a "fishing expedition," and results may not be easily interpretable or useful in guiding later drug development. The past decade has witnessed tremendous growth in understanding the roles specific mutations play in tumor biology, allowing specific pharmacogenomic hypotheses to be generated and tested as part of a wider precision medicine–based approach. As discussed in the gefitinib case, experiences and knowledge gained through its development course stimulated further research in EGFR mutations and acquired resistance. They subsequently facilitated the development of newer generation EGFR tyrosine kinase inhibitors that are specifically designed to overcome the acquired resistance from first-line therapy and evaluated in a prospectively selected patient population.

Outside of oncology, prospective pharmacogenomic approaches are beginning to be successfully implemented in the development of drugs targeting rare diseases, as illustrated by the following example of ivacaftor.

Cystic fibrosis (CF) is an inherited genetic disease caused by variants in the gene cystic fibrosis transmembrane conductance regulator (CFTR), which regulate chloride and water transport in the body. The disruption in chloride and water transport results in the formation of thick mucus in the lungs and digestive tract leading to severe respiratory and digestive problems, subsequent inflammation, and ultimately leading to an early death. Aprecision medicine–approach to treating CF is highly desirable as there are almost 2000 variants in CFTR associated with CF, each affecting receptor function in different ways [85]. The first CFTR variant to be successfully targeted was the G551D variant, present in approximately 4%–5% of CF patients, which causes loss of function by interfering with the gate that regulates chloride ion flow [86]. Following successful clinical trials in patients with at least one G551D variant, the FDA reviewed and approved ivacaftor for the treatment of G551D CF in only 3 months. Recently, the FDA expanded the approval of ivacaftor to include use in patients with one of 23 additional CFTR variants. This decision was heavily based on in vitro data suggesting that ivacaftor could exhibit receptor-potentiating effects in the presence of these variants, and further supported by observations from previous and ongoing clinical trials [87].

From an operational perspective, DNA samples must be collected before any pharmacogenomics work starts. The pharmaceutical industry has embraced DNA sample collection, with a recent survey showing that approximately 80% of companies routinely collect DNA samples from subjects in Phase I, II, and III studies; whereas approximately 20% of companies routinely collect DNA samples as a part of Phase IV studies [88]. However, support for pharmacogenomics sample collection is not universal. Principal investigators, Institutional Review Boards (IRBs), and ethics committees (ECs) often feel that due to the sensitive nature of genetic research, this research should be highlighted in a separate Informed Consent Form, and that DNA sample collection should be optional, especially in Phase II and III studies which are conducted in patients. Similarly, some industry researchers fear that the requirement of participation in genetic research will slow patient enrollment in late-phase trials.

There is also wide variability in DNA sample collection and handling across the globe. Some specific examples are provided as follows [89,90].

- DNA sample banking may not be allowed under any circumstances by certain countries or IRBs/ECs (including Brazil and Thailand).
- DNA sample banking may be allowed, but require prespecification of the genetic tests to be performed; thus limiting the ability of industry to initiate new genetic research in response to emerging information obtained during the course of drug development.
- Certain countries (including China) allow DNA sample collection, but prohibit DNA samples to be transported out of the countries.
- Some IRBs/ECs require that DNA genotyping results be provided to study investigator, the subject from which DNA sample was collected, and/or the IRBs/ECs themselves. Often this is expressed as a blanket requirement regardless whether the genotypes under investigation have any known clinical utility. The Sponsor may also be required by the IRBs/ECs to provide genetic counseling services.
- Most countries or IRBs/ECs require sample anonymization; whereas some IRBs/ECs have the opposite requirement (i.e., some IRBs/ECs in Italy and Brazil state that samples cannot be anonymized).
- Some IRBs/ECs have strict restrictions on the duration of sample storage, whereas many others do not. Tracking such different restrictions on storage time and ensuring that samples are destroyed in a timely manner creates additional operational burdens.

FUTURE PERSPECTIVES

Pharmacogenomics holds the promise to drive more efficient and effective drug development. Although this promise seems still far away, there is clear evidence that the conventional "one-size-fits-all" model is eroding in favor of precision medicine as the new paradigm. Like many other new technologies, incorporating pharmacogenomics into drug development is associated with a number of challenges; some are being addressed, whereas others still need to be addressed.

With the emergence of precision medicine, many new treatments are designed for specific patient subpopulations. Although large-scale, randomized, parallel group–controlled clinical trial is considered the gold standard, it is often difficult, if not impossible, to recruit enough patients when studying small genetically defined subgroups of patients. Hence, novel trial designs that allow smaller sample size and faster enrollment are needed. A number of alternative trial designs, such as adaptive randomization, delayed start, and early escape, have been proposed to overcome the limitations on sample size requirements and to facilitate the availability of new treatments targeting smaller patient populations. Each trial design comes with specific advantages and limitations, so careful selection of the appropriate study design is crucial [91].

At present, data analysis techniques are limited and only capable of revealing the most statistically predominant pharmacogenomic associations. However, it is believed that many important associations cannot be identified through current statistical methodology. Improved investment in pharmacogenomics data mining, and analytical techniques is needed. Currently, academic and industry researchers are exploring an array of novel sophisticated analytical approaches (i.e., multilocus, pathway analysis, and multivariant analysis) in combination with bioinformatics and biostatistics tools to achieve this goal.

Collaborations between industry, academia, and regulatory agencies are also essential for improving the success of applying pharmacogenomics in drug development. Several examples discussed earlier have highlighted the importance of indepth understanding of disease biology and genetics in implementing the precision

medicine–approach, reorientation of clinical development with a focus on genetically stratified trials, availability of validated real-time genetic diagnostics, and the establishment of a network for molecular screening of patients. Achievement of these would be impossible without a close interaction between scientists from industry, academia, and regulatory agencies.

The FDA is encouraging pharmacogenomic work and has taken a wide range of initiatives to put a regulatory framework around the integration of pharmacogenomics into drug development. Briefly, these included issuing white papers and guidances, organizing workshops, and developing online tools. The European Medicines Agency (EMA) has also been active in this area. Several EU regulatory guidances were released in recent years, including good pharmacogenomics practice, EU experience in oncology, inclusion of pharmacogenomics in pharmacokinetic/pharmacodynamic studies, use of pharmacogenomics in pharmacovigilance, and methodological issues associated with pharmacogenomics biomarkers. These documents, along with similar ones from the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan, provide a framework for using pharmacogenomic information throughout drug development and life-cycle management process.

For example, the FDA, EMA, and PMDA all expect that sponsors fully investigate the potential effect of polymoprhisms on any enzymes or transporters that play a major role in drug metabolism or clearance. The EMA takes this a step further and outlines specific trigger points for initiating pharmacogenomic studies. All three agencies also recommend examining the effect of genetic polymorphisms on pharmacodynamic endpoints. In general, it is recommended that if genotypes are predictive of drug effects in early trials, enrichment or stratification strategies should be considered to increase the representation of genotypes of interest in subsequent patient studies [92]. However, increased granularity in some of the guidelines and harmonization of regulatory frameworks among different regions are still needed. For example, although the guidelines on pharmacogenomic/pharmacokinetic research have matured rapidly over the last few years, those surrounding pharmacogenomic/pharmacodynamic assessment are considerably less well developed.

DISCUSSION QUESTIONS

1. How would pharmacogenomic investigations benefit different aspects of drug development?
2. What are the barriers for pharmaceutical industry to fully incorporate pharmacogenomics?
3. Are there any instances in which pharmacogenomic investigations provide no value for the industry?
4. How could industry and regulatory agencies work together to advance precision medicine through the use of pharmacogenomics?

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CHAPTER

4

Translating Pharmacogenomic Research to Therapeutic Potentials

Y. W. Francis Lam[1,2]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]College of Pharmacy, University of Texas at Austin, Austin, TX, United States

OUTLINE

OBJECTIVES

1. List the major steps involved in implementing pharmacogenomic testing in a clinical environment
2. Discuss the challenges associated with translating pharmacogenomic research findings to clinical practice
3. Delineate the differences between analytical validity, clinical validity, and clinical utility of a pharmacogenomic diagnostic test
4. Explain the potential roles of pharmacogenomics in all phases of drug development

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00004-8

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INTRODUCTION

The genetic basis of altered-drug pharmacokinetics and pharmacodynamics, as well as how these interindividual variabilities can potentially help optimize drug therapy in different disease states, are highlighted in Chapter 1 and other chapters devoted to specific therapeutic areas. However, most clinicians and researchers would agree that, although pharmacogenomic research findings are now utilized to different extents in clinical practice, meaningful implementation of pharmacogenomics is only available at a handful of major academic medical or research institutions [1–5]. Hence, we are still some years away from achieving the goal of broadly offered personalized therapy in healthcare that was envisioned decades ago.

Application of genomic data in clinical practice requires the use and interpretation of biomarker-based pharmacogenomic diagnostic tests. Although there are established genetic biomarkers that clinicians can use to predict drug efficacy and/or toxicity (e.g., human epidermal growth factor receptor 2 (Her2neu) testing for trastuzumab, *HLA-B*5701* for abacavir), the challenge is to address practical issues including whether the biomarkers should be used in patient assessment, as well as when and on whom to use the diagnostic tests. Establishing the clinical utility of the biomarker has been advocated to ensure that the use of the biomarkers is appropriate in patients, and the testing is cost-effective and ultimately improves clinical outcome.

Translation of the scientific knowledge into practice and integration within the healthcare system have been further hampered by commercial, economic, educational, legal, and societal barriers, each of which is fueled by stakeholders with different interests and goals. In addition, there is constant debate within the scientific community, with little agreement, as to how much data (replication studies, sample size) and the quality of data (retrospective cohort vs. randomized controlled clinical trial) are scientifically appropriate but at the same time realistically achievable [6]. This chapter will provide a perspective on the existing steps and challenges (Table 4.1) in the complex process for translating a pharmacogenetic biomarker that has been discovered to its clinical implementation as a test, as well as incorporating pharmacogenomics into drug development.

IMPLEMENTATION OF BIOMARKERS IN CLINICAL PRACTICE

Complexity of Genetic Variabilities and Nongenetic Influences

Although many biomarkers have been identified over the last decade, most of them have not advanced beyond the discovery phase. Exceptions to this are primarily in the area of oncology; however, there are also examples of biomarker use in neurology (*HLA-B*1502* testing to predict risk for severe skin reactions to carbamazepine), infectious disease (*HLA-B*5701* testing for hypersensitivity risk with abacavir), and cardiology (*CYP2C19* to predict clopidogrel effectiveness). The major issue has been the inconsistent results for replication of the genetic associations for most biomarkers, whether alone or in combination. The scientific challenge for study replication is magnified by our understanding that drug disposition and response phenotypes are more accurately predicted by multiple gene variations and not single-nucleotide polymorphisms (SNPs), population differences in prevalence of most genetic variants, as well as the accompanying sample-size requirement for statistical power in most pharmacogenomic studies. The atypical antipsychotic clozapine, with its complex pharmacological effects via the dopaminergic, serotonergic, adrenergic, and histaminergic receptors within the central nervous system, is a good example to illustrate the challenge of multiple gene variants,

TABLE 4.1 Major Steps and Challenges Involved in Implementation of Pharmacogenomic Testing in Clinical Practice

DISCOVERY AND VALIDATION OF PHARMACOGENOMIC BIOMARKERS IN WELL-CONTROLLED STUDIES
Challenge: Clinical validity and clinical utility: how to meaningfully translate a statistical genomic association between SNPs (or haplotypes) and drug response in a controlled, but usually underpowered, study to the real-world clinical environment.
REPLICATION OF GENE/DRUG ASSOCIATION
Challenge: Multiple non-genetic variables make it difficult to identify the most appropriate response phenotype for specific biomarker in most replication studies.
DEVELOPMENT AND APPROVAL OF COMPANION DIAGNOSTIC TESTS OR VALIDATION OF GENOTYPE RESULTS IN A CLINICAL LABORATORY PER QUALITY STANDARDS
Challenge: Financial incentive and resource for most small diagnostic companies. Approval process, especially in global markets, not completely delineated.
IDENTIFICATION OF APPROPRIATE PATIENT POPULATIONS FOR CLINICAL IMPLEMENTATION
Challenge: Continued debate whether routine genotyping for all patients versus reserving the test for selected patients.
DESIGNING AN EFFICIENT WORKFLOW STRATEGY FOR ORDERING AND RECEIVING GENETIC TEST RESULTS
Challenge: Reasonable turnaround time for point-of-care utility. Availability and documentation of test results. Electronic medical record not universally adopted in hospitals and clinics.
INTERPRETATION OF RESULTS AND ASSOCIATED ISSUES
Challenge: Not a simple normal vs. abnormal interpretation. Proper education is crucial to appropriate clinical use. Liability concerns for clinical decision based on pharmacogenomics information, including incidental findings.
EDUCATION OF CLINICIANS
Challenge: Educating current and future clinicians at a level such that the genomic information can be efficiently utilized.
ASSESSING CLINICIAN ACCEPTANCE OF GENETIC TESTING AND ADDRESSING BARRIERS TO CLINICAL IMPLEMENTATION.
Challenge: Most appropriate ways to address issues of lack of reimbursement from payers for most tests, privacy and discrimination concerns from patients, ownership of genetic information, health disparity, potential legal liability.

each accounting for a portion of the response variability. This is evident by conflicting data for association between clozapine response with either SNPs of each receptor subtype [7–10] or combinations of polymorphisms [11], suggesting that yet-to-be identified genes could account for additional variability in patients' responses to clozapine. The presence of different allele variants of, e.g., *CYP2D6*, *HLA-B*, *UGT1A1*, and serotonin transporter linked promoter region polymorphism (5-HTTLPR) of *SLC6A4*, among different ethnic groups (discussed in different chapters throughout the book) reminds investigators of the importance of ethnicity in pharmacogenomic studies [12–15]. As discussed in more detail in chapter 6 warfarin in particular is a good example of the need to include relevant ethnic-specific alleles in assessing pharmacogenomics study results as well as the clinical utility of genotyping implementation. In addition, it is well known that other factors additional to gene variants can impact drug therapy in different ways. Therefore, unless these variables are adequately addressed or controlled for, payers

might be reluctant to reimburse for the cost of pharmacogenomic testing.

The drug disposition and response phenotypes can further be affected by patient-specific and environmental variables. Concurrent therapy with potent CYP2D6 inhibitor such as fluoxetine or paroxetine could significantly reduce the CYP2D6 metabolic capacity of a genotypic extensive metabolizer to that of a poor metabolizer [16], thereby creating a genotype–phenotype discordance and affecting the ability to predict possible drug response based on genotype-guided dosing and achievable drug concentration. Another example is inflammation-mediated downregulation of drug-metabolizing enzymes. Using a transgenic mouse model of human *CYP3A4* regulation, Robertson et al. showed that presence of extrahepatic tumors elicited inflammatory response, including release of cytokines such as interleukin-6, and resulted in transcriptional downregulation of the human *CYP3A4* gene [17]. Therefore, literature report of lower docetaxel clearance in cancer patients could be related to tumor-associated inflammation and subsequent transcriptional repression of *CYP3A4*, potentially leading to unanticipated toxicity despite normal enzymatic activity in the patient. On the pharmacodynamic side, excessive vitamin K intake can override the effects of *VKORC1* genotype on warfarin dose requirements.

Currently, much less is known about the influence of environmental variables and gene–environment interactions on drug disposition and response phenotypes. There is an increasing appreciation that genetic heterogeneity alone cannot explain interindividual variations in drug responses, and epigenetic factors can result in changes in phenotype without DNA sequence alteration [18]. In the not too distant future, pharmacoepigenetics could provide the basis of studying the interaction among drugs, environment, and genes, and provide additional explanation of drug-response variations beyond the level of genetic polymorphisms.

Analytical Validity, Clinical Validity, and Clinical Utility

For evaluation of a pharmacogenomic biomarker test, regardless of whether it is to be developed as a companion diagnostic, both analytical and clinical validities of the test have to be considered. For the purpose of personalized therapy, a companion diagnostic for a drug can be defined as a biomarker that is critical to the safe and effective use of the drug. Analytical validity defines how well a diagnostic test measures what it is intended to measure, regardless of whether it is a mutation, protein, or an expression pattern. Clinical validity measures the ability of the test to differentiate responders from nonresponders, or to identify patients who are at risk for adverse drug reactions (Table 4.2). For practical implementation of the validated pharmacogenomic biomarker test, the clinical utility of the test also has to be determined. The clinical utility measures the ability of the test result to predict outcome in a clinical environment, and what value would be obtained compared to nontesting, i.e., standard empirical treatment. The analytical validity, clinical validity, clinical utility, and associated ethical, legal, and social implications (ACCE) Model Project [19] sponsored by the Office of Public Health Genomics, Centers for Disease Control and Prevention (CDC), has been advocated by some investigators to be the basis for evaluation of pharmacogenomic biomarker tests.

More than a decade ago, the CDC launched the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative, which aims to establish an evidence-based process for evaluating genetic tests and genomic technology that are being translated from research to clinical practice. In 2007, the EGAPP Working Group, incorporating the three levels of evaluations (analytical and clinical validity, and clinical utility), published their evidence-based review of the literature on the use of cytochrome P450 (CYP) enzyme genotyping for clinical

TABLE 4.2 Criteria for Establishing Utility of Pharmacogenomic Test

	Pertinent Questions to be Addressed	Comments
Analytical validity (i.e., test accuracy and precision)	Does the diagnostic test reliably measure the biomarker (e.g., a mutation)?	An important question would be how well does the test predict the pharmacokinetics and/or pharmacodynamics of the drug of interest.
Clinical validity	Is the biomarker capable of predicting clinical outcome (response and/or adverse drug reaction)?	Proof of clinical validity in studies with appropriate design and sample size does not necessarily translate into proof of clinical utility.
Clinical utility	Is measuring the biomarker with subsequent personalized therapy predictive of improved outcome for patients? How does personalized therapy compare to usual care in terms of value?	

management of depressed patients with the selective serotonin reuptake inhibitors (SSRIs). Based on strong evidence of analytical validity, possible demonstration of clinical validity, and lack of study data to support evaluation of potential clinical utility, the working group does not recommend the application of *CYP2D6* test for SSRI pharmacotherapy [20].

The analytical validity of most CYP-genotyping tests in detecting CYP450 gene variants is strong and to be expected, because their approval by the Food and Drug Administration (FDA) is dependent on technical performance. Nevertheless, there is no universal agreement as to which allele variant should be tested routinely, and ethnic variations in allele importance and distribution further complicate the picture. The weak evidence of association between the gene variants and SSRI metabolism, efficacy, and response are more likely related to most SSRIs relying on multiple enzymes for metabolism, some of which are not polymorphic, a flat dose–response relationship, and wide therapeutic index. The clinical validity of the test to differentiate response phenotypes is further limited by the CYP genotype–metabolic phenotype discordance that can occur because of drug–drug interactions or environmental influences. Given these pharmacokinetic and pharmacodynamic limitations as well as the lack of cost-effectiveness data, it is not surprising that the SSRIs are not good candidates for genotype-based pharmacogenomic therapy, and hence the recommendation of the EGAPP Working Group.

Traditional clinical studies aim at gathering evidence of drug efficacy and safety in large patient cohorts in an attempt to overcome statistical issues related to disease and population heterogeneities, placebo effects, inadequate understanding of disease etiologies, and, finally, drug-response variabilities per se. All too often, such studies result in achievement of small average benefit in the entire heterogeneous patient cohort. Nevertheless, given the current evidence-driven clinical environment, it is expected that any clinical trial to validate the clinical utility of pharmacogenomic biomarkers would have to be not only hypothesis driven, but also extensive in terms of time and sample size, and therefore costly. Although prospective, double-blind randomized clinical trials would provide the ideal evidence-based approach advocated by many investigators, a balance between the scientific demand of randomized clinical trials and the practical value of genotyping for patient care seems appropriate. In contrast to evidence-based practice, the emphasis and value of pharmacogenomics are more geared toward the outliers (the nonresponders, the poor metabolizers, or the ultrarapid metabolizers). Therefore,

to generate more robust evidence of efficacy, enrichment design or biomarker-stratified design clinical trials have been advocated by many investigators and sponsors to include patients who are more likely to respond or at least be stratified according to disease subtypes, and/or exclude patients who are highly susceptible to adverse drug reactions [21]. Even with the assumption of (and sometimes proven) association between genetic variabilities and drug response, both advantages and disadvantages exist for this study design (Table 4.3).

TABLE 4.3 Advantages and Disadvantages for Prospective Enrichment Design Studies

ADVANTAGES
• Substantial reduction in response (efficacy, side effects, disposition) variabilities
• Possibly can explore a greater dose range than otherwise achieved with entire population
• Smaller number of patients needed in pivotal phase iii trial
• Possible reduction of patients discontinuance (from less efficacy or increased side effects)
• Greater probability of successful trial
• Possible shorter duration of clinical trial
• Possible reduction in safety monitoring, including plasma concentration
DISADVANTAGES
• No opportunity to study excluded subjects in pivotal trial
• Information available only for a much narrower range of response variabilities
• Potential of overestimating drug efficacy in a highly selective group
• Possibly less inclination to monitor for safety in genotyped patients
• Resultant less information on short-term and long-term safety
ADDITIONAL CONCERN
• Any regulatory requirement (and may be ethical reasons) for studying safety in excluded patients?

For patient care, a good example for the need of balance between evidence-based medicine and precision medicine is clopidogrel. As discussed in Chapters 1 and 6, there is extensive evidence of clopidogrel efficacy linked to *CYP2C19* genetic polymorphism. However, the continued debate over the routine use of *CYP2C19* genotyping to guide clopidogrel therapy prevents more widespread use of the biomarker in individualized therapy, despite the significantly higher rates of stent thrombosis and the associated mortality rates in carriers of the reduced-function *CYP2C19*2* allele. Based on lack of outcomes data, the joint clinical alert originally issued in 2010 and updated in 2015 by the American College of Cardiology and the American Heart Association did not recommend routine genotyping, and suggested the need of large, prospective, controlled trials [22,23]. In the 2011 Practice Guideline for Percutaneous Coronary Intervention (PCI), routine clinical use of genetic testing to screen patients treated with clopidogrel who are undergoing PCI is not recommended. However, the guideline did suggest that genetic testing might be considered for patients at high risk for poor clinical outcomes [24]. Pending results from several ongoing multisite, randomized controlled clinical trials, including the Tailored Antiplatelet Therapy Following PCI trial (clinicaltrials.gov NCT01742117) and the Cost-effectiveness of Genotype Guided Treatment with Antiplatelet Drugs in STEM1 Patients: Optimization of Treatment (POPular Genetics) trial (clinicaltrials.gov NCT01761786) could provide additional insight regarding the effect of genotype-guided antiplatelet therapy. In the meantime, the questions then become: are we sacrificing patient care on the insistence of waiting for proof of value via the evidence-based approach? If none is available in the near future, should we focus on steps that can facilitate the genotyping implementation in clinical setting, and examine the cost-effectiveness of genotype-guided antiplatelet therapy with a variety of different approaches?

Another example of taking an alternative approach to evidence-based evaluation is tolbutamide. Based on pharmacokinetic study in

subjects genotyped for the *CYP2C9* polymorphism, the elimination of tolbutamide in carriers of the *CYP2C9*2* and *CYP2C9*3* variants were 50% and 84% lower, respectively, than in subjects with the *CYP2C9*1/*1* genotype [25]. However, there has not been any prospective controlled clinical study to evaluate whether dosage reduction in the order of 50% and 90% in patients with these two genotypes would be appropriate in clinical practice. Because tolbutamide efficacy can be easily monitored in the clinical setting, implementing these dosage reductions in clinics or physician office, in lieu of an expensive and time-consuming large-scale clinical trial, could constitute the first step to obtain information regarding the clinical utility of *CYP2C9* genotype in optimizing tolbutamide therapy.

Although certainly there is a need for a balanced approach to testing clinical utility, the abundance of pharmacogenomics research, including genome-wide association study (GWAS) studies, over the years have resulted in endless lists of potential and sometimes completely different sets of biomarkers for efficacy and or toxicity association. A good example is the multiple putative response biomarkers reviewed in chapter 7 that have been suggested to predict SSRI efficacy. Another example is *CYP4F2* identified via GWAS as another genetic variable for predicting warfarin dosing, despite its small contribution (1%–3%) compared to *CYP2C9* genotypes (10%–12%). On one hand, these examples definitely underscore the importance of understanding the multi-genic nature of drug efficacy. However, the ever-expanding list of variants not only adds to complex interpretation that can be challenging and frustrating to most practitioners, but also could be perceived by payers that the existing variants already included in test panels are not as important as they purport to be. Therefore, the unintended consequence of more data could be generation of scientific uncertainty for experts deciding on clinical guidelines for potential adoption of specific biomarkers in practice, and opportunity for payers to decline reimbursement for specific biomarkers, because they need to evaluate more clinical utility and cost-effectiveness studies that incorporate even more patients to account for the additional variant(s).

Evaluation of Cost-Effectiveness

For many healthcare facilities and systems, demonstration of cost-effectiveness of any test or procedure is critical prior to its implementation. Ideally, the pharmacogenomic biomarker not only will result in cost-effective improved clinical care in patients who will benefit from individualized therapy with the drug, but also will lead to avoidance of cost-ineffective treatment for patients who likely will not benefit from the drug, either because of lack of response or increased adverse drug reactions. Given the differences in revenue generation between a pharmacogenomic diagnostic companion test and a drug, conceivably, there could be much less incentive for pharmaceutical companies to include thorough cost-effectiveness analysis as part of drug development. However, demonstration of cost effectiveness of pharmacogenomics-based therapy can take different approaches. These approaches include comparing per-patient cost for specific clinical outcome between genotype-based regimen and standard regimen, as shown by the study of Furata et al. [26]; decision model-based study using simulated patient cohort as reported by Reese et al. for antiplatelet therapy [27]; and using real-world claims from clinical and/or pharmacy benefits databases to link health outcome with the use of pharmacogenomic testing. Examples of how claims and clinical data can be used to establish direct and indirect cost benefits are reviewed in chapter 14.

The economic impact and cost-effectiveness of screening can be affected by different variables.

To study the potential clinical and economic outcomes for pharmacogenomics-guided dosing of warfarin, two studies utilized modeling techniques in separate simulated patient cohorts. Despite the conclusion that the relatively high cost of *CYP2C9* and *VKORC1*–bundled test ($326 to $570) only resulted in modest improvements (quality-adjusted life-years, survival rates, and total adverse rates), the investigators also suggested that the cost-effectiveness can be improved in several ways. These could include cost reduction of the genotyping test by 50%, and applying genotype-guided warfarin-dosing algorithm in outliers (patients with out of range international normalized ratios [INRs] and/or those who are at high risk for hemorrhage) [28,29]. Dhanda et al. recently provided a unique perspective to compare sufficiency of evidence level for warfarin dosing based on pharmacogenomics (not recommended by clinical guidelines) *versus* warfarin dosing based on the known drug interaction between warfarin and amiodarone (recommended by clinical guidelines), and suggested a novel evidence quantification framework to guide decision-making for personalized warfarin therapy [30]. Finally, in addition, to using multiple approaches and/or models, it is also important to consider variables such as prevalence of a specific variant in a population (which impacts cost-effectiveness) and cost of alternative treatment approaches when evaluating cost-effectiveness.

Regulatory Approval of Pharmacogenomic Diagnostic Tests

Regulatory agencies, including the FDA and the European Medicine Agency (EMA), have for more than a decade acknowledged the importance of biomarkers and provided new recommendations on pharmacogenomic diagnostic tests and data submission [31,32]. These regulatory efforts and initiatives are further reviewed in Chapter 2. Based on pharmacokinetic mechanism relating drug disposition to pharmacological effects (efficacy and adverse reactions) and/or identification of specific gene(s) effect affecting the pharmacodynamics relationship, pharmacogenomic biomarker information for more than 180 drugs has been classified to date by the FDA. This classification comprises of three categories: (1) test required before the drug is prescribed, either for predicting efficacy or toxicity; (2) test recommended; and (3) test available only for information purposes. Table 3 in Chapter 2 lists up-to-date examples of drugs with pharmacogenetics-related information in their FDA-approved labeling. Unfortunately, the "for information only" classification is confusing to most practitioners. For example, even with an inclusion of a black-box warning of "reduced clopidogrel effectiveness for CYP2C19 PM" in the revised product label, clopidogrel remains within the list of drugs that do not require genetic testing. As such, expert committees do not endorse pharmacogenomics testing for drugs in the third category (primarily based on lack of sufficient supporting evidence and the regulatory classification of no mandatory testing), and such nonendorsement is extensively used by payers as the basis of non-reimbursement for genotyping.

The lack of clarity, in particular, had led to the conclusion by many payers that despite the scientific evidence, pharmacogenomics testing is "experimental" and, therefore, payers should not be responsible for experimental medical procedures. This reluctance of payment from payer includes even the Center for Medicare and Medicaid Service (CMS) for warfarin pharmacogenomics testing of *VKORC1* and *CYP2C9* variants after publication of the results from several randomized controlled clinical trials showing inconsistent data and inconclusive evidence [13,33,34]. However, these conflicting results highlight not only the importance of ethnic-specific variants discussed earlier, but also the lack of standardization of what are included in currently available pharmacogenomic tests,

and, more importantly, guidelines as to what are the relevant allelic variants to be tested. A recent study by Bousman et al. highlighted this issue of test standardization. They evaluated 20 pharmacogenetic panels for *CYP2C19* and *CYP2D6* polymorphisms, and reported that no two panels include the same combination of *CYP2C19* and *CYP2D6* alleles. In addition, most reporting from these panels did not provide sufficient information on variants, resulting in a distinct possibility that two panels measuring the same allele may report conflicting phenotypic prediction of metabolic status [35]. This highlights the limitation of most commercially available panels including only common *CYP2C19* alleles (**2, *3, *17*) and *CYP2D6* alleles (**2, *4, *5, *10, *17*), but not those that are specific for an ethnic group or population and those that occur less frequently, as well as a lack of reporting consistency. Both of these need to be addressed for better usability and certainly before wider adoption of CYP genotyping can be considered.

Within the United States, tests for a pharmacogenomic biomarker are performed either as a test developed by a clinical laboratory, or as an *in vitro* diagnostic device, each with its own regulatory oversight. Quality standards for clinical laboratory tests are governed by the Clinical Laboratory Improvement Amendments (CLIA). In addition, the laboratories are accredited either by the College of American Pathologists, the Joint Commission on Accreditation of Healthcare Organizations, or the Health Department of each individual state, that take into consideration CLIA compliance and laboratory standard practices that are in line with Good Laboratory Practice (GLP) regulations enforced by the FDA.

In contrast to clinical laboratories, the GLP regulations govern the testing of *in vitro* medical diagnostic devices. Although currently there is no formal regulatory process for submission of companion diagnostic tests, the well-established medical test and device regulatory process within the Office of *In Vitro* Diagnostic Devices seems amenable for application to approval of biomarkers. Despite original attempts to perform and market the AmpliChip CYP450 Test under CLIA regulations, the FDA decided that evaluation and approval as an *in vitro* diagnostic device was required. Note also that additional historical precedence had been set with the FDA fast-track approval of trastuzumab and the accompanying Hercep Test for detecting overexpression of Human Epidermal Growth Factor Receptor 2 (HER2) protein in breast cancer tissue in 2001, and for tests that utilize fluorescence in situ hybridization (FISH). Both vemurafenib and crizotinib were approved by the FDA with their respective companion diagnostic tests. Other drugs for which pharmacogenomic markers have been developed and approved by the FDA are listed in Table 3 of Chapter 2. Another example is approval of gefitinib by the EMA in June 2009. Subsequently, the EMA approved a companion diagnostic test for Human Epidermal Growth Factor Receptor 1 (*HER1*) mutations. These efforts by the FDA and foreign regulatory agencies not only increase the availability of companion diagnostic tests, but also provide an impetus of pharmacogenomic data submission for drug approval, and additional research to address the debate over the utility of the information incorporated in the revised labels, e.g., for clopidogrel [36].

Integration of Testing Within the Healthcare Environment

There are two practical aspects that need to be addressed before pharmacogenomic markers can be successfully utilized in any healthcare setting. Although most clinicians have a positive impression with the potential implication of pharmacogenomics testing for their practice [37,38], integration of the testing logistics and procedure within the existing workflow of specific healthcare practice poses a logistical challenge. Low volume of the diagnostic test may not justify inhouse testing in institutional

clinical laboratories. The ideal point-of-care performance for rapid decision-making at the bedside or within the clinic is not available at most hospitals. The inevitable outcome is longer turnaround time for test results coming from external clinical laboratories or research institutions. Although this might be acceptable in a relatively less "urgent" setting, e.g., *HER2* expression, or *CYP2C19* genotyping prior to scheduled Percutaneous Coronary Intervention (PCI), the contrary would be true for on-the-spot warfarin-dosing adjustment or when there is a need for emergency PCI. Nevertheless, progress has been made in this aspect. Several point-of-care *CYP2C19* genotyping devices, one of which could detect 11 *CYP2C19* variants, have shown the value of bedside genetic testing [39,40]. With a turnaround time of less than 3 h and with high degree of accuracy, incorporation of *CYP2C19* testing into clinical protocol for antiplatelet dosing is realistic and could pave the way for more widespread use of clopidogrel pharmacogenetics in clinical practice. However, unlike companion diagnostic tests, currently no point-of-care pharmacogenomic tests are FDA approved. An alternative approach would be the adoption of preemptive genotyping [2,41], which helps to optimize workflow in the clinic environment [41,42]. Ideally, all pharmacogenomics information and actionable results will be available in a robust system of patients' electronic medical records (EMRs) in which integrated clinical recommendations would alert the clinicians when the pertinent medications are ordered by physicians at point of care [43]. However, even with widespread adoption of EMRs [44], the implementation infrastructure for preemptive genotyping, including coordinated biobanking, clinical decision support, and integration of informatics with sufficient staff support [45], could pose a financial constraint on most healthcare systems. In addition, other practical challenges with EMR include assurance of data accuracy such as medication exposure and adherence pattern, both of which impact substantially on the interpretation of the pharmacogenomic test results, and hence their usability.

The second practical consideration relates to managing and using the information in a clinically relevant manner for patient care. A recent survey of primary care physicians at the Mayo Clinic revealed an overall lack of comfort with the clinical decision support in place [46], and underscores the importance of further refining the alert system [47]. With availability of test results comes the needs of interpretation and education of clinicians. Most pharmacogenomic diagnostic tests report genotype result, the interpretation of which is usually not difficult, especially for deciding the appropriateness of a specific drug for a patient. Examples include the presence of the *HLA-B*5701* variant for exclusion of abacavir therapy in patients with HIV-1 infection, and the use of gefitinib in patients with the epidermal growth factor receptor mutation. The interpretation is more complicated and challenging when the test result is used for dosing adjustment. For example, warfarin dosing is affected by many genetic (*CYP2C9*, *VKORC1*, *CYP4F2*, *calumenin*, as well as rs12777823 that is independent of *CYP2C9*2* and *CYP2C9*3*, and folylpolyglutamate synthetase (*FPGS*), which are more relevant for the African American populations) [14,48] and non-genetic (age, diet, drug–drug interaction, gender) variables that can affect its disposition and response.

As described in chapter 6, the availability of pharmacogenetic dosing algorithms helps utilize the patient's *CYP2C9* and *VKORC1* genotypes and other non-genetic factors (e.g., age, body size, concurrent interacting drug) in determining dosage. The algorithms based on the work of Gage et al. [49] and the International Warfarin Pharmacogenetics Consortium (IWPC) [50] are publicly available via the internet [51,52]. Nevertheless, although these algorithms can be useful, they are not without limitations. As with other CYP genotyping, the clinical utility is

mostly demonstrated in the outliers. For example, the IWPC showed that a pharmacogenetic dosing algorithm was most predictive of therapeutic anticoagulation in 46% of the patients cohort who required <25 mg/week or >49 mg/week. Although the algorithm approach has been successfully used in inpatients receiving multiple-drug therapy [53], most data have been primarily derived from outpatients receiving stable warfarin-dosage regimens. Not surprisingly, different, albeit not statistically significant, dosage requirements were obtained with various algorithms [54], likely reflecting the inconsistency in the choice of specific nongenetic variables among these algorithms. Most algorithms also do not include detection of *CYP2C9*8* or assessment of *CYP4F2* genotype. Although the contribution of *CYP4F2* genetic polymorphism only accounts for 1%–3% of the variability of warfarin dosing requirement, the exclusion of *CYP2C9*8* commonly found in African Americans likely would account for lower successful dose prediction associated with the use of these algorithms in this ethnic group. This limitation is similar to the challenge discussed earlier for deciding which *CYP* alleles or which *UGT1A1* alleles should be included in the diagnostic tests for these pharmacogenomic biomarkers.

In addition, to many practitioners, even understanding the role and implications of *VKORC1* haplotypes and *CYP2C9* genetic variants beyond that of the more common *2 and *3 appear to be far more perplexing. Additional challenge examples include drugs that rely on the P450 enzyme system for elimination, given the significant interindividual variabilities in activities of most of the isoenzymes and the possibility of phenocopying (or phenoconversion) with change in metabolic phenotype in the presence of drug–drug interaction [16,55]. This difference in interpretation complexity related to the intended use of the test is likely one of the reasons for the FDA to previously separate pharmacogenomic biomarkers into three categories. Nevertheless, this practical challenge of data interpretation can be mitigated with appropriate level of educational support and consultation [4,5].

Much like other clinical diagnostic tests, patients expect clinicians to be able to explain the pharmacogenomic diagnostic-test results and answer their questions. However, a survey of more than 10,000 physicians conducted in 2008 found that only 10% felt adequately trained to apply genetic information in clinical practice and only 26% had received pharmacogenomics education during their medical school or postgraduate training [56]. A more recent survey for the European pharmacogenomics implementation project "Ubiquitous Pharmacogenomics" identified similar self-identified lack of sufficient knowledge on pharmacogenomics from 40% of respondents [57]. This lack of training is unfortunate given 98% and 84% of those surveyed from these two studies agreed that patient's genetic profile could influence drug-therapy decisions in patients [56,57]. In their accreditation guidelines update (version 2, 1/23/2011), the Accreditation Council for Pharmaceutical Education listed pharmacogenomics as part of professional curriculum course work [58]. Although most pharmacy schools have pharmacogenomics courses or materials in place, this is not the case with medical schools [59,60]. The gap in knowledge can now be addressed through clinical guidelines and algorithms such as the guidelines available through the Clinical Pharmacogenetics Implementation Consortium (CPIC) for many drugs, including abacavir, carbamazepine, clopidogrel, codeine, fluoropyrimidines (5-fluorouracil and capecitabine), simvastatin, tamoxifen, and warfarin [61–69]. The CPIC guidelines help to narrow this educational gap and address this specific implementation barrier. Hopefully, additional clinical practice guidelines from diverse groups of organizations and expert panels would pave the way to greater extent of implementation. To that end, it is of note that regulatory guidance [70] has

been published to support the recommendation of the clinical practice guidelines. The current landscape of pharmacogenomics education and guidelines is further described in chapter 15.

The challenge of informing the patient is further amplified with the proliferation of available direct-to-consumer tests, especially within the psychiatry discipline. In this regard, the work by Mills et al. [71] in developing an educational video is notable in that it represents an example to address this "neglected" component of pharmacogenomics education. Such educational tools, upon further refinement, could help facilitate informed consent by the patient prior to discussion about the need of pharmacogenomic testing for specific drug. Further work in this area of "engaged patient care decision-making" would be much welcomed.

Reimbursement Issues

The successful implementation of pharmacogenomic biomarkers in clinical practice not only involves multidisciplinary coordination from physicians, pharmacists, and clinical laboratories, but also requires efforts from the payer. With the high cost of providing healthcare, the reimbursability of any particular test plays a significant role in deciding its implementation status in clinical practice. Reimbursement for diagnostic tests in the United States has been primarily linked to the Current Procedural Terminology (CPT) codes. Although the cost of testing for thiopurine S-methyltransferase is reimbursed according to CPT codes in some hospitals, that is not the case for most pharmacogenomic biomarker tests. Even with the revised product labeling information regarding the impact of CYP variants for drugs such as warfarin and clopidogrel, insurers are reluctant to reimburse the cost of the tests on the basis that (1) such tests are not medically necessary (because it has never been classified by the FDA as a required test), (2) there is no evidence of clinical utility (clinical utility is usually associated with endorsement by professional organizations), or (3) lack of cost-effectiveness analysis and/or comprehensive comparative effectiveness analysis. Nevertheless, it should be noted that even for trastuzumab, which is reimbursed by most insurers, there have been few cost-effectiveness analyses of HER2 protein expression and treatment with trastuzumab [72]. For most pharmacogenomic biomarkers, the ideal analysis might not be available until years after the diagnostic test is marketed. In addition, the recent change in the coding system with analyte-specific codes allows for identification of individual analyte in pharmacogenomic tests, greater transparency of what is billed, and increased payer's scrutiny as to how the information is used by the clinicians and whether an individual pharmacogenomic test should be reimbursed. Given the data of St. Sauver et al. that some clinicians overrode the recommendation of the clinical decision support [46], this additional insight into the actual level of pharmacogenomic tests use pattern by clinicians could become an additional implementation barrier as payers might be reluctant to provide reimbursement if the test results do not change prescribing behavior.

In an effort to provide coverage for promising technologies that have not met the level of evidence required for Medicare reimbursement standard, the CMS has conferred coverage with evidence development (CED) status to promising drugs, biologics, diagnostics, and devices [73]. With this designation, different studies utilizing real-world claims data from patients have been conducted with several commercially available pharmacogenomic tests, including those used for therapy with warfarin and psychotropic agents, to assess the clinical and economic impact of pharmacogenomic testing. Studies pertaining to psychopharmacogenomics are described in chapter 14. Even though with limitations associated with observational data, the results might provide a basis for more

payers to consider reimbursement for some of the pharmacogenomic biomarkers.

Despite the consistency in relying on existing conclusive evidence that links pharmacogenomic testing with health outcomes, there is significant variation among payers in reimbursement policy and the extent of coverage. In their survey of 12 payers, Cohen et al. [74] found that 67%–75% provide reimbursements to cover the costs of the required companion diagnostic tests for trastuzumab and cetuximab, even though a lower percentage (42%–50%) require documentation of testing prior to reimbursement. Likewise, 33% of payers will reimburse *CYP2C9* and *VKORC1* tests, but none require test documentation. Six of the eight study drugs included in the survey were antineoplastic agents. Reimbursements for the corresponding diagnostic tests are provided by most payers for these antineoplastic agents, with the exception of irinotecan, for which only two payers will reimburse for *UGT1A1* testing. Most payers consider conclusive evidence of link between the diagnostic test and health outcome to be much more important than evidence of test accuracy in identifying subpopulations of interest. Interestingly, the authors reported that most payers indicate test cost, medication adherence, and off-label use are not factors in their consideration for reimbursement.

It is clear that, currently, payers are reluctant to pay for the diagnostic tests (most costing ≤ $500), even though they will pay for the more expensive drugs. Such a stance would pose much less incentive for diagnostic companies to develop biomarkers, as they usually have less financial resources than pharmaceutical companies. An obvious solution to this would be codevelopment of proprietary drug and diagnostic tests that would be rewarding to both parties [75], and the example of FDA approval of crizotinib with a codeveloped diagnostic test might pave the way for further parallel development of drug and companion diagnostics.

Trastuzumab provides a good example of the paradigm shift in thinking about market share: the manufacturer's development of the drug along with the diagnostic device results in capturing 100% of the market share associated with breast cancer drug treatment in women overexpressing the HER2 protein. Therefore, trastuzumab is only used and reimbursed for patients with HER2 protein overexpression. Likewise, a similar paradigm shift might be applicable for reimbursement of companion diagnostics. Instead of reimbursing the same rate for every patient testing for a pharmacogenomic biomarker, it might be less of a financial burden for payer to institute a differential reimbursement based on indication (e.g., for CYP2C19 testing, a higher rate for high-risk PCI, a lower rate for a PCI that is not high risk, and none if no PCI was performed). This could provide additional incentive to use pharmacogenomic biomarkers as the equivalent of a differential diagnostic test to identify patients who will benefit most from genomics-guided personalized drug therapy. The financial cost of the one-time test should be easily covered through cost savings associated with not using the drug when it is ineffective or harmful in specific patient populations. Adopting this approach may provide a workaround to some payers' insisting on conclusive evidence of linking diagnostic tests to health outcomes [74].

Ethical, Legal, and Social Issues

Regardless of ethnicity, the public are, in general, receptive to genetic-based prescribing [76–80]. Although the benefit of pharmacogenomic testing lies in identifying individual patients with unanticipated response and/or adverse drug reactions, it also provides an opportunity to reveal information an individual's disease or medical condition to other parties, however unintended. As a result, concerns have been raised regarding individual right for privacy, as well as potential for discrimination and ineligibility for

employment and insurance [76–80]. In response, the United States Congress had passed the Genetic Information Nondiscrimination Act (GINA) in 2008. The GINA specifically prohibits the misuse of genetic information in determining employment decisions, insurance coverage and premium, as well as payers requiring individuals to submit genetic results prior to underwriting decisions.

Another intention of the GINA is to encourage individuals to participate in genetic research, although the issues of information sharing and confidentiality have not been addressed to the satisfaction of stakeholders, primarily patients. Of paramount importance are concerns regarding ownership of genetic materials, who have the right to access the information (reported as laboratory test value and usually stored as EMRs), and patient's awareness of the consequences of storing genetic materials and phenotypic data. In addition, to privacy issue, the bioethical implications of the ever-expanding genomic data, including data management concerns, have been amplified with different efforts to facilitate research collaboration among investigators and "Big Data" initiatives such as those detailed in Chapter 2.

Although the GINA is a well-intended legislation, there is a need to make some distinction in ethical and social concerns between tests that are primarily geared toward optimization of drug therapy *versus* those that are geared toward susceptibility to disease, which is much more relevant for privacy and discrimination. When discussing ethical, legal, and social implications of *genetic technology*, it is not uncommon for pharmacogenomic biomarkers to be grouped together with genetic tests predicting disease likelihood into a "generic" category of genetic tests. In terms of patient care, does consent need to be obtained from patients for tests designed to individualize their drug therapy (choice and/or dosage regimen), or should consent be only reserved for those tests that disclose disease susceptibility, which typically carry a much greater potential for abuse? As such, would it be reasonable to consider lessening regulatory restrictions for pharmacogenomic testing such that pharmacogenomic biomarker tests would not be treated with the same extent of scrutiny and protection as genetic testing for disease susceptibility? Doing so, *with careful consideration of what patient information should be restricted and kept confidential*, might facilitate the practical implementation of pharmacogenomics in the clinical environment. Currently, this issue of need for consent is very much open for further discussion and debate.

Social concerns also arise regarding potential challenges for healthcare systems. It is not unusual for insurance coverage that patients are required to pay for some of the cost of the medical service. Therefore, potential beneficial pharmacogenomic test information might be excluded because of an individual patient's socioeconomic status, thus exacerbating healthcare disparities. In addition, for those identified by pharmacogenomic tests either as nonresponders or at high risk of adverse drug reaction to a specific drug, the use of pharmacogenomic test as a "gatekeeper" of accessibility to drug treatment might pose a problem if there is no suitable alternative drug available. How should those patients be advised and treated? Is it ethical or appropriate if the patient and/or the physician opt for offlabel use of a drug regardless of the unfavorable response and/or risk associated with a specific genotype? These are relevant questions, because the clinical validity and utility of most pharmacogenomic tests have not been universally accepted in clinical practice. Another potential concern is liability on the part of the provider. If a genetic test (e.g., *CYP2C29*) is ordered to guide therapy with one drug (e.g., warfarin), but the patient is later prescribed another drug that is also affected by the gene previously tested (e.g., phenytoin), would the clinician be responsible for acting on the genotype results when dosing the second agent? If so, some point-of-care mechanism must be

universally in place, e.g., in an EMR containing the pharmacogenomics information for the clinician to readily determine that genetic test results relevant to the prescribed drug are available.

INCORPORATING PHARMACOGENOMICS INTO DRUG DEVELOPMENT

The scientific rationale and the applicable technical challenges for incorporating pharmacogenomics into drug development is discussed in chapter 3. This section will summarize the additional obstacles and considerations for the pharmaceutical industry.

The blockbuster drug concept and its financial impact on revenue have played a major role in pharmaceutical drug development. As such, the concept of pharmacogenomics and the resultant segmented (and smaller) market tailored to a subpopulation with specific genotype have been viewed unfavorably because of perceived lower revenue and decreased profit. However, as discussed earlier in this chapter, trastuzumab represents a paradigm shift in such perspective on revenue. With little or no competition, the perception of smaller market share in the entire population of patients with breast cancer can be overcome by 100% market share of all, albeit at a smaller number, of the patients with HER2 overexpression. There are additional drug-development advantages associated with this paradigm shift of product differentiation instead of market segmentation. Identifying patients likely to respond to participate in clinical trials would enable benefits to be shown in smaller number of patients, resulting in shorter Phase II and III studies, and reducing the cost of development. It could also screen out patients likely to have unfavorable side effects that only come to light in Phase IV post-marketing surveillance studies, and such events sometimes lead to the inevitable and unfavorable outcomes of postmarketing product recall and litigation. The litigation and financial burden could even be smaller if the pharmaceutical company can work with regulatory agencies to incorporate the pharmacogenomics information into a drug label that more accurately describes contraindications, precautions, and warnings. Finally, as indicated earlier in this chapter, proper partnership to develop and market a companion diagnostic test can also lead to additional revenue streams.

Nevertheless, relevant drug efficacy and safety data and issues that are important for regulatory decision-making were developed long before the era of pharmacogenomics, and it is unclear how traditional regulatory review would approach the inclusion of any pharmacogenomic data in a new drug-application (NDA) package. As described earlier and in Chapter 2, the FDA has developed multiple initiatives to encourage the use and submission of pharmacogenomic data by the pharmaceutical industry. However, concerns and questions remain regarding what type of pharmacogenomic data is necessary and when should they be incorporated in the NDA process. Such issues are summarized in Table 4.4.

High attrition rates in drug development is a well-known fact for the pharmaceutical industry, and a much less discussed and explored role of pharmacogenomics is the potential of "rescuing" drugs that fail clinical trials during drug development. The prime example for this benefit is gefitinib, which originally was destined to failure because only a small number of patients with small-cell lung cancer responded to the drug. However, in 2004, published results showed that tumor response to the drug was linked to mutations in the epidermal growth factor receptor (EGFR). Subsequently, development of pharmacogenomic biomarker tests for EGFR mutations in patients enables identification of responders for gefitinib. This example showed that investigational drugs found to be ineffective or unsafe during Phase II or III clinical trials might deserve a second look from the

TABLE 4.4 Issues for Pharmacogenomic Data Submission

VOLUNTARY GENOMIC DATA SUBMISSION AND VOLUNTARY EXPLORATORY DATA SUBMISSION
• How much data (all biomarkers and all genes or only those of interest) should be included?
• What is the requirement (type and extent) for biomarker validation?
• Are genome-wide analyses required?
• Would approval of biomarkers be required before they can be used in studies?
• Are data from retrospective subgroup analyses appropriate and sufficient?
• Are prospective case/control designs required?
• How should the issue of ethnicity be addressed?
REGULATORY REVIEW
• How would exploratory data be evaluated?
• What are the criteria that could change a voluntary submission to a required one?
• How would the extra time for additional studies affect the duration of exclusivity?
SPECIFIC CONCERNS
• If required (changed from voluntary) data are not sufficient, then what will happen?
• Are there unifying approaches to reviewing the data among regulatory agencies?
• If not, how would global drug development be affected?
• How would a company's intellectual property be affected by *voluntary* submission?

perspective of pharmacogenomics. Another example is lumiracoxib, a selective cyclooxygenase-2 inhibitor that was withdrawn from most global pharmaceutical markets because of hepatotoxicity. Subsequently, a strong association between patients with a human leukocyte antigen (HLA) haplotype and lumiracoxib-related liver injury has been identified [81]. Therefore, "failing" drugs can be further developed with a smaller target population with the genetic profile predictive of improved efficacy and/or reduced toxicity. This result can then be used for approval with appropriate product label containing the pharmacogenomic information. In reality, the possibility of such "drug rescue" with potential drug approval might not have sufficient incentive for the pharmaceutical company to spend additional cost to conduct another clinical trial, albeit in a smaller number of patients. Such incentive likely has to come from regulatory changes in the form of conditional drug approval with subsequent requirement of Phase IV trial. Without such changes, another incentive could take similar approach of drug development and approval under the Orphan Drug Act intended primarily for therapeutic agents in treatment of rare diseases.

CONCLUSION

Over the last few decades, significant achievements have been made in identifying variants in (or haplotypes linked to) genes that regulate the disposition and target pathways of drugs. Despite these advances, translating the pharmacogenomic results into clinical practice have been met with continued scientific debates, as well as commercial, economical, educational, legal, and societal barriers. Much work remains on addressing the logistics and challenges for fully incorporating pharmacogenomics into clinical practice and drug development. There are urgent needs to improve drug efficacy and safety for patient care, as well as the efficiency of the drug-development process, and that can only be achieved with all stakeholders in the field working together, and occasionally accepting a paradigm change in their current approach.

QUESTIONS FOR DISCUSSION

1. Why is evidence-based approach not necessarily the most appropriate means to evaluate the clinical utility of pharmacogenomic data?
2. What specific aspects of drug development can benefit from incorporation of pharmacogenomic evaluation?

3. How much pharmacogenomics information should be included in electronic medical record systems?
4. How important is the cost-effectiveness of a pharmacogenomic biomarker in decision regarding its implementation in clinical practice?

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CHAPTER

5

Pharmacogenomics in Cancer Therapeutics

Y. W. Francis Lam[1,2], *Stuart A. Scott*[3,4]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]College of Pharmacy, University of Texas at Austin, Austin, TX, United States; [3]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [4]Sema4, Stamford, CT, United States

OUTLINE

INTRODUCTION

Cancer is a multifactorial disease driven by genetic alteration in the somatic genome of the malignant cell. Subsequent proliferation, with or without additional genetic and/or epigenetic alterations that could further distort the genetic content of malignant cells, eventually leads to disruption of cellular machinery and signaling, and unregulated proliferation. These genetic alterations not only represent divergence from the original germline sequence, they also play a major role in determining the aggressiveness of the tumor, and more importantly, its resistance or sensitivity to specific therapy. Therefore, identifying somatic mutations that drive genetic alteration(s) is critical in assessing and predicting disease prognosis and treatment response.

Our expanding knowledge of the molecular characteristics of different tumors [1] over the past two decades has enabled a paradigm shift in anticancer drug development from the nonselective cytotoxic agents of the past to targeted therapies that are designed to ameliorate specific molecular and/or oncogenic abnormalities [2,3]. Most of these targeted drugs are

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00005-X

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TABLE 5.1 Selected Genetic Variants Affecting Treatment Outcomes in Lung Cancer

Genes	Allelic Variants	Treatment Phenotype Association
EGFR	Constitutive activating mutations of tyrosine kinase-binding domain within exons 18 to 21 of *EGFR*	Patients harboring the gain-of-function mutation have better clinical response to tyrosine-kinase inhibitors
ALK	Translocation of *EML4* (2p21) and *ALK* (2p23) results in the *EML4-ALK* fusion-type tyrosine kinase	Patients with *ALK* rearrangement show better response to ALK inhibitors
KRAS	Constitutive activation mutations of the RAS signaling pathway, primarily in codons 12 and 13 of exon 2 of *EGFR*	Limited data suggesting *KRAS* mutation as a negative predictive biomarker to tyrosine-kinase inhibitors

codeveloped with their associated predictive companion diagnostic tests, which usually empower clinicians to identify patients suitable for a given drug targeting tumor-specific genetic alteration, in contrast to empirically selecting cytotoxic agents solely based on cancer tissue of origin. Although targeted cancer therapy often focuses on somatically mutated genes, it should be noted that germline genetic variation associated with increased risk of cancer in carriers of mutated genes (e.g., mutations in tumor suppressor genes breast cancer 1 [*BRCA1*] and *breast cancer 2 [BRCA2]*) or altered drug sensitivity may also influence disease outcome and/or treatment responses.

In general, cancer biomarkers can be categorized as prognostic (associated with disease outcome) and predictive (associated with response to anticancer drug treatment). As a scientific discipline, pharmacogenomics evaluates genetic determinants of drug-response variability. For application within the field of oncology, pharmacogenomics should then be viewed as a clinical tool or scientific means to utilize the knowledge of the unique genetic makeup of the patient and his/her cancer, not only for identification of likely responders to specific targeted therapies but also to increase the overall clinical success rates. In this brief chapter, an overview of the role of cancer genomics and pharmacogenomics in precision oncology therapeutics, highlighting specific key examples, will be presented.

Therapy for Non-small-Cell Lung Cancer

Non-small-cell lung cancer is the most common type of lung cancer with several histological subtypes including adenocarcinoma, large-cell carcinoma, and squamous-cell carcinoma. The two most common mutated genes in patients with non-small-cell lung cancer are epidermal growth factor receptor (*EGFR*, *HER1*, or *c-ErbB-1*) and anaplastic lymphoma kinase (*ALK*), which usually occurs as a fusion product with another gene: echinoderm microtubule-associated protein-like 4 (*EML4*).

Activating *EGFR* mutation initiates a cascade of downstream EGFR signal transduction, increased tyrosine kinase activity, and cell proliferation (Table 5.1). In general, small molecule inhibitors of tyrosine kinase of EGFR (e.g., gefitinib, erlotinib) have shown significant activity in patients whose tumors harbor activating *EGFR* mutations [4]. The historical observation of limited clinical activity of gefitinib in a small subset of lung cancer and subsequent demonstration of significant response in lung cancer patients with somatic mutations in *EGFR* is described in more details in Chapter 3. Since that era of initial tyrosine kinase inhibitors, additional small molecules with more refined tyrosine kinase inhibition, e.g., afatinib and osimertinib, have been evaluated and approved.

The *EML4-ALK* fusion product is a mutation commonly found in about 4%–5% of non-small-cell lung cancer tumors (Table 5.1).

Activating mutations result in uncontrolled cell growth and differentiation as well as apoptosis inhibition [5]. In addition to being a positive predictive biomarker for tumor response in non-small-cell lung cancer patients harboring the mutation, *ALK* rearrangements are almost mutually exclusive with *EGFR* mutations, and limited data have suggested an association between *ALK* rearrangements and resistance to EGFR tyrosine kinase inhibitors [6]. Therefore, in contrast to patients with *EGFR* mutations who are typically treated with tyrosine kinase inhibitors, patients with *ALK* mutations are treated with specific ALK inhibitors. Crizotinib, ceritinib, and alectinib are examples of a rapidly expanding class of ALK inhibitors approved by the Food and Drug Administration (FDA) since 2011 [7].

Therapy for Chronic Myeloid Leukemia

Imatinib is another early example of the paradigm change in oncologic drug development for chronic myeloid leukemia. Imatinib competitively blocks the adenosine triphosphate (ATP)-binding site of B cell receptor–Abelson murine leukemia viral oncogene homolog (Bcr-Abl) kinase, which is the constitutively active product of *Bcr-Abl* fusion gene associated with the well-recognized Philadelphia translocation [8]. After its approval in 2002, it soon became apparent that imatinib therapy is associated with primary and secondary resistance in about one-third of the patients receiving the drug. Primary resistance occurs because of low systemic exposure that could be related to interindividual differences in activity of the organic cation transporter 1 that mediates imatinib influx into the leukemic cells [9]. The effect of polymorphism in the gene encoding the organic cation transporter 1 is also described in Chapter 3.

As importantly, secondary resistance to imatinib commonly occurs and is primarily related to acquired Bcr-Abl kinase domain mutations (e.g., D816V, two codon duplication in exon 9), leading to decreased drug sensitivity. Nevertheless, such resistance can be overcome by using higher doses of imatinib [10]. Therefore, testing for the mutational status can help in imatinib-dose optimization or determination of suitable patients for imatinib therapy [11]. Since the initial approval and experience with imatinib, newer Bcr-Abl tyrosine kinase inhibitors have been developed and include bosutinib, dasatinib, nilotinib, and ponatinib [12].

Therapy for Breast Cancer

The human epidermal growth factor receptor 2 (HER2/neu or ErbB-2) has been used as a biomarker for patient stratification in treatment of breast cancer. HER2 overexpression occurs in 15%–22% of breast cancers, and elevated level of HER2 is associated with a more-aggressive tumor type and adverse clinical outcome (poor prognosis and shorter survival) [13,14]. Trastuzumab is the first humanized monoclonal antibody developed and approved to target HER2. Clinical efficacy of trastuzumab in HER2-positive breast cancer was shown both as monotherapy and in combination with other anticancer drugs including paclitaxel and docetaxel [15–17]. In addition, clinical trial data showed that the treatment outcomes were positively correlated with the extent of HER2 overexpression. To avoid unnecessary toxicities in patients who would not benefit from the therapeutic benefits of the drug, use of trastuzumab is restricted to those who overexpress HER2, the level of which can be determined with a number of companion diagnostic tests (e.g., INFORM HER2 dual ISH DNA Probe Cocktail) [18,19] approved by the FDA. Clinical guidelines provided by professional organizations also endorse the testing of HER2 status [20,21]. Over the years, several other drugs that target HER2, e.g., ado-trastuzumab emtansine, lapatinib, and pertuzumab, have also been approved along with their companion diagnostic tests [22].

In addition to HER2, the estrogen receptor as a driver for breast tumor growth, serves as the pharmacological target for antiestrogenic compounds such as tamoxifen. The use of tamoxifen for patients with estrogen positive breast cancer is associated with up to 50% reduction in disease recurrence and 30% decrease in mortality. However, significant interindividual differences in tamoxifen response exist, which is at least partially related to variable extent of drug metabolism in tamoxifen-treated patients. Tamoxifen is extensively metabolized by multiple cytochrome P450 isoenzymes, including cytochrome P450 2D6 (CYP2D6), to two active metabolites: 4-hydroxy-tamoxifen and endoxifen [23,24]. The activity of CYP2D6, and hence the amount of endoxifen formed, can be modulated by *CYP2D6* polymorphism and/or concurrent administration of CYP2D6 inhibitors such as selective serotonin reuptake inhibitors [25–27].

Despite altered response rate and increased risk of cancer recurrence in CYP2D6 poor metabolizers [28], conflicting data from studies evaluating association between tamoxifen response and *CYP2D6* status have put a damper on the potential use of CYP2D6 genotyping to guide tamoxifen therapy [29,30]. Thus, the current FDA-approved tamoxifen label does not include a recommendation for *CYP2D6* testing. Although the current evidence does not support routine clinical testing, the study results of Irvin et al. and Kiyotani et al. suggest an increased tamoxifen dose could be an effective way to maintain an effective endoxifen concentration in patients who are carriers of decreased function or null alleles of *CYP2D6* [31,32]. Additional approach to provide further insight regarding *CYP2D6* genotyping for tamoxifen response was recently provided in a prospective clinical trial by Zembutsu et al. The investigators reported that the expression of a proliferation biomarker Ki-67 protein [33] was significantly associated with estrogen receptor expression level, and changes in Ki-67 expression could be potentially a useful surrogate biomarker for tamoxifen efficacy [34].

Therapy for Metastatic Colorectal Cancer

Over the years, molecular biomarkers have been identified that could provide both prognostic and predictive information to clinicians treating patients with colorectal cancer. Examples of these biomarkers include EGFR, v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), microsatellite instability, and thymidylate synthase (Table 5.2).

TABLE 5.2 Selected Genetic Variants Affecting Treatment Outcomes in Colorectal Cancer

Genes	Allelic Variants	Treatment Phenotype Association
KRAS	Constitutive activation mutations of the RAS signaling pathway, primarily in codons 12 and 13 of exon 2 of *EGFR*	Lack of response to anti-EGFR monoclonal antibodies in patients with KRAS mutation
Mismatch repair (MMR) genes	*MLH1, MSH2, MSH6, PMS1, PMS2*	Proficient MMR- or low MSI-status predicts response to 5-FU
TYMS	*TSER*2, TSER*3G, TSER*3C*	Homozygous carriers more likely to experience 5-FU toxicity
DYPD	*DYPD*2A*	Limited data on association between SNP with decreased DPD activity and 5-FU toxicity
UGT1A1	*UGT1A1*28, UGT1A1*6*	Homozygous carriers of either variant more likely to experience neutropenia and diarrhea with irinotecan treatment

Increased expression of EGFR has long been suggested to be a prognostic marker for a wide range of cancers, including colorectal cancer. The high expression is associated with poor clinical outcome and anti-EGFR monoclonal antibodies (e.g., cetuximab, panitumumab) had been investigated [35–38] and approved as targeted therapies for treatment of colorectal cancer since 2004. Nevertheless, EGFR-based drug regimens are only effective in a subset of patients that are related to the mutational status of the *KRAS* gene, a downstream conductor of *EGFR* signaling. *KRAS* mutations (in codon 12 and 13) are observed in approximately 30%–40% of colorectal cancer patients in the United States and act as an important prognostic biomarker [39]. More importantly, *KRAS* mutations allow tumor escape from EGFR regulation, and are predictive of resistance to cetuximab or panitumumab therapy [40,41]. To exclude patients who are not likely candidates to receive anti-EGFR monoclonal antibodies, testing for *KRAS* mutations are needed before starting therapy with cetuximab and panitumumab [42,43].

The antimetabolite 5-fluorouracil (5-FU) is commonly used in the treatment of advanced colorectal cancer. Microsatellite instability (MSI) associated with mismatch repair (MMR) genes (*MLH1, MSH2, MSH6, PMS1, or PMS2*) account for 15%–20% of sporadic colorectal cancer. Tumors with a *MMR* mutation have an absence of MMR protein expression, which, when coupled with high MSI, have been shown to be predictive biomarkers of decreased benefit from 5-FU-based chemotherapy in patients with Stage II and III disease [44–47].

In addition, thymidylate synthase is a folate-dependent enzyme and the inhibitory target of 5-FU. Polymorphisms in *TYMS*, the gene that encodes thymidylate synthase, have also been evaluated. Tandem repeat variants at the promoter enhancer region (TSER) (rs34743033) results in *TSER**2* (two copies of the 28-base pair tandem repeat [2R]) with low enzyme expression and *TSER**3* (three copies of the 28-base pair tandem repeat [3R]) with enzyme expression approximately 2.6× that of *TSER**2*. In addition, a G>C single-nucleotide polymorphisms (SNP) within the second repeat of *TSER**3* is associated with altered *TS* transcription [48]. Overexpression of thymidylate synthase has been linked to 5-FU resistance due to higher in vivo tumor thymidylate synthase activity [49,50] and thymidylate synthase SNPs have been associated with increased 5-FU toxicities in different populations [51,52]. Despite the attractiveness of *TSER* genotyping to better predict response to 5-FU-based regimen, current literature does not appear to provide sufficient evidence for measuring TYMS levels in tumors [53].

The enzyme dihydropyrimidine dehydrogenase (DYPD) mediates the metabolism of 5-FU, and genetic polymorphisms in the *DYPD* gene encoding DYPD result in DYPD-deficient phenotypes with an overall frequency of about 3%–5%. Of all known SNPs associated with grade 3- and grade 4-toxicities in 5-FU treated patients, the G>A point mutation within intron 14 (c.IVS14+1G>A, also known as rs3918290, or c.1905+1G>A) associated with the *DYPD**2A* allele results in a protein with no catalytic activity [54]. Homozygous and heterozygous carriers of this common variant allele of *DYPD* have a complete absence of and 50% reduced DYPD activity, respectively, resulting in significant and sometimes life-threatening 5-FU-related toxicities [55,56].

Additional Biomarkers for Drug Toxicity

Other than DYPD, additional germline mutations that have been shown to impact anticancer drug toxicities include thiopurine-S-methyl transferase and uridine-diphosphate glucuronosyltransferase. The pharmacogenetic relevance of these two Phase II metabolic enzymes is discussed in the first chapter and summarized in Table 5.3 of this chapter. Although genotyping for the uridine-diphosphate

TABLE 5.3 Genetic Variants in Drug Metabolizing Enzymes Affecting Toxicity Responses

Genes	Impact on Drug Exposure	Treatment Phenotype Association
DYPD	Increased level of 5-FU in carriers of *DYPD*2A*	Increased risk of neurological toxicities, grade 3 diarrhea, and possibly hand–foot syndrome
TPMT	Increased level of 6-MP in homozygous and heterozygous carriers of *TPMT* variants	Increased hematological toxicities in homozygote and heterozygote, requiring dosage reduction as recommended in clinical guidelines
UGT1A1	Significant increase in SN-38 concentrations in carriers of *UGT1A1*28* and treated with irinotecan	Homozygous carriers more likely to experience severe neutropenia and diarrhea with irinotecan treatment

glucuronosyltransferase 1A1*28 allele (*UGT1A1*28*) are not currently considered routine, clinical data do suggest the need for irinotecan-dose reduction to decrease the risk of severe diarrhea and neutropenia in patients who are homozygous carriers of the *28 allele. In contrast, thiopurine-S-methyl transferase is currently the only drug-metabolizing enzyme with widespread acceptance for genotyping and availability of clinical guideline through the Clinical Pharmacogenetics Implementation Consortium [57,58]. On the other hand, although thiopurine-S-methyl transferase gene variants have been reported to be associated with a higher risk of cisplatin-related ototoxicity, there is currently no recommendation for thiopurine-S-methyl transferase genotyping in cisplatin-treated patients.

The Path Forward for Implementation of Precision Oncology

At present, identification of patients who harbor *ALK* or *EFGR* mutations are included in current clinical guidelines and accepted as standard procedure for precision oncology practice in managing patients with non-small-cell lung cancer. However, the works by Chen et al. and Iorio et al. [59,60] are an indication that, through systematic expansion of our knowledge regarding cancer genomes, we will have additional insight about potential new biomarkers, therapeutic targets, and treatment options. Nevertheless, despite significant success of targeted therapies for management of several types of cancer (some of which are briefly reviewed in this chapter), similar progress has not been experienced in other areas of medical oncology.

In addition to continued effort to expand a well-supported and rigorous database of drug–target interactions [60], other potential barriers need to be addressed before large-scale adoption of implementation includes widespread availability of genomic and clinical data [61], and the need of different clinical trial designs to address genomics-based investigations [62–66]. In addition, viable infrastructure is needed to support the additional bioinformatics and laboratory resources deemed necessary for such refined clinical trials, along with practical assistance for practicing clinicians in using the abundant and complex information, and integration of validated bioinformatics tools and data platform into existing workflow.

In a way that is analogous to the concept of a medical consult service, molecular tumor boards and oncology practice models have been initiated at different cancer centers and academic institutions to assist in clinical decision and patient management. Experiences with these programmatic supports have been published in the literature [67–70]. A decision-support framework for genomics-guided cancer therapy has recently been developed to provide assistance for clinicians to make decisions in practicing precision oncology [71,72]. Further refinement of these two approaches and maybe even their integration with one another would enable clinicians to

benefit from both peer sharing of expertise and onsite support with fully incorporated bioinformatics for clinical decision-making.

Nevertheless, similar to implementation barriers in other medical specialties (discussed in Chapter 4, the major challenge lies with additional in-depth knowledge of tumor biology, and the level of evidence deemed necessary before any specific biomarker is considered "actionable" and subsequently utilized on a large scale. These challenges are "tied" to each other in that defining the functional significance of variant alleles pave the way to establishing the minimal level of evidence acceptable to all stakeholders, which would impact patient selection both for clinical trials and personalized treatment plan in practice. Clinical utility and practical implementation of precision oncology will also likely be shaped by future results from ongoing trials such as the National Cancer Institute-Molecular Analysis for Therapy Choice (ClinicalTrials.gov Identifier: NCT02465060), and the National Cancer Institute-Children's Oncology Group Pediatric Molecular Analysis for Therapy Choice (ClinicalTrials.gov Identifier: NCT03155620) [73].

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CHAPTER

6

Pharmacogenetics in Cardiovascular Diseases

Larisa H. Cavallari[1], *Kathryn M. Momary*[2]

[1]Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics, University of Florida, Gainesville, FL, United States; [2]Department of Pharmacy Practice, Mercer University, College of Pharmacy, Atlanta, GA, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00006-1

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OBJECTIVES

1. Provide examples of pharmacogenetic labeling for drugs used to manage cardiovascular disease.
2. Discuss guidelines for use of genetic information to guide therapy with cardiovascular agents.
3. Describe applications of pharmacogenetics in prescribing oral antiplatelet agents and warfarin.
4. Describe the potential applications of pharmacogenetics in the management of hypertension, heart failure, and drugs that influence cardiac conduction.

INTRODUCTION

Cardiovascular disease is the most common cause of death globally and is associated with significant productivity loss and healthcare costs [1,2]. Cardiovascular drugs, including antihypertensive medications and statins, consistently rank among the top 10 most commonly prescribed drugs in the United States [3]. Guidelines

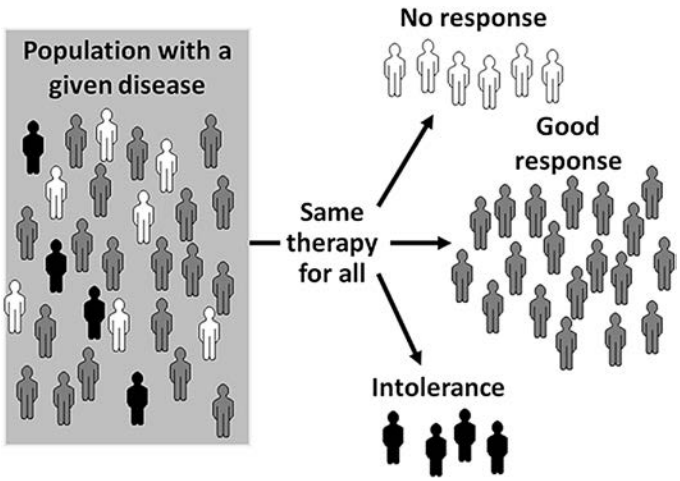

FIGURE 6.1 Current empiric approach of treating patients with cardiovascular disease. As shown, patients with a given cardiovascular disease are generally treated with similar therapy. The majority of patients will have a good response to such therapy. However, the problem with this approach is that a subset of patients will have little to no therapeutic response and another subset will develop intolerable adverse effects.

from expert consensus panels are available to guide the treatment for most cardiovascular diseases, including hypertension, heart failure, dyslipidemia, and ischemic heart disease [4–9]. These guidelines are based on data from large, randomized, placebo-controlled clinical trials demonstrating significant improvements in clinical outcomes with certain medications in clinical trial populations. As illustrated in Fig. 6.1, for some cardiovascular diseases, the same drug or drug combination is recommended for all affected persons, regardless of individual characteristics. Such is the case with renin angiotensin system inhibitors and β-blockers, which are recommended for all patients with left ventricular dysfunction in the absence of a contraindication [9]. However, although these treatments were efficacious in clinical trial populations as a whole, there is no guarantee that they will be safe or effective in an individual patient. In fact, there is significant interpatient variability in response to angiotensin-converting enzyme (ACE) inhibitors and β-blockers, with some patients deriving no benefit and other patients experiencing intolerable adverse effects with these agents. Currently, it is difficult if not impossible to predict how a patient will respond to a cardiovascular agent based on clinical factors alone.

It is now well recognized that an individual's genotype impacts his or her response to cardiovascular drugs. As of August 2017, genetic information was included in the Food and Drug Administration (FDA)-approved labeling for at least 12 drugs used to treat cardiac and vascular disorders (Table 6.1). Genotype primarily influences cardiovascular drug response by affecting drug disposition in the body (pharmacokinetics) or a patient's sensitivity to a drug (pharmacodynamics), as described in detail in Chapter 1.

This chapter reviews the pharmacogenetics of various cardiovascular agents. The strongest evidence exists for clopidogrel, warfarin, and simvastatin, and thus the most indepth discussion is devoted to these drugs. This chapter also provides an overview of pharmacogenetic application for dosing tacrolimus after cardiac transplant and the potential for pharmacogenetics to improve prescribing of antihypertensive agents, heart-failure medications, and drugs that influence cardiac conduction. Challenges and opportunities with bringing cardiovascular pharmacogenetics to the clinical arena are also highlighted.

TABLE 6.1 Cardiovascular Drugs With Genetic Labeling

Drug Class/Drug	Biomarker	Location of Label Information	Context
STATINS			
Atorvastatin	LDL receptor	Indications/Dosage and Administration/Clinical Studies	Among other indications, atorvastatin is indicated in patients with familial hypercholesterolemia that is due to mutations in the LDL receptor gene.
Pravastatin	Genotype APOE E2/E2 and Fredrickson Type III dysbetalipoproteinemia	Clinical Studies	Response to pravastatin in patients with genotype E2/E2 and Fredrickson Type III dysbetalipoproteinemia is shown.
BETA-BLOCKERS			
Carvedilol	CYP2D6	Drug Interactions/Clinical Pharmacology	Reduced carvedilol metabolism in poor metabolizers.
Metoprolol	CYP2D6	Drug Interactions/Clinical Pharmacology	Reduced metoprolol metabolism in poor metabolizers.
Propranolol	CYP2D6	Clinical Pharmacology	Reduced propranolol metabolism in poor metabolizers.
ANTIPLATELETS			
Clopidogrel	CYP2C19	Boxed Warning/Warnings and Precautions/Clinical Pharmacology	Reduced clopidogrel efficacy in poor metabolizers.
Prasugrel	CYP2C19	Use in Specific Populations/ Clinical Pharmacology/ Clinical Studies	No effect of CYP2C19 genotype on prasugrel efficacy.
Ticagrelor	CYP2C19	Clinical Pharmacology	No effect of CYP2C19 genotype on ticagrelor efficacy.
ANTICOAGULANTS			
Warfarin	CYP2C9/VKORC1	Dosage and Administration/ Drug Interactions/Clinical Pharmacology	Lower warfarin doses needed with the CYP2C9*2, CYP2C9*3, and VKORC1 -1639A alleles.
ANTIARRHYTHMICS			
Propafenone	CYP2D6	Dosage and Administration/ Warnings and Precautions/ Drug Interactions/Clinical Pharmacology	The recommended dose is the same in slow and extensive metabolizers.
Quinidine	CYP2D6	Precautions	Quinidine can convert extensive metabolizers to poor metabolizers of CYP2D6 substrates.
MISCELLANEOUS			
Hydralazine	NAT	Clinical Pharmacology	Fast acetylators have lower hydralazine exposure.

PHARMACOGENETICS OF ANTIPLATELET AGENTS

Background on Antiplatelet Agents

Antiplatelet therapy plays a major role in cardiovascular risk reduction. Antiplatelet therapy began with aspirin monotherapy and has advanced to include multiple oral antiplatelet drugs affecting different mechanisms of platelet function [10]. In addition to aspirin, currently approved oral antiplatelet drugs include ticlopidine, clopidogrel, prasugrel, and ticagrelor. Ticlopidine is rarely used because it increases the risk for neutropenia and thrombotic thrombocytopenic purpura. As such, the discussion will be limited to the other agents.

Clopidogrel has long been available; ticagrelor and prasugrel were more recently approved by the FDA. Although these agents have different pharmacokinetic and pharmacodynamic properties and indications, they all share the common mechanism of blocking the platelet $P2Y_{12}$ receptor, resulting in attenuation of adenosine diphosphate (ADP)–mediated platelet activation and aggregation. Thus, they are all classified as $P2Y_{12}$ receptor inhibitors.

Overview of Clopidogrel Metabolism and Pharmacodynamics

Clopidogrel is indicated in combination with aspirin for patients with an acute coronary syndrome (ACS) who are medically managed or undergo percutaneous coronary intervention (PCI) based on data that it reduces morbidity and mortality in these patient populations [11–13]. The combination of clopidogrel and aspirin also reduces the risk for coronary-stent thrombosis following PCI [14]. There is significant interpatient variability in clopidogrel pharmacokinetics and pharmacodynamics [15]. Clopidogrel is a prodrug requiring bioactivation by multiple cytochrome P450 (CYP450) enzymes. As shown in Fig. 6.2, clopidogrel is a p-glycoprotein substrate, and once absorbed, the majority of clopidogrel is eliminated via esterases. The remaining drug requires conversion via a two-step process to its active form. Genetic variation in pathways involved in clopidogrel absorption and bioactivation has been investigated for its effects on clopidogrel disposition and effectiveness.

Clopidogrel responsiveness can be characterized via drug effects on either platelet aggregation or clinical outcomes. Platelet aggregation tests involve ex vivo exposure of platelets to aggregating agents, including ADP. Decreased response to clopidogrel, as demonstrated by insufficient attenuation of platelet aggregation, has been linked to an increased risk of adverse cardiovascular events [15,16]. Investigators have also used clinical events, such as myocardial infarction (MI), stroke, or coronary artery stent thrombosis, as measures of clopidogrel response [16,17]. Genetic determinants of both measures of response will be discussed in this section.

CYP2C19 Genotype and Clopidogrel Responsiveness

Various isoenzymes of the CYP450 system, including cytochrome P450 3A4 (CYP3A4), cytochrome P450 3A5 (CYP3A5), and cytochrome P450 2C19 (CYP2C19), are involved in clopidogrel metabolism. However, polymorphisms within the gene for CYP2C19, which is involved in both steps of the clopidogrel bioactivation pathway and serves a major role in converting clopidogrel to its active form, have the greatest implications for clopidogrel response. In contrast, no consistent associations have been found between the cytochrome P450 3A (CYP3A) genotypes and clopidogrel pharmacokinetics or clinical response [16,18–20]. The CYP2C19 gene is located on chromosome 10q23.33. The CYP2C19*2, *3, *4, *5, *6, *7, and *8 alleles are nonfunctional (loss of function) alleles associated with absent or reduced CYP2C19 function compared to the CYP2C19*1 (normal function)

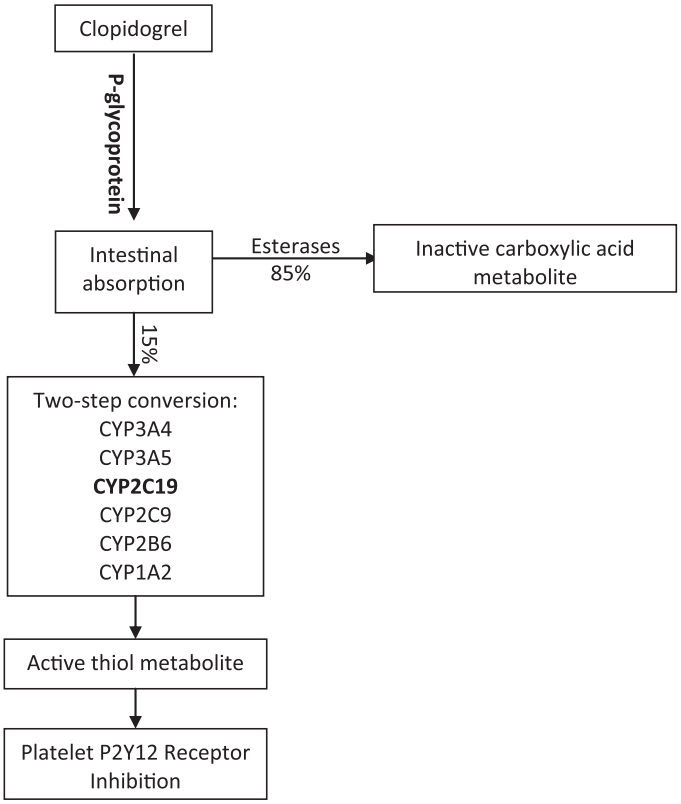

FIGURE 6.2 Proteins involved in the absorption and metabolic activation of clopidogrel. Genes for proteins shown in bold contain polymorphisms linked to clopidogrel responsiveness. *CYP*, cytochrome P450.

allele [21]. In contrast, the CYP2C19**17* allele is associated with increased CYP2C19 function. The CYP2C19**2* allele is by far the most common nonfunctional CYP2C19 variant; however, its frequency differs by ancestral origin (Fig. 6.3), with a higher frequency in Asians (approximately 30%) compared to Caucasians (13%) and African Americans (18%) [21,22]. The CYP2C19**3* allele also occurs commonly in Asian populations (~10%) but is rare in individuals of other ancestral backgrounds (<1%). Approximately 14% of Asians, 2% of Caucasians, and 4% of African Americans are CYP2C19 poor metabolizers (with two nonfunctional alleles), and 50%, 25%, and 30%, respectively, are intermediate metabolizers (with one nonfunctional allele).

In individuals with one or two nonfunctional CYP2C19 alleles, there is decreased production of the active clopidogrel metabolite and reduced clopidogrel effectiveness [23]. Studies have consistently shown that possession of a CYP2C19 nonfunctional allele increases the risk of cardiovascular events with clopidogrel (Fig. 6.4) [16,20,24–28]. In a metaanalysis of nine studies and 9685 total patients, the majority of whom underwent PCI (91%) and had an ACS (54%), carriers of at least one CYP2C19 nonfunctional allele had a higher risk of adverse cardiovascular events, with a hazard ratio of 1.57 (95% confidence interval [CI]: 1.13–2.16) compared to noncarriers (i.e., the risk for adverse cardiovascular events was approximately 1.5-fold greater in nonfunctional allele carriers) [29]. The hazard ratio for stent thrombosis was 2.81 (95% CI: 1.81–4.37) for nonfunctional allele carriers compared to noncarriers. Similarly, another metaanalysis

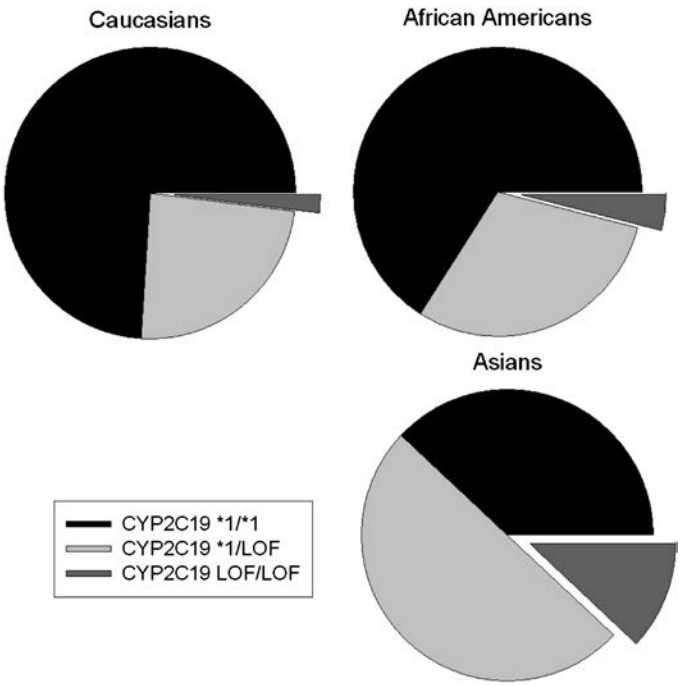

FIGURE 6.3 Cytochrome P450 2C19 (CYP2C19) allele frequencies among ethnic groups. *LOF*, loss-of-function.

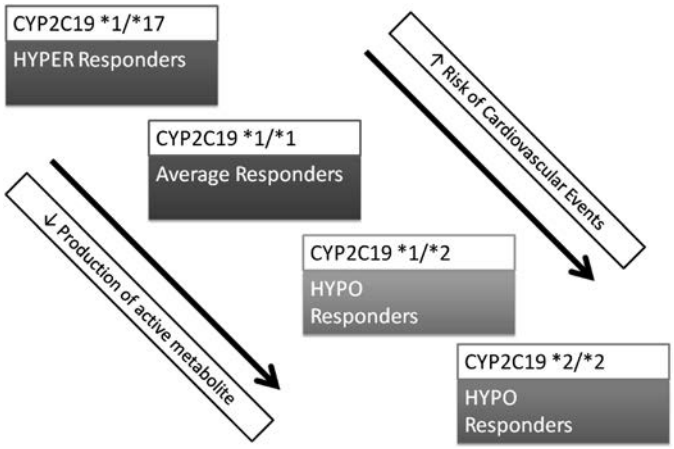

FIGURE 6.4 Effect of cytochrome P450 2C19 (CYP2C19) genotype on clopidogrel pharmacokinetics and efficacy.

of nearly 12,000 patients reported that carriers of the CYP2C19*2 allele had increased risk for major adverse cardiovascular events (odds ratio [OR]: 1.29; 95% CI: 1.12–1.49) and stent thrombosis (OR: 3.45; 95% CI: 2.14 to 5.57) compared to noncarriers (i.e., the odds of adverse events were 1.29 times greater, and the odds for stent thrombosis were more than three times greater for carriers vs. noncarriers) [30]. In contrast, two metaanalyses including more heterogeneous

patient populations found no association between CYP2C19 genotype and adverse events with clopidogrel [31,32]. However, these latter analyses have been criticized for including studies of lower-risk patients, such as those with atrial fibrillation or with an ACS managed medically (vs. with PCI). A more recent metaanalysis examined outcomes separately in patients who underwent PCI and those who did not [33]. Among clopidogrel-treated patients who underwent PCI, there was a significantly higher risk for adverse cardiovascular events in those with a CYP2C19 nonfunctional allele compared to those without a nonfunctional allele. However, no association by genotype was observed in non-PCI patients. The majority of data demonstrates that CYP2C19 nonfunctional genotype significantly impacts formation of the active clopidogrel metabolite, ex vivo inhibition of platelet aggregation with clopidogrel, and clopidogrel's effectiveness in preventing adverse cardiovascular events, particularly among patients undergoing coronary artery stent placement.

The effect of the CYP2C19*17 increased function allele on clopidogrel responsiveness has also been examined; however, the results from these studies have been inconsistent. This allele has been associated with increased production of the clopidogrel active metabolite and greater inhibition of platelet aggregation with clopidogrel [34,35]. There is some evidence that CYP2C19*17 carriers may be at greater bleeding risk [35]. However, the CYP2C19*17 and *2 alleles are in linkage disequilibrium (LD) such that the CYP2C19*17 single-nucleotide polymorphism (SNP) is not known to occur on the same allele as *2, thus complicating interpretation of effects observed in CYP2C19*17 allele carriers. Given that an independent effect of the CYP2C19*17 allele has not been clearly established, this allele is not accompanied by a specific therapy change recommendation in the currently available CYP2C19 genotype-directed practice guidelines [22,36].

ABCB1 Genotype and Clopidogrel Responsiveness

P-glycoprotein is encoded by the ATP-binding cassette, subfamily B, member 1 (ABCB1) gene. The most commonly studied ABCB1 variant is the synonymous c.3435C>T polymorphism, located in a region that encodes for a cytoplasmic loop in the transporter [37]. A lower-peak plasma concentration (Cmax) and total area under the plasma concentration–time curve (AUC) of clopidogrel and its active metabolite were noted after single 300 and 600-mg doses in subjects who were homozygous for the variant ABCB1 3435T allele [38]. Of note, increasing the clopidogrel dose to 900 mg overcame the effect of genotype on drug concentrations. Several studies have also assessed the association between ABCB1 genotype and clinical response to clopidogrel with varying results [17,19,20,24,39]. The inconsistent results from these studies render it difficult to apply ABCB1 testing to patients starting clopidogrel.

Paraoxonase-1 (PON1) Genotype and Clopidogrel Responsiveness

Paraoxonase-1 (PON1) is an esterase that has been shown to facilitate the activation of clopidogrel in vitro [41]. A nonsynonymous polymorphism in the coding region of PON1, p.Q192R, has been evaluated for its role in clopidogrel responsiveness. The 192Q allele was associated with increased clopidogrel activation in vitro in one study [41]. The same study showed that possession of a 192Q allele was associated with decreased risk of stent thrombosis. However, in contrast to most previous data, the investigators found no association between CYP2C19 genotype and stent thrombosis risk. Several studies have since demonstrated no association between *PON1* genotype and clopidogrel responsiveness [28,42–44]. Because of the lack of replication with the PON1 genotype, PON1 genotyping is not currently recommended.

Genome-Wide Association Study (GWAS) of Clopidogrel Responsiveness

Investigators for the Pharmacogenomics of Antiplatelet Intervention-1 (PAPI-1) study conducted a genome-wide association study (GWAS) of ex vivo platelet aggregation with clopidogrel in a cohort of generally healthy subjects from the Old Order Amish population ($n = 429$) [19]. Each subject was given a 300-mg clopidogrel loading dose, followed by a dose of 75 mg/day for 6 days, and platelet aggregation was measured before and after clopidogrel administration. Between 500,000 and 1 million variants were assessed for each subject to identify genetic associations with clopidogrel responsiveness based on ex vivo platelet aggregation. A cluster of 13 highly correlated variants on chromosome 10 in the genetic region encoding CYP2C18, CYP2C19, CYP2C9, and CYP2C8 were associated with clopidogrel response. These variants were in strong LD with the CYP2C19*2 allele and explained 12% of the interindividual variation in platelet aggregation. Of note, no association was seen with CYP2C19*17 or with polymorphisms in the genes encoding CYP3A, ABCB1, or PON1. In a replication cohort of 227 patients undergoing nonemergent PCI and treated with clopidogrel, the investigators found that, similar to most previous data, CYP2C19*2 was associated with residual platelet aggregation and an increased risk for cardiovascular events or death at 1 year, with a hazard ratio of 2.4 (95% CI 1.18 to 4.99), indicating a nearly 2.5-fold greater risk for events or death with the CYP2C19*2 allele [19].

Alternative Treatment Approaches in Patients With a CYP2C19 Nonfunctional Allele

Several studies have addressed whether clopidogrel dose escalation overcomes the effects of CYP2C19 nonfunctional alleles. In a multicenter, double-blind clinical trial, patients with cardiovascular disease and the CYP2C19*1/*2 or *2/*2 genotype were randomized to receive clopidogrel at varying doses (75, 150, 225, and 300 mg), each for a 14-day period. Platelet function testing was conducted with each dose, and results were compared with those from noncarriers of the CYP2C19*2 allele receiving clopidogrel 75 mg [45]. For carriers of a single CYP2C19*2 allele, a clopidogrel dose of 225 mg/day resulted in levels of platelet inhibition similar to that attained with a 75 mg/day dose in noncarriers. However, in CYP2C19*2 homozygotes, not even the 300-mg/day dose resulted in platelet inhibition comparable to the 75-mg/day dose in noncarriers. Similarly, among patients with an acute MI receiving a clopidogrel loading dose of 300 mg, significantly lower inhibition of platelet aggregation was observed in both heterozygous and homozygous carriers of the CYP2C19*2 allele compared to noncarriers [46]. A 900-mg loading dose was sufficient to inhibit platelet aggregation in heterozygotes, but not in homozygotes. A study in healthy volunteers found that poor metabolizers (CYP2C19*2/*2 or *2/*3 genotypes) receiving a clopidogrel loading dose of 600 mg, followed by a maintenance dose of 150 mg/day for 5 days, had similar inhibition of platelet aggregation compared to normal metabolizers (CYP2C19*1/*1 genotype) receiving a 300-mg loading dose and 75 mg/day dosing [47]. Intermediate metabolizers (CYP2C19*1/*2 or *1/*3 genotype) had a similar response as normal metabolizers with all clopidogrel doses tested. In contrast, a study of patients undergoing PCI after an ACS found that doubling the maintenance dose of clopidogrel in CYP2C19*2 carriers was not effective in overcoming reduced inhibition of platelet aggregation [48]. Another study of ACS patients undergoing PCI found that providing up to three additional 600-mg clopidogrel loading doses to CYP2C19*2 carriers, according to the degree of platelet reactivity, was successful in overcoming reduced response with standard 600-mg dosing in some patients [49]. However,

12% of these patients never reached the desired level of inhibition of platelet aggregation. This latter study demonstrates that although titrating clopidogrel dosing based on platelet aggregation testing in carriers of a CYP2C19 nonfunctional allele may be a viable approach to optimizing the clopidogrel loading dose for some patients, it is not an effective approach for all patients. The inconsistent results among studies are most likely due to differences in study populations (i.e., healthy subjects vs. patients with an acute cardiac event undergoing PCI).

A more effective approach to antiplatelet therapy based on CYP2C19 genotype is to treat nonfunctional allele carriers with an alternative antiplatelet agent, namely prasugrel or ticagrelor. Like clopidogrel, prasugrel is a thienopyridine that binds covalently and irreversibly to the $P2Y_{12}$ receptor and is a prodrug requiring bioactivation [50]. In contrast, ticagrelor is administered in its active form and more reversibly binds to the $P2Y_{12}$ receptor to change its conformation.

Prasugrel is currently FDA-approved for use in patients with ACS undergoing PCI. Like clopidogrel, prasugrel is a p-glycoprotein substrate that is converted to the active metabolite via multiple enzymes, and CYP3A, CYP2B6, CYP2C9, and CYP2C19 in particular. However, unlike clopidogrel, esterases convert prasugrel to an intermediate metabolite (rather than an inactivated metabolite), and the CYP450 bioactivation occurs in a single step (rather than two steps). Likely because of prasugrel's unique bioactivation pathway, common genetic variants in CYP450 enzymes do not affect the pharmacokinetics or clinical efficacy of prasugrel [51]. There is also no association between ABCB1 genotype and prasugrel pharmacokinetics, possibly because prasugrel is more rapidly metabolized compared to clopidogrel [17]. Ticagrelor is indicated for ACS, regardless of whether patients undergo PCI. The CYP3A4 enzyme is the primary enzyme responsible for ticagrelor metabolism. Similar to prasugrel, there is no evidence that common genetic variation affects ticagrelor pharmacokinetics or efficacy [40]. The data demonstrate that prasugrel and ticagrelor provide greater inhibition of platelet aggregation and greater protection against cardiovascular events compared to clopidogrel in CYP2C19 nonfunctional allele carriers [17,23,40,52,53]. In a prospective evaluation of genotype-guided antiplatelet prescribing, patients with a CYP2C19 nonfunctional allele treated with prasugrel were shown to have lower platelet reactivity compared to nonfunctional allele carriers treated with clopidogrel [54]. However, it is important to note that prasugrel use is contraindicated in patients with a history of stroke or transient ischemic attack, and its use is not recommended in patients 75 years of age or older because of increased bleeding risk.

Clopidogrel Labeling Revisions

Based on substantial data supporting an association between CYP2C19 nonfunctional alleles and reduced clopidogrel responsiveness, the FDA approved the addition of genetic information to the clopidogrel labeling in March 2010 [55]. These label changes include a boxed warning about diminished antiplatelet response to clopidogrel in CYP2C19 poor metabolizers (with two nonfunctional alleles). The labeling further states that genetic testing is available and advises consideration of alternative therapy in poor metabolizers. Although these labeling updates highlight the importance of CYP2C19 genotype in clopidogrel responsiveness, they provide no guidance on when or whom to genotype and little guidance on how to manage poor metabolizers. Further, they do not address CYP2C19 intermediate metabolizers.

Guidelines for the Clinical Use of CYP2C19 Genotyping With Clopidogrel

Several statements by expert consensus panels address CYP2C19 genotyping to determine clopidogrel responsiveness [22,55]. In 2010,

TABLE 6.2 Phenotype Classification and Therapeutic Recommendations From the CPIC Based on CYP2C19 Genotype For Patients Requiring Dual Antiplatelet Therapy. After Acute Coronary Syndrome and Percutaneous Coronary Intervention [22]

CYP2C19 genotype	Phenotype Classification	Therapeutic Recommendation	Classification of Recommendation
*1/*1	Normal metabolizer	Clopidogrel should be effective at the label-recommended dosage and administration	Strong
*1/*17 or *17/*17	Ultra-rapidmetabolizer	Clopidogrel should be effective at the label-recommended dosage and administration	Strong
*1/*2	Intermediate metabolizer	Prasugrel or ticagrelor if no contraindication	Moderate
*2/*2	Poor metabolizer	Prasugrel or ticagrelor if no contraindication	Strong

the American College of Cardiology and the American Heart Association Foundation issued a joint response to the addition of genetic information to the clopidogrel labeling, which is summarized in their guidelines for management of PCI patients [7,55]. They indicated that the available data were insufficient to recommend routine use of genetic testing for patients undergoing PCI, specifically citing the lack of outcomes data with genetic testing from large randomized control trials. However, they further stated that CYP2C19 genotyping may be considered in patients who are at moderate to high risk for poor cardiovascular outcomes, such as those undergoing elective high-risk PCI for extensive and/or very complex disease and others at the clinician's discretion. In these patients, alternative therapy (e.g., prasugrel or ticagrelor) is recommended. These recommendations are designated as Class IIb based on Level C evidence, meaning that the benefit of genotyping may be slightly greater than or equivalent to not genotyping (note to the reader: definitions of evidence levels are provided in the guidelines).

Guidelines by the Clinical Pharmacogenetics Implementation Consortium (CPIC) do not address whether to order CYP2C19 testing for patients undergoing PCI, leaving this to the discretion of the physician [22]. Rather, they provide recommendations for therapy based on available genotype results. The guidelines focus on patients with an ACS who undergo PCI. For these patients, either prasugrel or ticagrel is recommended in the presence of a CYP2C19 nonfunctional allele, in the absence of contraindications. For patients without a nonfunctional allele, clopidogrel is expected to be effective (Table 6.2).

Randomized Controlled Trials Examining Outcomes With CYP2C19-Guided Antiplatelet Therapy

Two large randomized controlled trials are addressing the efficacy of genotype-guided clopidogrel use. The Tailored Antiplatelet Therapy Following PCI (TAILOR-PCI) trial is examining the effect of genotype-guided antiplatelet therapy on adverse cardiovascular events (ClinicalTrials.gov Identifier: NCT01742117). Patients undergoing PCI are randomized to either genotype-guided antiplatelet therapy or to treatment with clopidogrel. In the genotype-guided arm, patients with a nonfunctional allele are prescribed ticagrelor, and those without a nonfunctional allele are prescribed clopidogrel. The primary outcome is composite of nonfatal myocardial infarction, nonfatal stroke, cardiovascular mortality, severe recurrent ischemia, or stent thrombosis during the 12 months following PCI. The trial is targeting 5270 patients and began in 2012, with anticipated completion in 2020.

In the Cost-effectiveness of Genotype Guided Treatment with Antiplatelet Drugs in STEMI Patients: Optimization of Treatment (POPular Genetics) trial (ClinicalTrials.gov NCT01742117), patients with ST-segment elevation myocardial infarction who undergo PCI are randomized to a genotype-guided group or control group. Patients in the genotype-guided group with a nonfunctional CYP2C19 allele are treated with prasugrel or ticagrelor, whereas clopidogrel is prescribed to patients without a nonfunctional allele. Patients in the control arm are prescribed either prasugrel or ticagrelor. The primary endpoint is death, recurrent myocardial infarction, stent thrombosis, stroke, or major bleeding at 1 year. The trial is targeting an enrollment of 2700 patients and expected to be completed in 2019.

Two smaller trials in Chinese patients also examined a genotype-guided approach to antiplatelet prescribing. In one trial, 600 patients who underwent PCI were randomized to clopidogrel without genotyping or to genotyping, with high-dose clopidogrel prescribed for IMs and high-dose clopidogrel plus cilostazol prescribed for PMs [56]. The genotype-guided group had a significantly lower risk for the composite endpoint of death, MI, or stroke at 6 months. The second trial included 628 patients and had similar treatment arms except that ticagrelor was prescribed for PMs [57]. Similar to the first trial, there was a significantly lower risk for the composite endpoint of death, MI, stroke, or target vessel revascularization in the genotype versus conventional treatment arm.

Clinical Implementation of CYP2C19-Guided Antiplatelet Therapy

Based on the strong and consistent evidence of reduced clopidogrel effectiveness in patients with a CYP2C19 nonfunctional allele, a number of institutions have starting offering CYP2C19 genotyping to assist with antiplatelet prescribing decisions for patients undergoing PCI [58–62]. Some institutions are taking a preemptive approach whereby patients undergoing cardiac catheterization or at high risk for cardiovascular events are genotyped so that genotype is readily available in the event that the patient requires PCI. Other institutions are genotyping in a more reactive manner at the time of PCI. Regardless of the approach, most institutions are following CPIC guidelines and recommending alternative antiplatelet therapy with prasugrel or ticagrelor for patients with the PM or IM phenotype. Many sites have built clinical decision support rules into their electronic health record so that in the event that clopidogrel is prescribed for a patients with a PM or IM phenotype on record, then the physician is alerted to the genotype result and risk for poor response to clopidogrel. Examples of clinical decision-support tools are available through the National Institute of Health sponsored Implementing Genomics in Practice (IGNITE) website (https://ignite-genomics.org/).

Outcome data are beginning to emerge from pragmatic and observational studies of clinical implementation of CYP2C19 genotyping. Unlike randomized controlled trials, which often have strict eligibility criteria and occur in controlled settings that limit the generalizability of results, pragmatic studies provide data in the context of routine clinical practice, thus reflecting the effectiveness of an intervention in a real-world setting and maximizing generalizability [63]. There is less control for sources of bias in pragmatic studies, and propensity score matching and other statistical techniques are often required to account for differences between treatment groups. As part of the National Institutes of Health (NIH)-funded IGNITE Network, investigators from seven institutions in the United States pooled data on cardiovascular events for over 1800 patients who underwent either emergent or elective PCI and were genotyped as part of clinical care [64]. The median time from PCI to genotype results being available across sites was 1 day, demonstrating the feasibility of providing genotype-guided antiplatelet therapy.

Alternative antiplatelet therapy was recommended for patients with one or two CYP2C19 nonfunctional alleles (e.g., IM or PMs), but the ultimate choice of antiplatelet therapy was left to the decision of the prescriber. Approximately 30% of patients had a nonfunctional allele, and 60.5% of these were prescribed alternative therapy. In contrast, 85% of patients without a nonfunctional allele were prescribed clopidogrel. After propensity scoring to account for differences between groups, the risk for major adverse cardiovascular events (defined as the composite outcome of death, myocardial infarction, or ischemic stroke) over the 12-month follow-up period after PCI was significantly higher in carriers of a nonfunctional allele prescribed clopidogrel versus alternative therapy (adjusted hazard ratio 2.26, 95% CI 1.18–4.32). In contrast, there was no difference in outcomes between carriers of a nonfunctional allele prescribed alternative therapy and those without a nonfunctional allele.

Similar results were observed in a Dutch study that included over 3200 patients who underwent elective PCI. In contrast to the U.S. study, recommendations for alternative antiplatelet therapy were confined to PMs [59]. Over the follow-up period of up to 18 months, 31% of PMs treated with clopidogrel versus 5% of PMs treated with alternative therapy had an adverse cardiovascular event ($P = .003$).

An additional study was conducted in Spain and compared outcomes between approximately 300 patients who received genotype-guided antiplatelet therapy and approximately 400 historical controls who underwent PCI prior to genotype implementation [65]. Both the CYP2C19 and ABCB1 genotypes were determined in the genotype group, with alternative therapy prescribed to those with a CYP2C19 nonfunctional allele or the ABCB1 TT genotype. Most of the patients in the control group were treated with clopidogrel. The investigators reported a significantly lower risk for cardiovascular death, MI, or stroke in the genotype group compared to historical controls.

Opportunities and Challenges With Clopidogrel Pharmacogenetics

The data supporting CYP2C19 genotype associations with clopidogrel response have accumulated to the extent that institutions have started offering genotyping as part of clinical practice. Data from small randomized controlled trials and observational and pragmatic studies demonstrate improved outcomes with a genotype-guided approach to antiplatelet therapy after PCI. However, current guidelines for the management of patients with PCI do not recommend CYP2C19 genotyping to guide antiplatelet therapy because of the lack of data from large randomized controlled trials. Data from recent pragmatic studies or the ongoing TAILOR-PCI and POPular-Genetics trials may lead to changes in future PCI guidelines, prompting adoption that is more widespread of genotype-guided antiplatelet therapy after coronary intervention.

In addition to the evidence barrier, another factor hindering genotype adoption is concern that genetic testing may disrupt workflow in the busy cardiac catheterization laboratory. The availability of rapid genetic testing helps to overcome this concern [60]. Preemptive testing, so that results are available at the time of PCI, is another approach to limiting workflow disruption [61].

WARFARIN PHARMACOGENETICS

Challenges With Warfarin

Warfarin is an oral anticoagulant indicated for the prevention and treatment of venous thrombosis and thromboembolic complications associated with atrial fibrillation or heart-valve replacement. Even with the availability of newer oral anticoagulants, warfarin remains commonly prescribed, especially for individuals unable to tolerate or afford newer agents or with indications not covered by newer agents. Although in use for over 60 years, warfarin remains a difficult drug to manage primarily because of its narrow

therapeutic index and the wide interpatient variability in the dose required to obtain optimal anticoagulation. For most indications, warfarin is dosed to achieve an international normalized ratio (INR, a measure of its anticoagulant activity) of 2–3. Failure to achieve optimal anticoagulation significantly increases the risk for adverse sequelae. Specifically, subtherapeutic anticoagulation increases the risk for thromboembolism, and supratherapeutic anticoagulation (particularly when the INR exceeds 4) increases the risk for bleeding [66,67]. Because of the difficulty in achieving therapeutic anticoagulation with warfarin, warfarin consistently ranks among the leading causes of serious drug-related adverse events, prompting a boxed warning in its FDA-approved labeling. Achieving therapeutic anticoagulation in an efficient manner is, therefore, a priority for clinicians managing warfarin therapy.

The warfarin dose required to achieve an INR within the therapeutic INR range varies by as much as 20-fold among patients [68]. There are also significant differences in warfarin-dose requirements by ancestral origin, with African Americans generally requiring higher doses and Asians requiring lower doses compared to those of European descent [69]. Thus, a major challenge with initiating warfarin therapy is predicting the dose that will produce therapeutic anticoagulation for a particular patient. Traditionally, warfarin is initiated at a similar dose for all patients, typically 5 mg/day, with the dose adjusted according to INR results. The problem with this trial-and-error dosing approach is that it often leads to overanticoagulation during the initial months of therapy when the risk for bleeding is greatest [70]. Alternatively, for patients requiring doses higher than 5 mg/day, it can delay attainment of therapeutic anticoagulation. Clinical factors, including age, body size, diet, medications that interfere with warfarin metabolism, and renal and hepatic function, influence warfarin-dose requirements [71–73]. However, clinical factors alone account for only 15%–20% of the overall variability in warfarin dose, and considering these factors alone is often insufficient to predict the dose of warfarin a patient will require [74,75].

Genes Affecting Warfarin Pharmacokinetics and Pharmacodynamics

It is widely recognized that genotype significantly influences the pharmacokinetics and pharmacodynamics of warfarin and contributes to the interpatient variability in warfarin-dose requirements [76,77]. The major genes influencing warfarin pharmacokinetics and pharmacodynamics are cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1), respectively. As shown in Fig. 6.5, CYP2C9 metabolizes the *S*-enantiomer of warfarin to the inactive 7-hydroxy warfarin protein. The *S*-enantiomer possesses approximately three to five times the anticoagulant effects of *R*-warfarin [70]. The VKORC1 gene encodes for the target site of warfarin. Specifically, warfarin inhibits vitamin K epoxide reductase complex 1

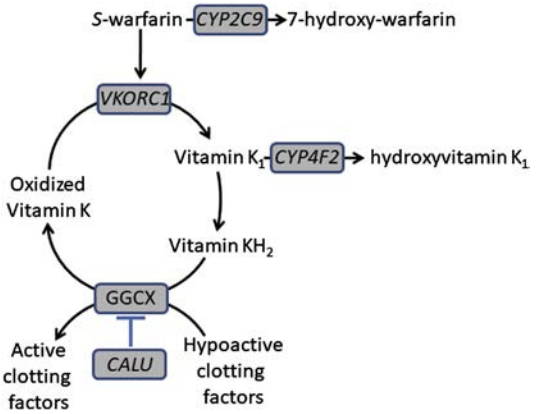

FIGURE 6.5 Genes involved in warfarin pharmacokinetics and pharmacodynamics. The CYP2C9 gene influences the drug's pharmacokinetics; other genes affect warfarin's pharmacodynamics. CYP2C9, cytochrome P450 2C9; VKORC1, vitamin K epoxide reductase complex subunit 1; GGCX, gamma-glutamyl carboxylase; CALU, calumenin; CYP4F2, cytochrome P450 4F2; vit, vitamin.

(VKORC1), thus preventing formation of vitamin K hydroquinone, a necessary cofactor for the gamma-carboxylation and activation of clotting factors II, VII, IX, and X.

Data from numerous candidate gene and GWASs consistently show that the CYP2C9 and VKORC1 genotypes affect warfarin-dose requirements [74–76,78,79–82]. There are additional data that the rs12777823 G > A polymorphism on chromosome 10 near the CYP2C18 gene influences warfarin-dose requirements in African Americans [83]. Other genes, including CYP4F2 and calumenin, produce lesser effects on warfarin pharmacodynamics and provide minor contributions to the variability in warfarin-dose requirements [84–87]. These genes are described in detail in the following sections. The major goal of warfarin pharmacogenetics is to improve the accuracy of warfarin dosing and, consequently, to reduce the risk for adverse sequelae with warfarin therapy.

CYP2C9

The CYP2C9 gene is located on chromosome 10q24.1, and approximately 60 CYP2C9 alleles have been described, as detailed in Chapter 1. The CYP2C9*2 and *3 alleles are the most extensively studied and result from variants in the coding regions of the gene, as shown in Table 6.3. The CYP2C9*2 amino acid substitution occurs on the outer surface of the enzyme, and the *3 substitution occurs internally [88,89]. Neither substitution appears to affect substrate binding. Rather, evidence suggests that the

TABLE 6.3 Nucleotide Base Pair Or Amino Acid Substitution, Location, and Minor Allele Frequencies of Variants Associated With Warfarin Dose Response in Various Populations [69,78,85,100,101,230,231]

			Minor Allele Frequency			
Polymorphism	**Base Pair or Amino Acid Substitution**	**Location**	**Caucasian**	**African American**	**Asian**	**Egyptian**
CYP2C9						
*2 (rs1799853)	p.R144C	Exon 3	0.13–0.14	0.01–0.02	<0.01	0.12
*3 (rs1057910)	p.I359L	Exon 7	0.06–0.11	0.01	0.02–0.04	0.09
*5 (rs28371686)	p.D360E	Exon 7	<0.01	0.01	<0.01	0.01
*6 (rs9332131)	10601delA	Exon 5	<0.01	0.01	<0.01	NR
*8 (rs7900194)	p.R150H	Exon 3	<0.01	0.05–0.07	0.01	<0.01
*11 (rs28371685)	p.R335W	Exon 7	<0.01	0.01–0.04	<0.01	NR
VKORC1						
rs9923231	c.1639G>A	Promoter	0.39	0.11	0.91	0.46
rs9934438	c.1173C>T	Intronic	0.40	0.10	0.90	NR
CYP4F2						
rs2108622	p.V433M	Exon 11	0.24	0.07	0.23	0.42
CALU						
rs339097	c.A>G	Intronic	<0.01	0.14	0.02	0.02

APOE, apolipoprotein E; *CALU*, calumenin; *CYP*, cytochrome P450; *VKORC1*, vitamin K epoxide reductase complex subunit 1.

CYP2C9*2 and *3 alleles disrupt formation of intermediate compounds in the CYP2C9 catalytic cycle leading to significant reductions in enzyme activity [90]. As a result, the clearance of *S*-warfarin is reduced approximately 40% with the CYP2C9*1/*2 genotype, up to 75% with the *1/*3 genotype, and up to 90% with the *3/*3 genotype [77,91–93]. Accordingly, individuals with the CYP2C9*1/*2 or *1/*3 genotypes require dose reductions of 30%–47%, respectively, compared to those with the CYP2C9*1/*1 (wild-type) genotype [77]. Individuals with the CYP2C9*3/*3 genotype need up to 80% lower warfarin doses than CYP2C9*1 homozygotes [91,92].

The CYP2C9*2 and *3 alleles are the most common variant CYP2C9 alleles in Caucasians but are much less prevalent among Asians and African Americans, as shown in Table 6.3. The CYP2C9*8 allele is one of the most common variant CYP2C9 alleles in African Americans but is virtually absent in other populations [94]. The CYP2C9 *5, *6, and *11 alleles also occur almost exclusively in African Americans but at much lower frequencies than the *8 allele. The CYP2C9*5, *8, and *11 alleles result from nonsynonymous variants in gene coding regions, whereas CYP2C9*6 results from a nucleotide deletion (Table 6.3). Decreased enzyme activity and clearance of CYP2C9 substrates have been reported with the CYP2C9*5, *6, *8 and *11 alleles [95–98]. However, allele effects appear to be substrate specific. For example, CYP2C9*8 decreases enzyme activity toward warfarin and phenytoin, increases enzyme activity toward tolbutamide, and has no effect on losartan metabolism [95,96,98,99]. The CYP2C9*8 allele decreases clearance of *S*-warfarin by 25%–30% [98]. This decrease coincides with about a 20% reduction in warfarin-dose requirements with the CYP2C9*8 allele [78]. Similarly, lower warfarin-dose requirements have been reported in individuals with a CYP2C9*5, *6, or *11 allele [78,80,100].

In addition to affecting warfarin-dose requirements, the CYP2C9 genotype is associated with the risk of overanticoagulation and bleeding during warfarin therapy [91,101,102]. Specifically, warfarin-treated patients with a CYP2C9 variant allele have about a twofold greater risk for bleeding compared to CYP2C9*1 homozygotes [101,103]. Although the risk for bleeding with a CYP2C9 variant allele is highest during the initial months of warfarin therapy, there is evidence that it persists during chronic therapy [101]. Thus, patients with a CYP2C9 variant allele should be monitored closely for signs and symptoms of bleeding throughout warfarin therapy.

VKORC1

The VKORC1 gene is located on chromosome 16p11.2 and was initially discovered in the context of warfarin resistance, in which exceptionally high doses of warfarin (e.g., >20 mg/day) are needed to achieve therapeutic anticoagulation [104]. Warfarin resistance is due to nonsynonymous (or missense) mutations in the VKORC1 coding region. Variants contributing to warfarin resistance are commonly referred to as "mutations" because they are rare in most populations. An exception is in the Ashkenazi Jewish population, in which individuals have a relatively high prevalence (8%) of the VKORC1 p.D36Y variant, leading to a higher prevalence of warfarin resistance in this population [105].

In 2005, investigators identified common VKORC1 variants, c.1639G>A (rs9923231) and c.1173C>T (rs9934438), that contribute to warfarin-dose variability across the general population [76,106]. These variants occur in VKORC1 regulatory regions and are in near complete LD [69]. In vitro studies in liver tissue showed that the -1639G>A and 1173C>T variants were associated with twofold allelic mRNA expression imbalance (e.g., twofold lower gene expression) [107]. Numerous studies have consistently demonstrated that the -1639A and 1173T alleles are associated with significantly lower warfarin-dose requirements in these populations [68,69,74,75,78,80,108–110]. On average, the -1639

AA, AG, and GG genotypes predict warfarin maintenance doses of 3, 5, and 6 mg/day, respectively. The -1639G>A and 1173C>T SNP are equally predictive of dose requirements [69]. Thus, only one of these Variants needs to be considered for warfarin-dosing decisions. This greatly simplifies genotype-guided warfarin dosing compared to dosing based on VKORC1 haplotype because only one SNP needs to be genotyped.

As shown in Table 6.3, the frequency of the VKORC1 -1639A allele differs significantly by ancestry, with a greater frequency in Asians and lower frequency in African Americans compared to Caucasians. Approximately 50% of Caucasians have the -1639AG genotype, associated with intermediate VKORC1 sensitivity and usual (i.e., 5 mg/day) warfarin-dose requirements. The -1639 AA genotype is the most common genotype in Asians and is associated with high VKORC1 sensitivity and low warfarin-dose requirements. The most common genotype in African Americans is -1639 GG, which is associated with lower VKORC1 sensitivity and high-dose requirements. The difference in VKORC1 genotype distribution among ancestral groups contributes to the higher mean warfarin maintenance dose in African Americans and lower mean dose in Asians, compared to Caucasians, independent of the effects associated with CYP2C9 genotype [69].

CYP4F2

The CYP4F2 enzyme is responsible for metabolizing vitamin K_1 to hydroxyvitamin K_1, as shown in Fig. 6.5 [111]. This process results in less vitamin K_1 being available for reduction to vitamin KH_2, which is necessary for clotting-factor activation. Thus, increased CYP4F2 activity leads to reduced clotting-factor activation. The CYP4F2 p.V433M SNP in exon 11 leads to lower CYP4F2 protein concentration and consequently to greater vitamin K availability [111].

In an initial study of three independent Caucasian cohorts, the CYP4F2 433M/M genotype was associated with approximately 1 mg/day higher warfarin-dose requirements compared to the V/V genotype, with heterozygotes requiring intermediate doses [84]. Subsequent studies in Caucasians and Asians confirmed the association between V433M genotype and warfarin-dose requirements [81,82,112–114]. The CYP4F2 V433M genotype explains approximately 1%–3% of the overall variability in warfarin dose in these populations [82,112]. Interestingly, the association between CYP4F2 genotype and warfarin-dose requirements was not observed in African Americans, Indonesians, Egyptians, or children [78,115–117]. The lack of association in African Americans is likely due to the low frequency of the 433M allele in individuals of African ancestry. However, the 433M allele is common in Indonesians and Egyptians, and the explanation for the negative association in these groups is unclear. Body size provides a greater contribution to warfarin-dose variability in children versus adults, potentially explaining the negative findings with the CYP4F2 genotype in a pediatric population.

CALU

Calumenin inhibits gamma-carboxylation of vitamin K–dependent proteins, suggesting that CALU may influence warfarin-dose requirements [118]. The CALU variant, rs339097 A>G, was associated with warfarin maintenance dose in a diverse patient cohort [87]. Specifically, the minor rs339097G allele was significantly overrepresented among patients requiring high (mean dose of 13 mg/day) versus low (mean dose of 2.6 mg/day) warfarin doses. The association between the rs339097 variant and warfarin-dose requirements was validated in a separate diverse cohort and in a cohort of African Americans [87]. In a pooled analysis of 241 African Americans, the G allele was associated with an 11% higher warfarin dose than predicted based on clinical factors, CYP2C9, and

VKORC1. The correlation of the rs339097G allele with higher warfarin doses was confirmed in a separate study of Egyptian patients, in whom the variant allele was associated with 14 mg/week higher dose requirements [116]. The rs339097G allele is common among African Americans but rare in other populations, as shown in Table 6.3.

Genome-Wide Association Studies

Several GWASs with warfarin have been completed in varying populations and confirm that the CYP2C9 and VKORC1 genes are the primary contributors to warfarin-dose requirements [79,81,82]. In an initial GWAS, investigators surveyed over 538,000 Variants in a discovery cohort of 181 Caucasians and 2 independent replication cohorts consisting of 374 Caucasians taking warfarin [79]. An SNP in complete LD with the VKORC1 -1639G>A variant had the most significant effect on warfarin dose in the index population and explained approximately 25% of the overall variance in dose requirements. The CYP2C9*2 and CYP2C9*3 alleles provided modest contributions to warfarin dose and explained an additional 9% of the variability. These associations were validated in the replication cohort. The combination of VKORC1, CYP2C9, and clinical factors (age, sex, weight, amiodarone use, and losartan use) explained 47% of total variance in warfarin maintenance dose [79].

In a second GWAS, over 325,000 variants were tested for their association with warfarin dose in 1053 Swedish patients [82]. Similar to the first GWAS, the VKORC1 locus had the strongest association with warfarin dose, followed by variants clustered around CYP2C9. After adjustment for VKORC1, CYP2C9, age, and gender, the only other SNP reaching genome-wide significance with warfarin dose was CYP4F2 V433M, which explained an additional 1%–2% of the variability. Results were confirmed in a replication cohort of 588 Swedish patients.

A third GWAS was conducted in Japanese patients [81]. Similar to the studies in Caucasians, VKORC1 was found to provide the greatest contribution to warfarin maintenance dose, with CYP2C9 and CYP4F2 providing lesser contribution.

A fourth GWAS was conducted in African Americans and identified a novel association between the rs12777823G>A polymorphism in the CYP2C cluster on chromosome 10 and warfarin-dose requirements [83]. In addition, to its association with lower warfarin-dose requirements, the rs12777823A allele was correlated with lower *S*-warfarin clearance. Approximately 40% of African Americans carry the rs12777823A allele. Although it is also common in Europeans and Asians, the rs12777823G>A polymorphism has not been associated with warfarin-dose requirements in these populations, suggesting that it does not directly influence warfarin response, but rather may be in linkage disequilibrium with a functional variant or variants influencing warfarin response in African Americans.

Warfarin Pharmacogenetics Dosing Algorithms

There are a number of published algorithms to assist clinicians with warfarin dosing when genotype is known [74,75,109,119–124]. Most contain the VKORC1 -1639G>A or 1173C>T SNP, CYP2C9 *2 and *3 alleles, and clinical factors, including age, body size, and amiodarone use. The two algorithms derived from the largest populations and most commonly cited are those by the International Warfarin Pharmacogenetics Consortium (IWPC) [75] and by Gage and colleagues [74]. The latter is commonly referred to as the warfarindosing.org algorithm. It was derived from a population of 1015 warfarin-treated patients, 83% of whom were Caucasian, and validated in 292 patients with similar characteristics. The IWPC was formed by members of the Pharmacogenomics Knowledge Base (PharmGKB) in collaboration with investigators from the international community with the initial purpose of creating a dosing equation that would

have global clinical utility (see Chapter 3 for further information about the PharmGKB) [75]. Researchers from 21 groups representing 11 countries and four continents pooled genotype and phenotype data for over 5000 chronic warfarin-treated patients (55% Caucasian, 30% Asian) [69,75]. Data from 4043 patients were used to derive the IWPC algorithm, with validation in the remaining 1009 patients.

The Gage et al. and IWPC algorithms include clinical factors and the CYP2C9*2 and *3 and VKORC1 -1639G>A genotypes and provide similar dose estimations. Both are freely available through the www.warfarindosing.org website. The algorithm available through the www.warfarindosing.org. Website allows for refinement of dose estimation based on INR response to previous warfarin doses and thus may be preferred over the use of other algorithms when genotype results are not immediately available [125].

The Gage et al. and IWPC algorithms explain between 30% and 60% of the variability in warfarin dose requirements in Caucasians but less of the variability in African Americans and Asians [69,123]. They are superior to other dosing methods, especially for patients requiring low (≤3 mg/day) or high (≥7 mg/day) warfarin doses [75,126]. However, warfarin pharmacogenetic algorithms have several limitations. First, they estimate doses within 20% of the actual dose only about 50% of the time [127–129]. Pharmacogenetic algorithms do not include all of the factors known to affect warfarin-dose variability, such as vitamin K intake and many of the drugs known to interact with warfarin. In addition, most algorithms, including the www.warfarindosing.org and IWPC algorithms, do not contain genetic variables that are common or specifically affect dose in African Americans (e.g., CYP2C9*8, rs12777823), likely contributing to lesser accuracy in this population [129,130]. Also, many algorithms do not include genetic variants associated with warfarin resistance and are thus less accurate at predicting higher than usual doses [131]. Finally, pharmacogenetic algorithms may overestimate doses in elderly patients (>65 years) who often require warfarin doses of less than 2 mg/day [132]. As such, pharmacogenetic algorithms are useful to reduce uncertainty about initial warfarin doses. However, they should not replace routine INR monitoring and clinical judgment.

Warfarin Labeling Revisions

In August 2007, the FDA approved the addition of pharmacogenetic data to the warfarin labeling. The pharmacogenetic content of the label was further revised in January 2010, with the addition of a dosing table based on the CYP2C9*2 and *3 alleles and VKORC1 genotypes (Fig. 6.6). The table may be used to estimate initial warfarin dose when genotype is known, with subsequent dose adjustment based on INR results. An advantage of the table over dosing algorithms is its ease of use. However, it does not include clinical factors that influence dose variability and has been shown to be less accurate at predicting warfarin-dose requirements compared to pharmacogenetics algorithms [126].

Early Studies of Genotype-Guided Warfarin Dosing

A comparative effectiveness study showed that patients who were offered free CYP2C9 and

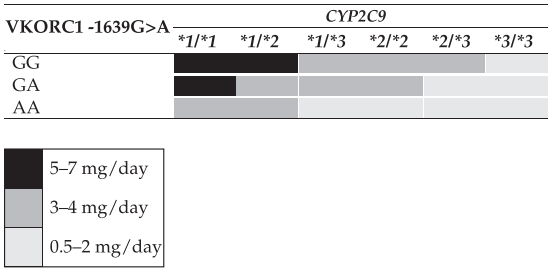

FIGURE 6.6 Warfarin dose by CYP2C9 and VKORC1 genotypes, as reproduced from the FDA-approved warfarin label.

VKORC1 genotyping, with results provided to their physician with an interpretive report, had fewer hospitalizations for any cause and fewer hospitalizations for bleeding or thromboembolism during the initial 6 months of warfarin therapy compared to historical controls [133]. In contrast, two small, randomized trials showed no benefit with a genotype-guided approach over traditional dosing [131,134]. In particular, both trials showed that the percent of time spent within the therapeutic range, which is often used as a marker of bleeding or thrombotic risk, was similar between patients dosed based on genotype plus clinical factors or clinical factors alone. However, these trials were small in size, including only 206 to 230 patients. In addition, an exploratory analysis of one trial, called the CoumaGen-I trial, showed a benefit with pharmacogenetic dosing for two groups of patients: those with more than one variant allele and those with the wild-type genotype (VKORC1 -1639 CC and CYP2C9 **1/*1*) [131]. In contrast, single-variant allele carriers appeared to have no benefit from genotype-guided dosing, likely because patients with a single variant usually require a warfarin dose of about 5 mg/day, which is the dose commonly started in patients new to warfarin. In contrast, those with multiple variant alleles usually require lower doses (e.g., 3–4 mg/day), and those with the wild-type genotype usually require higher doses (6–7 mg/day). Thus, starting a dose of 5 mg/day in individuals with multiple or no variant allele would probably result in over- and undercoagulation, respectively.

The subsequent CoumaGen-II involved (1) a blinded, randomized comparison of two pharmacogenetic dosing algorithms; and (2) a clinical effectiveness comparison of genotype-guided warfarin dosing ($n = 504$) versus standard dosing ($n = 1911$) [135]. For the comparison of dosing algorithms, a modified version of the IWPC algorithm (taking into account smoking status and different INR targets) was compared to a three-step algorithm in which the CYP2C9 genotype was not taken into account until day 3, and a dose-revision algorithm was used starting on day 4, taking into account warfarin dosing history and INR. The three-step algorithm was found to be noninferior, but not superior, to the modified IWPC algorithm in terms of the percent of out-of-range INR values at one and 3 months. Thus, the two pharmacogenetic dosing approaches were combined for comparison with standard dosing. Genotype-guided therapy (using either algorithm) was superior to standard warfarin dosing in reducing the percent of out-of-range INRs and the percent of INRs greater than or equal to 4 or less than or equal to 1.5. An additional analysis suggested that there were fewer serious adverse events at 3 months in the genotype-guided arm.

Large Randomized Clinical Trials of Genotype-Guided Warfarin Therapy

Two multicenter, randomized trials assessing the clinical efficacy of genotype-guided warfarin dosing were published in 2013 with differing results. The details of these trials are shown in Table 6.4. The European Pharmacogenetics of Anticoagulation Therapy (EU-PACT) trial was conducted in a homogenous European population and randomized participants to genotype-guided warfarin dosing, with use of a pharmacogenetic algorithm, or to a traditional fixed-dose approach (e.g., 5 mg/day) [136]. The trial showed significantly greater time in therapeutic range, the primary endpoint, with use of a pharmacogenetics algorithm. The Clarification of Optimal Anticoagulation through Genetics (COAG) trial was conducted in a diverse U.S. population and randomized participants to dosing with a pharmacogenetics algorithm, including both genotype and clinical factors or to dosing with a clinical algorithm, containing clinical factors only [137]. In contrast to the EU-PACT trial, the COAG trial showed no difference in the primary endpoint of time in therapeutic range between dosing strategies. Among African

TABLE 6.4 Clinical Trials that Assessed the Clinical Utility of Genotype-Guided Warfarin Dosing

Trial Name or Acronym	Intervention	Outcomes	Study Population	Findings
Clarification of Optimal Anticoagulation Through Genetics (COAG) [137]	Genotype guided dosing with a pharmacogenetics dosing algorithm (including the CYP2C9*2 and *3, VKORC1 -1639G>A) variants versus clinically guided dosing with a dosing algorithm including clinical factors only	Time spent within the therapeutic INR range in the first 4 weeks	n = 1015 patients	Mean percent of time in range was 45.2% in the genotype-guided group and 45.4% in the clinically-guided group ($P = .91$) Among blacks, the mean time in range was 35.2% in the genotype-guided group versus 43.5% in the clinically guided group ($P = .01$).
European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) [136]	Genotype-guided warfarin dosing, with use of a pharmacogenetic algorithm (including the CYP2C9*2 and *3, VKORC1 -1639G>A> variants) versus standard dosing consisting of 10 mg on day 1 (5 mg for patients older than 75 years), 5 mg on days 2 and 3, then dosing according to usual practice	Percent of time in an INR range of 2.0–3.0 during the initial 12 weeks	n = 455 patients (61% male, 98.5% white, mean age 67 years)	Primary: Mean percent of time in therapeutic range was 67.4% in the genotype-guided group and 60.3% in the control group ($P < .001$)
Genetics Informatics Trial (GIFT) of Warfarin to Prevent DVT [139]	Genotype guided dosing with a pharmacogenetic dosing algorithm (including the CYP2C9*2 and *3, VKORC1 -1639G>A>, and CYP4F2 Val 433Met variants) versus clinically guided dosing with a dosing algorithm including clinical factors only	Primary: Composite of death, venous thromboembolism, major bleeding, and INR ≥ 4 during the initial 4–6 weeks	N = 1597, age ≥65 years, 64% women, 91% Caucasian, undergoing elective knee or hip replacement surgery	Event rate was 14.7% in the clinical arm and 10.8% in the genotype-guided arm, representing a 27% reduction in the primary endpoint with genotype-guided dosing

DVT, deep vein thrombosis; *GI*, gastrointestinal; INR, international normalized ratio; *PE*, pulmonary embolism.

Americans, who composed 27% of the population, pharmacogenetic dosing resulted in less time in therapeutic range and more time with an INR over 3. The occurrence of INRs ≥4 and the composite secondary outcome of any INR ≥4, major bleeding, or thromboembolism was similar between groups in the population overall as well as in the African American subset. Although there was no difference between groups in the individual secondary endpoint of major bleeding, there were numerically more bleeds in the clinically dosing arm, and the difference between groups was statistically significant at 6 months (4% vs. 1%, $P = .021$).

There were several differences between the two trials that might have contributed to the disparate results, including differences in the comparator arm, differences in patient populations, and lack of a loading dose in the COAG trial. Dosing in the genotype-guided arm of both trials was based on the CYP2C9*2, *3, and VKORC1 -1639G>A genotypes. These are the primary genotypes influencing warfarin dose in persons of European ancestry, and thus appropriate for the EU-PACT trial. Additional variants, namely the CYP2C9*5, *6, *8, *11 and CYP2C rs12777823 variants, contribute to warfarin-dose requirements in African Americans. Data published since the COAG trial show that failure to account for these variants leads to significant overprediction of warfarin doses in African Americans, likely contributing to the greater likelihood of supratherapeutic anticoagulation with genotype-guided dosing among African American participants of the COAG trial [130]. Additional data suggest that loading doses may be especially important to efficiently attain therapeutic anticoagulation for patients with one or no genetic variants associated with decreased warfarin-dosing requirements [138]. Most patients of European or African ancestry would fall into this category. Thus, failure to use loading doses in the COAG trial may have contributed to the inability to detect differences in time in therapeutic range in the initial weeks of therapy.

The primary endpoint for both the EU-PACT and COAG trials was time in therapeutic range, which is a surrogate marker for risk of venous thromboembolism or bleeding (Table 6.4). In contrast, the more recent Genetics InFormatics Trial (GIFT), which enrolled more patients than the EU-PACT and COAG trials combined, was powered to examine venous thromboembolism and major bleeding with genotype-guided dosing [139]. GIFT included older patients requiring prophylaxis for venous thromboembolism after hip or knee arthroplasty. Similar to the COAG trial, participants in GIFT were randomized to dosing with use of a pharmacogenetics versus clinical algorithm. Patients in the pharmacogenetic dosing arm spent significantly more time in the therapeutic INR range through the first 4 weeks of therapy. The investigators reported a 27% reduction in the composite endpoint of venous thromboembolism, major bleeding, INR ≥4, and death with pharmacogenetic versus clinical dosing. This was driven mainly by a reduction in supratherapeutic INR values with pharmacogenetic dosing. Genotyping in GIFT was similar to that for the COAG and EU-PACT trials with the addition of the CYP4F2 Val433Met genotype. The majority of GIFT participants (91%) were of European ancestry, and thus the exclusion of CYP2C variants common in African Americans unlikely had a significant impact on the results. However, refinement of warfarin-dosing algorithms through the inclusion of additional variants influencing dose requirements across populations would be expected to further improve dosing accuracy and hence clinical outcomes with genotype-guided dosing.

Pharmacogenetic Guidelines

The CPIC guidelines for dosing warfarin based on genotype were originally published in 2011, and were updated in 2017 to reflect the more recent data with genotypes important for African Americans [121]. Similar to CPIC guidelines for clopidogrel, the warfarin CPIC guidelines do not address when to order genotyping, leaving that to the discretion of the clinician. Based on the strong and consistent evidence that genotype influences warfarin-dose requirements, the guidelines recommend dosing warfarin based on genotype when appropriate genotype information is available. Separate recommendations are provided for patients of African and non-African ancestry, as outlined in Fig. 6.7. For those of non-African ancestry, the recommendation is to dose warfarin based on the VKORC1 -1639G>A (or 1173C>T), CYP2C9*2, and CYP2C9*3 genotypes using one

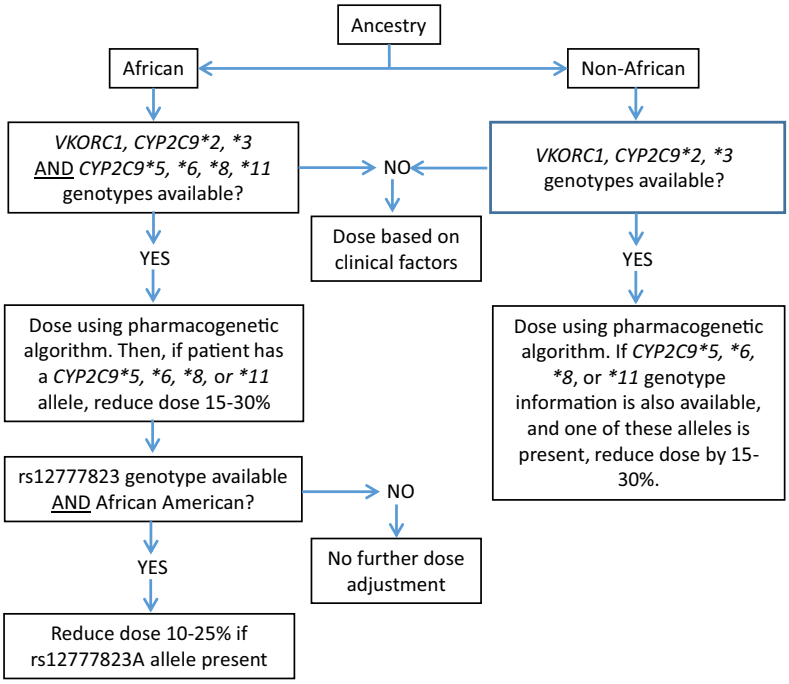

FIGURE 6.7 Clinical Pharmacogenetics Implementation Consortium Guidelines for genotype-guided warfarin dosing.

of the published pharmacogenetic dosing algorithms. Recognizing the importance of the CYP2C9*5, *6, *8, and *11 variants in persons of African ancestry, the recommendation for this population is to only use genotype information to dose warfarin when these additional variants are also tested. Otherwise, the recommendation is to dose warfarin based on clinically factors alone. For African Americans, an additional dose reduction is recommended when genotyping includes the rs12777823G>A variant, and the rs12777823A allele is present.

Opportunities and Challenges for Warfarin Pharmacogenetics

Genotype-guided warfarin dosing has the potential to improve time to reach therapeutic anticoagulation and reduce the risk for adverse events during the warfarin initiation period. Thus, despite the inconsistent clinical trial data, some institutions have started to offer genotyping to guide warfarin initiation based on the large body of evidence supporting genetic associations with warfarin-dose requirements and bleeding risk [58,140]. However, several challenges remain that limit broader initiation at present. One of the biggest challenges is the lack of reimbursement for genetic testing by most insurers. In particular, the Centers for Medicare and Medicaid Services has announced that coverage for genetic testing to guide warfarin therapy would be denied unless testing is provided in the context of a controlled clinical study. Whether recent outcomes from GIFT will alter this position remains to be determined. Cost-effectiveness data are important for policy decisions, and studies have demonstrated the cost-effectiveness of genotype-guided warfarin dosing in the setting of atrial fibrillation [141]. In cases in which genotyping is done during hospitalization, which is often for patients newly

starting warfarin, coverage for the cost of testing may fall to the hospital. In these cases, it may be important to demonstrate the benefits of genotyping to hospital administrators, in terms of effects on time to therapeutic INR or clinical outcomes (e.g., bleeding or thrombotic events). This is especially important during the initial 30 days following discharge when hospitals may not be reimbursed for readmissions under the Center for Medicare and Medicaid Services Hospital Readmissions Reduction Program.

Another challenge is obtaining timely genotype results, ideally before the first dose of warfarin. This would require either rapid genotyping or preemptive genotyping ahead of the need for warfarin. With a preemptive approach, genotype results may be placed in the medical record so that they are available in the event that warfarin is needed. Another approach that has been used is to base the initial warfarin dose on clinical factors alone, and then obtain genotype results prior to the second dose [140].

TRIALS AND TRIBULATIONS OF PHARMACOGENETICS OF AGENTS USED TO TREAT DYSLIPIDEMIA

Overview of Statin Pharmacokinetics and Pharmacodynamics

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, are commonly prescribed to reduce low-density lipoprotein (LDL) cholesterol. Multiple randomized, placebo-controlled, clinical trials have demonstrated that statins reduce the relative risk of major coronary events [142]. However, there is substantial variability in LDL cholesterol lowering and clinical outcomes with statin therapy [143,144]. In addition, although these medications are well tolerated, a small percentage of patients can experience the serious adverse event of rhabdomyolysis. Candidate genes associated with the pharmacokinetics and pharmacodynamics of statins have been studied for their contribution to this variability.

Table 6.5 shows the various enzymes involved in statin transport and metabolism. The CYP3A4 enzyme plays an important role in the metabolism of lovastatin, simvastatin, and atorvastatin; fluvastatin and rosuvastatin are metabolized primarily by CYP2C9 [145]. Pravastatin is primarily eliminated unchanged in the feces and urine, and pitavastatin is a substrate for UGT1A3 and UGT2B7. Most statins are transported by OATP1B1 into hepatocytes, in which they are competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme involved in cholesterol synthesis. All statins share this uniform mechanism of action.

Pharmacogenetics of Statin Safety

Statins are generally well tolerated but can facilitate myopathies in some individuals, with symptoms ranging from mild myalgias to life-threatening rhabdomyolysis. In clinical trials, the reported incidence of statin-associated myalgias is 3%–5%, with greater risk with the use of high-dose statin therapy [146]. Fatal rhabdomyolysis is rare, occurring in an estimated 1.5 patients per 10 million prescriptions [146].

The mechanism underlying statin-associated myopathies is unknown but likely is related to increased statin concentrations [146]. Statin concentrations are affected by extensive first-pass uptake into hepatocytes and the rate of metabolism by hepatic CYP450 enzymes. Hepatic uptake appears to be necessary for statin clearance. Genetic variants for hepatic uptake and statin metabolism have been associated with altered statin concentrations and risk for myopathy [145].

The strongest genetic association with statin-induced myopathy has been detected with genes involved in statin hepatic uptake. Statins are transported into hepatocytes by OATP1B1, which is encoded by the SLCO1B1 gene. Organic anion transporting polypeptides or solute carrier

TABLE 6.5 Drug Metabolizing Enzymes and Transporter Proteins for Various Statins

Statin	Metabolizing CYP450 Enzymes	Active Metabolite	Transporter Proteins
Atorvastatin	3A4, 3A5, 7A1	Yes	OATP1B1, ABCG2
Fluvastatin	2C9, 3A4, 2C8	No	OATP1B1, ABCG2
Lovastatin	3A4, 3A5, 2C8	Yes	OATP1B1, ABCB1
Pitavastatin	2C9		OATP1B1, ABCB1
Pravastatin	None	No	OATP1B1, ABCB1, ABCG2, ABCC2
Rosuvastatin	2C9, 2C19	Yes	OATP1B1, ABCG2
Simvastatin	3A4, 3A5, 2C8	Yes	OATP1B1, ABCB1, ABCG2

Approximately 10% of rosuvastatin is metabolized by CYP2C.

organic (SLCO) anion transporters are vital for drug uptake into tissues and organ systems. These transporters are found in the liver, intestine, and central nervous system. All statins are transported by this mechanism into hepatocytes.

A genome-wide analysis in participants of the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) study demonstrated an association between SLCO1B1 genotype and myopathy risk with statin therapy [147]. More than 300,000 variants were genotyped in 85 patients who developed confirmed myopathy (cases) and 90 patients who did not develop myopathy (controls) during treatment with simvastatin 80 mg/day. The only variant reaching genome-wide significance for association with statin-induced myopathy was rs4363657, a noncoding SNP located within the SLCO1B1 gene on chromosome 12. The rs4363657 variant was in near complete LD with the nonsynonymous rs4149056 (c.521T>C, p.V174A) variant. The odds ratio for myopathy was 4.5 (95% CI: 2.6 to 7.7) with a single rs4149056C allele and nearly 17 (95% CI: 4.7 to 61) with the CC versus TT genotype. In a replication cohort of patients who received simvastatin 40 mg/day as part of the Heart Protection Study, rs4149056 remained associated with statin-induced myopathy (OR: 2.6, 95% CI: 1.3 to 5.0).

The haplotypes containing the SLCO1B1 521C allele include SLCO1B1*5, **15* and **17* [148]. The 521C allele is associated with low OATP1B1 activity and increased plasma concentrations of relevant substrates [148]. Consistent with previous data, in a study of patients receiving atorvastatin, simvastatin, or pravastatin, SLCO1B1*5 was associated with increased adverse effects from statins, defined as statin discontinuation for any side effect, myalgia, or creatinine kinase greater than three times the upper limit of normal [149]. The association between SLCO1B1*5 and statin-induced myopathy was further validated in two additional studies [150,151]. Data from one of these studies suggest the association may be stronger for simvastatin than atorvastatin [150]. In contrast, there is little evidence that SLCO1B1 rs4149056 is associated with myopathy for pravastatin or rosuvastatin [149,152].

The CPIC published guidelines related to simvastatin dosing when SLCO1B1 genotype results are available [148]. Regardless of genotype, the simvastatin 80-mg dose should be avoided. For heterozygous (CT genotype) and homozygous variant carriers (CC genotype), CPIC recommends using a lower simvastatin dose or considering an alternative statin (e.g., pravastatin or rosuvastatin) and considering routine creatine kinase (CK) surveillance. The guidelines also states that factors other

than genotype are implicated in statin-induced myopathy and should be considered. These factors include increased statin dose, advanced age, small body-mass index, female gender, metabolic comorbidities (e.g., hypothyroidism), intense physical exercise, and Asian or African ancestry.

Variants in other transporter genes have also been found to be associated with statin-induced myopathy. A ABCC2 variant was associated with simvastatin discontinuation and dose reduction [153]. There is also a theoretical role for ABCB1 in statin myopathy. In addition, some genetic variants in the CYP450 system have been associated with statin-induced myopathy, but this relates specifically to the metabolic pathway of each statin [154].

The role of genetics in antibody-mediated myopathy with statin therapy has also been evaluated [154–156]. This form of myopathy is far less common but does persist even after statins have been discontinued. The HLA Class II DRB1*11:01 allele was associated with this unique form of myopathy; however, it is unclear if statin exposure is the sole trigger for this disease state. Until further evidence is available, genotyping to predict this unique form of statin myopathy is not recommended.

Genetic Contributors to Plasma Lipid Levels

Plasma lipid levels are highly heritable traits, with over 50% of the interindividual variation in LDL cholesterol levels attributed to genetic factors [157]. Mutations in single genes with severe functional consequences contribute to Mendelian lipid disorders (also referred to as familial hypercholesterolemia); polymorphisms in multiple genes, each with fairly weak to moderate effects, contribute to variation in lipid levels across the general population. Among the most notable discoveries from Mendelian studies were genetic mutations in the LDL receptor that cause significantly elevated LDL cholesterol and premature coronary heart disease [158]. Information about variants in the LDL receptor gene and other variants associated with Mendelian lipid disorders are included in the product labeling for some statins (Table 6.1).

As evidence of multigenic contributions to cholesterol levels across the population, a large GWAS examining approximately 2.6 million variants in over 100,000 individuals identified variants at 95 loci associated with lipid levels [159]. In addition, to genotype, lipid levels are also affected by lifestyle, diet, and other environmental factors, thus underscoring the complexity of dyslipidemia [160]. This complexity renders it difficult to identify the genetic factors that influence statin response.

Pharmacogenetics of Statin Efficacy

Given the important role of statins in reducing cardiovascular disease risk, pharmacogenetic studies of statins are plentiful. The majority of data are related to statin efficacy. There are two major outcomes in these studies: LDL cholesterol lowering or clinical event risk lowering with statin therapy. The efficacy-related studies follow either a candidate-gene approach (single or multiple genes) or GWAS. There are several plausible candidate genes that have been well studied for their role in statin response. These include genes encoding for HMG-CoA reductase (HMGCR), the target of statin therapy; apolipoprotein E (ApoE), which transports cholesterol through the bloodstream; and organic anion transporting polypeptide 1B1 (OATP1B1), which transports statins to the liver [161–167]. However, the data with these genotypes are inconsistent. In addition, a large metaanalysis of GWASs was published [168]. The authors analyzed two separate cohorts of patients from randomized controlled trials and observational studies of statin therapy via two steps of genome-wide analysis. The first and second cohorts included 18,596 and 21,975 patients, respectively. Metaanalysis of the first cohort

found three loci with 13 variants that reached genome-wide significance ($P < 5 \times 10^{-8}$) for association with low-density lipoprotein cholesterol (LDL-C) response to statin treatment. The three loci were in the genes encoding apolipoprotein E (ApoE), lipoprotein (a) (LPA), and the rapamycin-insensitive companion of mammalian target of rapamycin (mTOR) (RICTOR). The association with APoE and LPA loci persisted in the second cohort and two new loci (SORT1/CELSR2/PSRC1 and SLCO1B1) were detected. The authors also performed a genome-wide conditional analysis of these polymorphisms to detect combined effects. They found 14 variants that were independently associated with LDL-C response to statin therapy including those from LPA, APoE, SLCO1B1, and SORT1/CELSR2/PSRC1. Those 14 variants explained approximately 5% of the variability in LDL-C response to statin therapy. The majority of genes identified in this study were associated previously with statin efficacy. These results further underscore that variation in LDL-C reduction from statin therapy is genetically complex.

Despite the many studies assessing the pharmacogenetics of statin responsiveness, no concrete genotype associations with statin efficacy have been made. There are several reasons why genetic association studies with statins are difficult. First, each statin has its own specific metabolic process. Therefore, genetic variation in a particular metabolizing enzyme will not affect response to all statins. In addition, baseline lipid levels are affected by many factors beyond genetics. Thus, the effect of statin therapy on lipid levels is laid over the backdrop of an already complex physiology. Because each study assesses a different statin and a different patient population, with varying underlying pathophysiologies, it is difficult to find genotypes that consistently affect statin response. A composite of variants from several genes and clinical factors, each explaining some small portion of statin response, will likely be necessary to truly predict statin response.

Ezetimibe Pharmacogenetics and NPC1L1 Genotype

Ezetimibe lowers LDL-C by blocking the Niemann–Pick C1-like 1 (NPC1L1) intestinal cholesterol transporter. The first genetic association reported with ezetimibe was in a treatment-resistant patient who was found to have rare nonsynonymous NPC1L1 gene mutation [169]. The gene was subsequently sequenced in additional patients, and 140 variants and five insertion/deletion polymorphisms were identified.

Multiple studies have assessed the association between NPC1L1 genotype and LDL-C response to ezetimibe. The first study found a haplotype, consisting of three variants (1735C, 25342A, and 27677T), associated with the percent of LDL-C reduction from baseline [170]. Specifically, subjects possessing at least one copy of the NPC1L1 haplotype had smaller LDL cholesterol reduction from baseline with ezetimibe ($-23.6 \pm 1.6\%$ vs. $-35.9 \pm 4.0\%$, $P < .01$). The second study also used three NPC1L1 variants to create haplotype groups, albeit different variants from the previous study [171]. They found that possession of the haplotype −133A/-18A/1679G was associated with greater ezetimibe-induced LDL-C lowering. However, because each study found different NPC1L1 variants and haplotypes to be associated with ezetimibe response, it is yet unclear which polymorphism(s) is actually underlying altered LDL-C response. In addition, there were impressive differences in the allele frequencies for studied variants by ancestral origin. Thus, whether ancestral differences exist in the genotype–ezetimibe response association is unclear.

Other variants in NPC1L1 have been associated with baseline cholesterol absorption and lipid profile [172,173]. The exons of NPC1L1 were resequenced in 7364 patients with coronary heart disease and 14,728 controls of varied ancestry, and 15 distinct variants were identified [173]. Heterozygous carriers of inactivating mutations had a mean LDL-C level that was

12 mg/dL lower than noncarriers, which was a statistically significant difference. In addition, carrier status was associated with a relative reduction in coronary heart-disease risk of 53%. This makes it difficult to distinguish between the baseline and pharmacogenetic effects of these variants. Another group of investigators discovered similar associations between NPC1L1 genotype and cardiovascular events that persisted after controlling for total cholesterol, LDL-C, and other cardiovascular risk factors [174].

At this time, because of these issues, regular genotyping for NPC1L1 polymorphisms to predict ezetimibe response cannot be recommended. In addition, as discussed with statins, lipid homeostasis involves several pathways with many different genes. Therefore, a polygenetic approach will likely be necessary to assess ezetimibe response.

Opportunity in Pharmacogenetics: Potential to Improve Management of Dyslipidemia

In clinical trials, statins have been shown to reduce the risk for adverse cardiovascular events in patients with established cardiovascular disease as well as those at high risk for cardiovascular disease [8]. However, not all patients derive protection against cardiovascular events with statins, and some patients experience intolerable (e.g., myopathy) and potentially life-threatening (e.g., rhabdomyolysis) adverse effects. Pharmacogenetics offers the potential to identify patients who will either not benefit from statin therapy or who are at high risk for experiencing adverse statin-induced effects, in whom a statin may be avoided. At present, the evidence most strongly supports a genetic determinant of adverse statin-induced effects for simvastatin (e.g., SLCO1B1 genotype and statin-induced myopathy), and several institutions have implemented SLCO1B1 genotyping into clinical practice, either as a standalone test or as part of a comprehensive genotype panel, to predict risk for simvastatin-induced myopathy [175,176].

TACROLIMUS PHARMACOGENETICS

Tacrolimus is a widely prescribed immunosuppressant indicated after solid organ transplant, including heart transplant. Tacrolimus has a narrow therapeutic index, with subtherapeutic blood concentrations increasing the risk for organ rejection and supratherapeutic concentrations increasing the risk for hypertension, nephrotoxicity, and other adverse drug effects. CYP3A4 and CYP3A5 are involved in the metabolism of tacrolimus, and CYP3A5 genotype has been consistently associated with variability in tacrolimus blood concentrations [177].

The CYP3A5*3 allele is a nonfunctional allele that creates an aberrant splice site in intron 3. Approximately 85% of individuals of European ancestry are homozygous for the *3 allele and have no CYP3A5 activity. These individuals are deemed CYP3A5 nonexpressers. However, individuals with the CYP3A5 *1/*1 or *1/*3 genotype are CYP3A5 expressers, with partial to full CYP3A5 activity. African Americans and Asians are more likely than Caucasians to be CYP3A5 expressers.

Following FDA-label recommended dosing of tacrolimus, lower blood concentrations have been reported in CYP3A5 expressers compared to nonexpressers, placing expressers at an increased risk for organ rejection [177]. Although no data are available in heart-transplant recipients, a randomized controlled trial in kidney-transplant recipients showed that a genotype-guided approach to tacrolimus dosing with higher doses started in individuals with the CYP3A5 *1/*1 or *1/*3 genotype, decreased time to achieve therapeutic drug concentrations compared to a traditional (nongenotype-guided) dosing approach [178]. CPIC guidelines addressing tacrolimus dosing based on CYP3A5 genotype were published in 2015 and recommend increasing the tacrolimus dose by 1.5–2 times the label recommended dose in CYP3A5 expressers [177]. However, the total daily dose should not exceed 0.3 mg/kg day

given the risk for serious adverse effects with supratherapeutic concentrations.

PHARMACOGENETICS OF ANTIHYPERTENSIVES

Hypertension is the most common chronic disease in the United States, affecting more than 85 million Americans [1]. Thus, agents to treat hypertension are among the most commonly prescribed drugs in the United States and other countries. There is significant interpatient variability in response to antihypertensive agents, and factors underlying this variability are not well understood [179,180]. Clinicians currently treat hypertension with a largely trial-and-error approach. It is often necessary to try several agents or combinations of agents before achieving adequate blood pressure control with acceptable tolerability for a given patient. The ability to predict antihypertensive response may allow for earlier initiation of effective antihypertensive therapy, thus reducing the time to adequate blood pressure control and potentially reducing the risk for adverse sequelae from prolonged untreated hypertension. In addition, it may also help to decrease adverse event risk with antihypertensive therapy. With this idea in mind, a number of investigators are searching for genetic determinants of antihypertensive responses. However, in contrast to pharmacogenetic data with warfarin and clopidogrel, pharmacogenetic data with antihypertensives are often inconsistent and even conflicting, which is particularly true with agents that antagonize the renin–angiotensin system. Thus, the potential for improving blood pressure control with pharmacogenetics is largely unrealized.

The International Consortium for Antihypertensive Pharmacogenomics Studies (ICAPS) was formed in 2012 to assist in replication of previously identified genetic variants and the discovery of new variants [179]. ICAPS includes 29 cohorts with more than 345,000 participants from 22 different research groups based in 10 countries on three continents. The work from this group is facilitating the identification and validation of pharmacogenetic markers in hypertension, and some of the data will be summarized in this section.

The following section discusses only the most consistently replicated genetic associations with blood-pressure-lowering effects with antihypertensive agents. In addition, emerging data on genetic determinants of clinical outcomes and adverse drug effects with antihypertensive agents will be discussed.

Genetic Determinants of β-Blocker Response

β-blockers are indicated for the treatment of a number of cardiovascular disorders, including hypertension, coronary artery disease, heart failure, and cardiac arrhythmias. Many β-blockers are metabolized to some degree by CYP2D6 to inactive metabolites, and all β-blockers exert their therapeutic effects by primarily antagonizing the β1-adrenergic receptor, encoded by the ADRB1 gene. Both the CYP2D6 and ADRB1 genes are highly polymorphic and can have significant effects on β-blocker plasma concentration and therapeutic effects, respectively [179,181].

Metoprolol is the β-blocker most extensively metabolized by the CYP2D6 enzyme. A description of the CYP2D6 genetic variants is provided in Chapter 1. The clinical relevance of alterations in β-blocker plasma concentration due to CYP2D6 polymorphism is questionable, given that β-blockers have a wide therapeutic index. Nonetheless, investigators have reported a higher risk of adverse effects with β-blocker therapy among CYP2D6 poor metabolizers compared to normal metabolizers, with normal CYP2D6 function [182]. Specifically, in a cohort of more than 700 metoprolol users, the PM phenotype was associated with a significantly lower heart rate and diastolic blood pressure and a nearly fourfold higher risk of bradycardia compared to the normal metabolizer phenotype [182].

Common variants in the ADRB1 gene, p.S49G and p.R389G, have been correlated with blood pressure lowering effects of β-blocker therapy. These variants are in strong LD, as described in detail in Chapter 1. The R389 allele has been associated with hypertension in multiple large studies [180]. In addition, the majority of studies have shown greater blood pressure reduction with β-blocker therapy with the homozygous RR389 genotype and the S49-R389 haplotype [183–188]. This change in blood pressure response is likely due to an increased coupling of the β1-adrenergic receptor to the second messenger adenylyl cyclase with the R389 allele [180]. Data also suggest that the S49 allele encodes for a receptor that undergoes less internalization resulting in greater downstream signaling.

Given that blood pressure is a surrogate marker and the ultimate goal of antihypertensive therapy is to reduce hypertension-related morbidity and mortality, genetic associations with clinical outcomes have particular relevance. The influence of ADRB1 genotype on the incidence of death, nonfatal myocardial infarction, or nonfatal stroke was examined in participants in the International VErapail SR/Trandolapril (INVEST) study [189]. Patients in this trial had both hypertension and coronary heart disease and were assigned to either atenolol- or verapamil-sustained release (SR)-based treatment. The ADRB1 S49-R389 haplotype was associated with an increased risk for death among patients randomized to verapamil but not those randomized to atenolol. These data suggest that atenolol exerts a protective effect in individuals with hypertension, coronary heart disease, and the S49-R389 genotype. Another study found that polymorphisms in the promoter region of ADRB1 were associated with increased risk of adverse cardiovascular events in patients taking β-blockers [190]. Lastly, R389 and S49-R389 haplotype have been associated with improved clinical outcomes in patients receiving β-blocker therapy for the treatment of atrial fibrillation, ventricular arrhythmias, and heart failure [191–193].

The ability to predict response to β-blockade based on genotype could have important clinical implications. Specifically, in the absence of compelling indications for β-blocker therapy, β-blockers could be reserved for hypertension management in individuals expected to have a good blood pressure response to this drug class based on ADRB1 genotype. Alternative antihypertensive agents could be used in those expected to have little to no blood pressure reduction with β-blockade. β-blockers could also be used as first-line therapy for hypertensive patients with coronary heart disease and the ADRB1 genotype predictive of poor survival. However, further confirmatory data are necessary before genotype will be used clinically for antihypertensive therapy.

Genetic Determinants of Response to Thiazide Diuretics

Thiazide diuretics exert their effect by blocking the reabsorption of sodium and chloride in the distal tubule and therefore an accompanying amount of water. NEDD4L encodes the NEDD4-2 protein, which plays a role in controlling receptor expression of the epithelial sodium channel, ENaC, and potentially other sodium transporters [180]. There is a common functional variant in the NEDD4L gene, rs4149601G>A. The rs4149601G allele increases expression of the ENaC and has been associated with salt-sensitive hypertension with lower plasma renin activity and increased cardiovascular mortality [194,195]. A genetic substudy of the Nordic Diltiazem (NORDIL) study examined the impact of the rs4149601 variant on blood pressure response to diuretic and β-blocker therapy, given the effects of these drugs on inhibiting sodium reabsorption and renin release, respectively. The investigators found that carriers of the NEDD4L rs4149601G allele treated with either a thiazide diuretic or β-blocker had greater systolic and

diastolic blood pressure reduction than similarly treated patients with the AA genotype [196]. A subsequent study confirmed the association of the G allele with blood pressure lowering with thiazide diuretics, but not with β-blockers [195].

The *NEDD4L* rs4149601G allele has also been evaluated for its association with treatment-related clinical outcomes. In the NORDIL genetic substudy, patients with the G allele treated with a β-blocker and/or diuretic had a significant reduction in the risk for MI or stroke compared to those with AA genotype [196]. Consistent with this finding, carriers of the G allele in the International Verapamil SR Trandolapril (INVEST) Study who were not taking a thiazide diuretic had a significantly higher risk of cardiovascular disease than those with AA genotype [195]. Taken together, the data suggest that the *NEDD4L* rs4149601G allele is associated with worse cardiovascular outcomes and that thiazide diuretics and β-blockers may ameliorate this risk. These data suggest that there may be a role for *NEDD4L* genotype in predicting blood pressure response to thiazide diuretics and cardioprotective effects of both diuretics and β-blockers.

Opportunity in Pharmacogenetics: A Look to the Future of Hypertension Management

There are a number of antihypertensive agents available, and it is often difficult to choose which agent to prescribe for a particular patient. Even when following guideline recommendations, there is no guarantee that the prescribed drug will effectively lower blood pressure and prevent adverse outcomes in a given patient. The ability to predict response to antihypertensive therapy based on genotype could eliminate the trial and error approach to hypertension management. Further, an improved understanding of genetic contributions to the mechanisms underlying hypertension could lead to the development of novel therapies to combat the disease.

PHARMACOGENETIC POTENTIAL IN HEART FAILURE

Current Approach to Heart-Failure Management

As shown in Fig. 6.8, standard therapy for heart failure generally consists of an ACE inhibitor or an angiotensin receptor blocker (ARB) (or combination of an ARB plus neprilysin inhibitor) and a β-blocker for morbidity and mortality reduction, with the addition of a diuretic for symptom control. Other agents, including aldosterone antagonists and the combination of isosorbide dinitrate (ISDN) and hydralazine have been shown to further improve outcomes when added to the standard heart-failure drug regimen in select patients [9,197]. Thus, patients may require three to four or more medications for their heart failure alone, in addition, to therapy needed to treat any concomitant diseases.

There are several limitations with our current approach to heart-failure treatment. First, patients often have difficulty adhering to the multidrug regimens that have become the norm in heart failure. Second, many patients cannot safely take target doses of all recommended heart-failure therapies because of low blood pressure. Thus, clinicians must decide which drug to uptitrate and which drug to continue at suboptimal doses or abandon all together. Third, although data from multiple randomized trials demonstrate reductions in morbidity and mortality with vasodilators and β-blockers in overall heart-failure study populations, not all study participants derived benefits from these agents, and some experienced serious adverse effects requiring drug discontinuation. For example, approximately 13% of enalapril-treated subjects in the Studies of Left Ventricular Dysfunction (SOLVD) discontinued the drug because of worsening heart failure or adverse drug effects [198]. Similarly, 14% of patients in the β-blocker arm of the Metoprolol Controlled Release/Extended Release (CR/XL) Randomized Intervention

Current Approach to Heart Failure Treatment			Potential of Pharmacogenetics
Place in Therapy	**Drug Class**	**Limitations**	
Recommended for all patients (in the absence of contra-indications) to reduce morbidity and mortality	ACE inhibitors	• Does not improve outcomes in all patients. • Produces serious (angioedema, hyperkalemia) or intolerable (cough) side effects in some patients.	
	B-blockers	• Does not improve outcomes in all patients. • Worsens symptoms in some patients.	
Useful in many patients to control symptoms	Diuretics	• Significant inter-patient variability in response. • Increases risk for electrolyte derangements and renal dysfunction with supra-therapeutic doses.	
	Digoxin	• Increases risk for ventricular arrhythmias.	
Appropriate in select patients to reduce morbidity and mortality	Aldosterone antagonists	• Does not improve outcomes in all patients. • Produces hyperkalemia and worsening renal dysfunction in some patients.	Streamline treatment based on genetic signatures to include the combination of medications most likely to improve outcomes without causing toxicity for a given patient.
	Hydralazine/ nitrates	• Does not improve outcomes in all patients • Produces significant hypotension in some patients	
	Angiotensin receptor blockers	• Does not improve outcomes in all patients. • Produces serious side effects (angioedema, hyperkalemia) in some patients.	
	Angiotensin neprilysin receptor inhibitors	• Does not improve outcomes in all patients. • Produces serious side effects (angioedema, hypotenion) in some patients.	

FIGURE 6.8 Current approach and potential of pharmacogenetics in the treatment of heart failure.

Trial in Congestive Heart Failure (MERIT-HF) discontinued the drug prematurely because of poor tolerability [199]. Thus, although ACE inhibitors and β-blockers improved outcomes in clinical trial populations as a whole, there is no guarantee that they will improve outcomes without causing harm in an individual patient. Currently, there is no reliable method of predicting response to heart-failure medications, and all patients are treated with a similar "cocktail" of medications. Pharmacogenetics in heart failure aims to identify the combination of drugs most likely to be of benefit without causing harm for a particular patient based on genotype.

Pharmacogenetics of ACE Inhibitors in Heart Failure

The genes discussed thus far in this chapter primarily influence drug response by altering drug pharmacokinetics or pharmacodynamics. However, there are also examples of genes associated with disease prognosis, in which the adverse consequences attributed to a gene are modified by drug therapy. One such example is the ACE gene. Most studies of the ACE gene have focused on a 287-bp insertion/deletion (I/D) polymorphism in intron 16 of the gene, which occurs commonly in persons of European and

TABLE 6.6 Minor Allele Frequencies for Genes Associated With Responses to Hypertension and Heart-Failure Therapies [218,232,233]

Gene	Variant	Caucasians	African Americans
ACE	I/D	0.42	0.56
ADRB1	S49G	0.15	0.13
	R389G	0.27	0.42
ADRA2C	Del322-325	0.04	0.40
NOS3	E298D	0.37	0.14

African descent (Table 6.6). The ACE D allele has been consistently correlated with higher ACE activity and has been shown to confer increased risk for cardiac transplant or death in heart-failure patients, likely because of the deleterious effects of the renin–angiotensin system on heart-failure progression [200–204]. Inhibition of the renin–angiotensin system appears to attenuate the detrimental effects of the ACE D allele. For example, a study of patients with systolic heart failure showed that the adverse effect of the ACE D allele on transplant-free survival was greatest among patients who were not taking β-blockers or were taking less than or equal to 50% of the recommended target ACE inhibitor dose (dose associated with mortality reduction in clinical trials) [202]. Both ACE inhibitors and β-blockers attenuate the renin–angiotensin system. Use of β-blockers and higher ACE inhibitor doses, defined as doses greater than 50% of the target dose, attenuated the detrimental effects of the ACE D allele. A subsequent study in diastolic heart failure revealed similar findings [202].

In contrast to candidate gene studies linking the ACE I/D genotype to adverse outcomes in heart failure, a GWAS examining over 2.4 million variants in nearly 21,000 Caucasians and 3000 African Americans found no association between the ACE gene and heart-failure prognosis [205]. However, the investigators did not account for heart-failure treatment, which has been shown to modify the effect of ACE genotype on outcomes. Nonetheless, it is certainly premature to suggest that ACE inhibitors may not be necessary in individuals without an ACE D allele. However, if a patient is known to carry the ACE D allele, it may be particularly beneficial to use ACE inhibitors at recommended target doses to potentially ameliorate adverse consequences of this genotype.

ADRB1 Genotype: A Case for Targeted β-Blocker Therapy?

β-blockers are well recognized to reduce morbidity and mortality in heart failure by inhibiting the excessive sympathetic nervous system activity that propagates heart-failure progression. However, not all patients benefit from β-blocker therapy [206]. In addition, because β-blockers inhibit cardiac contractility, they must be started in very low doses with careful uptitration to help prevent worsening heart failure. Nonetheless, some patients still suffer cardiac decompensation during β-blocker initiation.

The ADRB1 gene has been extensively studied for its effects on β-blocker response. Although the data are not always consistent, the ADRB1 R389G genotype is associated with the degree of improvement in left ventricular ejection fraction with either metoprolol or carvedilol, with the greatest improvement observed with the RR389 genotype [207,208]. The RR389 genotype has also been associated with greater survival benefits with the β-blocker, bucindolol. Unlike metoprolol, carvedilol, and bisoprolol, which significantly improved survival in heart-failure clinical trials, bucindolol was shown to have a neutral effect on survival [209,210]. However, unlike clinical trials with the other β-blockers, the trial with bucindolol included a larger number of African Americans, and a subgroup analysis revealed improved survival with bucindolol in Caucasians but not African Americans [209]. A subsequent genetic analysis showed that response to bucindolol was dependent on ADBR1 genotype, with a reduced risk

for hospitalization and death with bucindolol in RR389 homozygotes, but not G389 allele carriers [193]. The G389 allele is more common among African Americans than Caucasians (Table 6.6), potentially accounting for the negative effects of bucindolol in persons of African descent. Other studies demonstrate RR389 homozygotes have a significant decrease in all-cause mortality or cardiac transplantation, new onset atrial fibrillation, and ventricular tachycardia and fibrillation burden when treated with bucindolol [191,192,211].

In contrast to data with bucindolol, the ADRB1 genotype was not associated with clinical outcomes with metoprolol or carvedilol [212–216]. However, there are important pharmacological differences among β-blockers, including a sympatholytic effect with bucindolol, which may contribute to differential genotype interactions with response to various drugs. Based on the pharmacogenetic data with bucindolol, ARCA Biopharma sought FDA approval of bucindolol in patients with the ADRB1 RR389 genotype. However, their initial request was denied.

Pharmacogenetics of Nitrates/Hydralazine

In the African-American Heart Failure Trial (A-HeFT), the addition of isosorbide dinitrate (ISDN)/hydralazine to standard therapy with an ACE inhibitor plus/minus a β-blocker significantly improved the primary composite endpoint of death, hospitalizations for heart failure, and quality of life compared to placebo [217]. Based on these data, the ISDN/hydralazine combination was FDA-approved for the treatment of heart failure in self-identified African Americans. Because the effects of adjunctive ISDN/hydralazine therapy has been examined only in African Americans, the benefits of the combination in individuals of other descents are unknown. Consistent with the FDA-approved labeling, current joint guidelines from the American College of Cardiology and American Heart Association recommend ISDN/hydralazine for African Americans with continued symptoms despite optimal treatment with ACE inhibitors, β-blockers, and diuretics [9,197].

Ancestral origin is a poor and controversial marker of drug response. Because any difference in drug response may be attributable, at least in part, to genotype, investigators have attempted to identify a genetic marker for response to ISDN/hydralazine in the African American heart-failure trial (A-HeFT) population. ISDN/hydralazine is believed to exert its beneficial effects by increasing nitric oxide availability, and as such, several variants in the endothelial nitric oxide synthase (eNOS) gene have been examined for their effects on ISDN/hydralazine response. Of these, only the p.E298D variant in exon 7 was found to influence response to ISDN/hydralazine [218]. The EE298 genotype is more common in African Americans than Caucasians and was associated with a greater improvement in the study's composite endpoint with ISDN/hydralazine, an association largely driven by the improvement in the quality-of-life score with the EE298 genotype. An additional study focused on the guanine nucleotide-binding protein β-3 subunit (GNB3) genotype, which is involved in adrenergic receptor signaling. The c.C825T polymorphism, which occurs more commonly in African Americans, was associated with greater response to ISDN/hydralazine, with the greatest benefit observed with the TT genotype [219]. These data suggest that eNOS and GNB3 genotypes, rather than ancestral background, may be useful as predictors of response to ISDN/hydralazine therapy. In the future, studies determining outcomes by self-reported race will likely be replaced by genetic studies. Until then, studies will continue to collect both race and genetic information in the hopes of effectively predicting drug response.

Opportunity in Pharmacogenetics: Potential to Streamline Heart-Failure Therapy

At this point, data are insufficient to support withholding any heart-failure therapy because

of a potential lack of benefit based on genotype. However, future prospective studies evaluating the benefits of pharmacogenetic-based prescribing compared to traditional prescribing of heart-failure medications are conceivable. Ultimately, results of pharmacogenetic research efforts could lead to genotype-guided prescribing of heart-failure therapy. Specifically, rather than initiating the same "cocktail" of drugs for all patients, regimens might be tailored according to each individual's genetic predisposition for obtaining benefit or experiencing harm from a particular drug. Drugs predicted to be of minimal to no benefit could be avoided, thus simplifying drug regimens and reducing the associated costs, while potentially improving overall patient outcomes.

GENETIC INFLUENCES OF DRUG-INDUCED ARRHYTHMIA

Cardiac arrhythmias are potentially fatal if not treated appropriately. However, the drugs used to treat arrhythmias are themselves arrhythmogenic. Thus, there has been significant study of the genetics of cardiac arrhythmia and the pharmacogenetics of antiarrhythmic therapy to improve drug effectiveness and limit proarrhythmic effects.

Antiarrhythmic Medications

Antiarrhythmic agents in general have a narrow therapeutic index. Thus, they are often susceptible to drug–drug interactions and can cause significant adverse events. Polymorphisms in the genes encoding drug-metabolizing enzymes have been examined for their role in antiarrhythmic efficacy and toxicity. The highly polymorphic CYP2D6 enzyme metabolizes propafenone and quinidine. Propafenone is a class IC antiarrhythmic that exerts its effects by blocking the fast-inward sodium current in addition to having some β-receptor blocking properties at higher concentrations. Propafenone is primarily metabolized by CYP2D6, though CYP1A2 and CYP3A4 also contribute to its metabolism. Genetic classification of CYP2D6 activity is complex and can be determined via genotyping or phenotyping. Patients are generally classified as poor, intermediate, normal, or ultrarapid metabolizers. Patients who are classified as CYP2D6 poor metabolizers have decreased propafenone clearance, which leads to an increase in propafenone serum concentrations. However, because this increase is balanced by a decrease in production of an active metabolite, the recommended dosing regimen is the same regardless of CYP2D6 phenotype.

The greater variability in plasma concentrations of propafenone and its metabolites in CYP2D6 poor metabolizers does require that propafenone be titrated carefully and the echocardiogram (ECG) be monitored for evidence of toxicity [220]. Importantly, the additional β-blockade seen in CYP2D6 poor metabolizers can potentially lead to adverse events in asthmatic patients. In addition, although the data are not entirely consistent, there is evidence that subjects with paroxysmal atrial fibrillation classified as CYP2D6 poor metabolizers are more likely to maintain normal sinus rhythm with propafenone compared to normal metabolizers [221].

In contrast, quinidine is not a CYP2D6 substrate. However, the prescribing information for quinidine contains information on CYP2D6 pharmacogenetics because quinidine is a potent CYP2D6 inhibitor even at a subtherapeutic dose (Table 1). Quinidine can convert patients who are normal metabolizers to poor metabolizers. Therefore, it is important to monitor for adverse events when quinidine is coadministered with CYP2D6 substrates. Although pharmacogenetic studies have been done for several other antiarrhythmic medications, no strong and reliable associations have been observed.

Pharmacogenetics of Drug-Induced Long QT Syndrome

The proarrhythmic effects of medications (both those used to treat arrhythmia and those

used for other indications) have been well studied given that they can be life threatening and require a significant amount of patient monitoring. Proarrhythmia is generally defined as the worsening of the arrhythmia being treated or generation of a new arrhythmia with drug therapy [222,223]. Genetic studies have focused on drug-induced increases in the QT interval on the ECG and the life-threatening arrhythmia, torsades de pointes. The knowledge gained from many years of studying and evaluating congenital long QT syndromes has aided the study of genetic factors associated with drug-induced prolonged QT intervals.

The QT interval on an ECG represents the action potential of ventricular myocytes. The ventricular action potential is made up of several currents produced by different ion channels. The action potential is prolonged when there is either increased inward current or decreased outward current. The heart has significant built-in redundancy, as several ion channels participate in the ventricular action potential. This redundancy is termed "repolarization reserve." Thus, variation in one ion channel will not necessarily lead to an increase in the QT interval. A combination of factors is generally necessary for patients to exhibit congenital or drug-induced long-QT syndromes.

Genetic variation in ion channels associated with ventricular action potential has been well studied in congenital long-QT syndromes because of the possible effect on "repolarization reserve" [224]. Mutations found in genes encoding potassium (KCNQ1, KCNH2, KCNE1, and KCNE2) and sodium (SCN5A) voltage-gated channels have been associated with risk for congenital long QT syndrome. In addition, medications can prolong the QT interval by blocking the ion channel pore, inducing conformational changes in the ion channel pore, and/or decreasing production of the proteins encoding the ion channels. The amino acid structure of KCNH2 appears to make this ion channel pore particularly susceptible to drug blockade. Polymorphisms in the gene encoding KCNH2 may affect its susceptibility to drug binding and contribute to risk of long-QT syndrome [224]. In the FDA guidance for industry for screening non-antiarrhythmic drug potential to cause delay in cardiac repolarization, they recommend considering genotyping for cardiac ion channel mutations for patients who experience marked QT-interval prolongation or torsades de pointes in early clinical trials [225].

GWASs have identified additional genes associated with congenital long-QT syndrome. Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP), which encodes for an accessory protein for the nitric oxide synthase type 1 gene, was initially linked to variability of QT interval across the normal population [226]. Variants in NOS1AP have also been linked to arrhythmia risk, sudden cardiac death, congenital long-QT syndrome, and the QT-interval prolonging effects of verapamil and amiodarone, though the mechanism underlying these associations remains unclear [226,227].

Clinical factors, such as hypokalemia, recent conversion of atrial fibrillation, and advanced heart disease may potentiate risk of drug-induced long-QT syndromes. The risk of drug-induced long-QT syndrome may also be increased if the clearance of a drug is decreased via either a drug interaction or genetic variants in hepatic enzyme systems. Clinicians should be particularly vigilant in monitoring for drug interactions with medications known to prolong the QT interval. In addition, if genetic variability in hepatic enzyme systems for a patient is known, this should be considered as well [228].

Currently, using genetic information to predict drug-induced long-QT syndrome cannot be recommended. However, evidence on this topic is growing rapidly, and with validated genetic markers, genotyping may in the future be clinically useful. However, it is unlikely that polymorphisms in a single gene or a single clinical risk factor will be sufficient to predict risk because of the redundancy in the system. Predicting

drug-induced long-QT syndrome will likely require a complex combination of multiple polymorphisms and clinical and environmental information. In support of this, a combination of 61 genetic variants was recently found to predict risk of QT prolongation with multiple drugs [229].

Opportunities in Pharmacogenetics: Potential to Resurrect Old Drugs

One of the primary reasons that drugs in development do not succeed or that approved drugs are withdrawn from the market is because of proarrhythmic effects. The ability to predict risk for proarrhythmia with a drug could potentially revive some agents, particularly if few other treatment options are available. In this case, genetic testing for the "at-risk" variant(s) would likely be required prior to drug use. The drug could then be avoided in patients genetically at risk for drug-induced proarrhythmia.

CONCLUSION

After nearly two decades of research in the area of cardiovascular pharmacogenetics, the evidence has accumulated to the extent of informing clinical implementation of genotype-guided prescribing for several drugs. An increasing number of institutions are implementing clinical pharmacogenetics programs for cardiovascular drugs, with clopidogrel being the primary focus to date [175]. One approach to pharmacogenetic implementation is to genotype variants related to a specific drug at the time of drug prescribing. Another approach is to genotype a panel of variants influencing responses to numerous drugs preemptively. This way, genetic information is available at the time various drugs are prescribed. Although fewer variants need to be tested with the former approach, there are substantial personnel costs associated with the need to obtain genotype results efficiently. For example, in the case of CYP2C19 testing for clopidogrel, genotype results need to be obtained quickly to inform antiplatelet therapy early after the coronary intervention when the risk for adverse cardiovascular events is highest. Dedicated personnel are needed to efficiently process the sample with preemptive panel-based testing. Multiple samples can be batched and run at one time, because there is no urgency in obtaining genotype results, significantly reducing personnel time and associated costs. Both approaches are being used in clinical practice, and guidelines by the CPIC are available to assist with translating genotype results into actionable prescribing decisions for drugs with the greatest evidence supporting genotype-guided decisions. Research continues for other drugs to identify variants influencing risk for toxicity or likelihood of therapeutic response. With efforts such as the NIH-funded eMERGE and IGNITE Networks, the incorporation of genotype data into drug-therapy decisions is expected to be increasingly utilized to optimize treatment.

DISCUSSION POINTS

1. Contrast the effect of a poor drug-metabolizer phenotype on response to warfarin versus clopidogrel.
2. Describe feasible approaches to implement warfarin and clopidogrel pharmacogenetics into clinical practice.
3. Describe the data supporting use of genetic data to assist with clopidogrel, warfarin, simvastatin, and tacrolimus dosing.
4. Describe CPIC guideline recommendations for genotype-guided clopidogrel, warfarin, simvastatin, and tacrolimus prescribing.

QUESTIONS FOR DISCUSSION

1. What are examples of novel pharmacogenetic findings from genome-wide association studies?

2. What are barriers to the clinical implementation of pharmacogenetics to manage cardiovascular disease?
3. How might pharmacogenetic findings lead to new drug development for cardiovascular disorders?

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CHAPTER

7

Pharmacogenomics in Psychiatric Disorders

Y. W. Francis Lam[1,2], *Toshiyuki Someya*[3]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]College of Pharmacy, University of Texas at Austin, Austin, TX, United States; [3]Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00007-3

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OBJECTIVES

1. Discuss the utility of *CYP* genotyping in psychopharmacology
2. List and define the rationale of different drug targets for psychopharmacogenomic investigations
3. Discuss alternative approaches to pharmacogenomic studies in psychopharmacology
4. Describe how pharmacogenomics may play a role in minimizing adverse effects of antipsychotics

INTRODUCTION

Evaluations and prediction of treatment response and potentials of adverse drug reactions in psychiatric patients have in the past been partially limited by the patients' subjective reports and the subjective elements in clinicians' assessments. Despite the availability of different clinical rating scales, there remains no reliable biological marker of response. Since the completion of the Human Genome Project in 2003 and in an effort to improve outcome, the implications of pharmacogenomics in psychiatry have been increasingly evaluated. Many candidate genes have been identified, with the hope that they can be utilized to improve patient outcome. However, so far, applications of pharmacogenomics have been primarily more successful in predicting adverse drug reactions than treatment response. This chapter will review the pharmacogenetic findings, discuss the evidence and challenges of genotyping biomarkers in psychopharmacotherapeutics, and address the future potentials of applying pharmacogenomics in psychopharmacology. Because a comprehensive review of all research in psychiatric pharmacogenomics is beyond the scope of this chapter, affective disorder and schizophrenia will be the focus to highlight the principles and issues in this emerging field.

POLYMORPHISMS IN PROTEINS THAT AFFECT DRUG CONCENTRATIONS

Genes Encoding Drug-Metabolizing Enzymes

Antidepressants

Several polymorphic cytochrome P450 isoenzymes, notably CYP2D6 and CYP2C19, are involved in metabolism and elimination of tricyclic antidepressants (TCAs) and the selective serotonin reuptake inhibitors (SSRIs) (Table 7.1) [1,2]. The lack of therapeutic response even with standard-dosage regimens of the TCA nortriptyline provided one of the earliest clinical examples of how altered expression of *CYP2D6* can impact drug response in patients who have multiple copies of the *CYP2D6**2 allele. The original clinical observation [3] was followed up with additional studies that elucidated the molecular basis [4] and the gene–dose relationship in nortriptyline pharmacokinetics [5] in patients with the ultrarapid metabolizer (UM) phenotype for CYP2D6. In their report of antidepressant dose recommendations based on pharmacokinetics and pharmacogenetics relationships, Kirchheiner et al. [6] suggested increased dose requirement in UMs receiving nortriptyline (up to 230% of

TABLE 7.1 Major CYP Isoenzymes and Transporters Responsible for Metabolism and Efflux of Selected Psychotropics

	Antidepressants	Antipsychotics
CYP1A2		Olanzapine Clozapine
CYP2C9	Not a major isoenzyme, but provides the only secondary pathway for fluoxetine	
CYP2C19	Amitriptyline, citalopram, clomipramine, doxepine, escitalopram, imipramine, nortriptyline, sertraline	Clozapine
CYP2D6	Amitriptyline, desipramine, doxepin, duloxetine, fluoxetine, imipramine, mirtazapine, nortriptyline, olanzapine, paroxetine, trazodone, venlafaxine	Aripiprazole, chlorpromazine, clozapine, haloperidol, iloperidone, olanzapine, perphenazine, pimozide, risperidone, thioridazine
ABCB1	Amitriptyline, nortriptyline, paroxetine, venlafaxine	Risperidone

the usual dose), desipramine (up to 260%), and mianserin (up to 300%). The number of literature reports of lower efficacy in UMs is significantly less for the SSRIs, which is expected given their flatter dose–response curve. Kawanishi et al. [7] showed in a small pilot study a preponderance of UMs (10%) in 81 nonresponders who received at least 4 weeks of standard recommended-dosage regimens of TCAs and SSRIs that are CYP2D6 substrates. In addition to potential impact of metabolic polymorphism on efficacy, Penas–Lideo et al. also suggested an association between discontinuance of amitriptyline and fluoxetine with the CYP2D6 phenotype. In their study of 100 patients with major depressive disorder, all four UMs discontinued drug treatment within the first 4 weeks, whereas no PMs did so after 12 weeks of therapy [8].

The clinical use of SSRIs has expanded over the years to include other psychiatric conditions such as obsessive–compulsive disorders and generalized anxiety disorder. Although *CYP2D6* polymorphism has been shown to influence the plasma fluoxetine-to-norfluoxetine concentration ratio [9,10], the correlation with clinical response has been less robust in patients with major depressive, panic, and anxiety disorders. This may be due to the additional contribution from the polymorphic *ABCB1*, which influences the extent of SSRI entry via the blood–brain barrier (discussed in a later section) [9], as well as that from polymorphisms in the genes encoding the serotonin transporter [11,12] and the serotonin 2A receptor [13] (both are discussed in a later section).

Both citalopram and escitalopram are metabolized significantly by the polymorphic CYP2C19. As expected, a recent study in 2,087 patients showed that the *CYP2C19* genotypes significantly affect escitalopram exposure. Compared to patients with *CYP2C19 *1/*1* genotype, the serum drug concentrations in homozygous and heterozygous carriers of the null allele (defined as *2, *3, *4) were significantly increased by 3.3-fold and 1.4–1.6-fold, respectively. Homozygous and heterozygous carriers of the *CYP2C19*17* allele showed 20% and 10% decreases in concentrations, respectively. This is, by far, one of the largest studies to document the relationship between metabolic genotypes and systemic drug exposure. In addition, therapeutic failure (defined as switching from escitalopram to another antidepressant within 1 year after serum drug-concentration monitoring) were 3.3, 1.6, and 3.0 times more frequent among patients with *CYP2C19 null/null* genotype, patients with *CYP2C19 *1/*17* genotype, and patients with *CYP2C19 *17/*17* genotype, respectively. Switching, presumably due to either insufficient pharmacologic response or occurrence of adverse drug reactions, occurred

in a larger proportion of patients with extreme genotypes (*CYP2C19 null/null* and *CYP2C19 *17/*17*) [14]. Therapeutic failure to citalopram has also been described in a case report [15].

Mrazek et al. studied a cohort of 1,074 citalopram-treated patients and reported CYP2C19 PMs tolerated citalopram less so than other patients [16]. On the other hand, Serretti et al. reported that the CYP metabolic genotypes have no correlation with either response to antidepressants or remission of depression, although most of the 197 nonresponders received antidepressants that depend on multiple CYP enzymes for metabolism [17]. Another report also reported a lack of association between *CYP2C19* genotype or citalopram concentration and treatment response in 223 citalopram-treated patients [18]. These negative association data concur with reports by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group [19], and results of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial [20].

Despite evidence that *CYP2D6* and *CYP2C19* polymorphisms are correlated with the pharmacokinetics of several antidepressants, the effect of genetics on CYP2D6 and CYP2C19 enzyme activity, or the pharmacokinetics of their respective substrates, can be further modulated by other variables such as drug dose [21,22], treatment duration [23,24], patient-specific factors such as presence of concurrent CYP2D6 or CYP2C19 inhibitors (the phenoconversion phenomenon) [25], smoking status [26,27], diet, medication adherence, and ethnicity. The effect of *CYP2D6* variants (**5* and **10*) on fluvoxamine and paroxetine pharmacokinetics are shown only in patients treated with the lower doses of 50 and 10 mg/day, respectively. This is most likely a result of CYP2D6 being a low-capacity enzyme, with saturation of its metabolic capacity occurring with higher-dosage regimens: 100–200 mg/day of fluvoxamine and 20–40 mg/day of paroxetine, respectively [21,22], thus effectively diminishing the impact of the genetic polymorphism at higher dosages.

Difference in treatment-response phenotypes was reported to be evident during the second to fourth week, but not during the eighth week, of antidepressant therapy [23]. A similar finding of better early-treatment response to escitalopram in CYP2C19 PM was also reported in a prospective, open-label observational study of Chinese patients with panic disorder [24]. Because fluvoxamine is a CYP1A2 substrate, the effect of the *CYP2D6* genotype on fluvoxamine pharmacokinetics is further modulated by smoking, which together accounted for 23% of the variance in fluvoxamine concentrations for patients treated with the low-dose regimen of 50 mg/day [26]. Tsai et al. reported polymorphisms in *CYP2D6* and *CYP2C19* impact on the therapeutic outcome and serum concentrations of S-citalopram [28]. Because multiple *CYP2D6* and *CYP2C19* alleles occur at variable frequencies among different ethnic groups, and the patient population in the STAR*D trial [20] included primarily Caucasians (78.1%) and African Americans (16.1%), the conflicting study results [20,28] underscore the importance of heterogeneity of not only study design, but as importantly, study populations and the need of defining ethnicity in pharmacogenomic research. This issue of ethnicity is further discussed in relevant sections of different chapters throughout the book.

Although the use of the TCAs has declined over the years, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has released guideline recommendations for these drugs in patients with different *CYP2C19* and *CYP2D6* genotypes [1]. Even though dosage adjustment recommendations appear in some package inserts, no clear guidance exists on dosing from the Food and Drug Administration. In addition, little evidence exists that prior dosing recommendations [29,30] for CYP-dependent psychotropic medications are widely implemented. This finding is consistent with a report from the Dutch Pharmacogenetics Working Group [30].

In summary, current evidence suggests that the utility of CYP-based pharmacogenetic testing for antidepressants lies more in

anticipating adverse drug effects than in predicting their therapeutic efficacy. In a study of 1,198 elderly patients treated with antidepressants, PMs of CYP2D6 were five times more likely to show significant adverse effects with the use of CYP2D6-dependent TCAs [31]. Rau et al. also reported a predominance of PMs in 28 patients who were treated with CYP2D6-dependent TCAs and SSRIs [32], and patients with the intermediate metabolizer (IM) phenotype for CYP2D6 were found unable to tolerate venlafaxine doses larger than 75 mg [33]. These earlier study results were replicated in a more recent study conducted in an acute psychiatric unit, in which longer hospitalization associated with greater side effects were reported in CYP2D6 PMs compared with other genotypes [34]. With implementation of *CYP2D6* and *CYP2Ç19* genotypes in clinical practice, Muller et al. also confirmed usefulness of genotyping primarily for PMs and IMs [35]. Similar to the results of Penas–Lideo et al. [8], a recent study also reported an almost four times higher antidepressant discontinuance in pregnant women who are PMs or IMs of CYP2D6, suggesting that knowledge of the *CYP2D6* genotype might help identify pregnant patients at risk for antidepressant discontinuance [36].

Antipsychotics

Although literature data provide good evidence that polymorphic *CYP2D6* plays a role in determining pharmacokinetic profiles of different antipsychotics (primarily risperidone, aripiprazole, chlorpromazine, haloperidol, perphenazine, and thioridazine; and to a lesser extent clozapine, olanzapine, and quetiapine, Table 7.1), little evidence exists for a role of *CYP2D6* genotypes in determining antipsychotic efficacy. In a prospective study, Pollock et al. reported no significant differences in improvement of psychotic symptoms between five CYP2D6 PMs and 40 extensive metabolizers (EMs) treated with perphenazine for 17 days [37]. Even though a trend of lower haloperidol efficacy in UMs and higher efficacy in the PMs was shown in the study of Brockmoller et al. [38], the significant overlap in the haloperidol daily doses among the four metabolic groups: with 14 ± 10 mg in UMs versus 13 ± 9 mg in the PMs, preclude the possibility of any useful genotype-based dose recommendation. In 235 patients with schizophrenia or schizoaffective disorder who failed to respond to typical antipsychotics, subsequent *CYP2D6* genotyping showed the presence of the UM phenotype in less than 1% of the patients, suggesting that the UM genotype was not a major contributing factor to the therapeutic failure [39]. In the Clinical Antipsychotics Trials of Intervention Effectiveness (CATIE), Grossman et al. reported little evidence of difference in efficacy of either perphenazine or risperidone in patients with different *CYP2D6* genotypes, although there were no UMs included in the study [40]. Likewise, two other studies with risperidone showed that *CYP2D6* genotypes did not predict clinical improvement [41,42]. Another issue is that very few published studies separated UMs from EMs, which likely would negate any possible difference in efficacy between the UM and other *CYP2D6* genotypes. Finally, although Kim et al. suggested genetic polymorphism of *CYP3A5*3* is associated with the pharmacokinetics of quetiapine [43], there is little evidence that CYP3A polymorphism impacts the dosing of this atypical antipsychotic.

In their report of no difference in perphenazine efficacy between 40 EMs and 5 PMs, Pollock et al. also found that PMs experienced more severe adverse effects, including oversedation and parkinsonism, than EMs during the first 10 days of treatment, although there were no drug-concentration measurements performed [37]. Several studies have also shown PMs and IMs to have a higher incidence of adverse drug reactions, including extrapyramidal side effects, and drug discontinuance associated with the use of antipsychotic agents [38,44–53], whereas the evidence of a role of CYP2D6 in the etiology of tardive dyskinesia in PMs was less clear, with studies reporting both positive [51,54–57] and negative associations [58–61]. Without good data to suggest a concentration-dependent

relationship, it is not surprising that tardive dyskinesia might not be related to the *CYP2D6* genotype. *CYP2D6*10*, a predominant allele in Asian IMs, had been reported to be associated with weight gain in risperidone-treated Chinese patients [62], although it is not known whether plasma concentration correlates with weight gain. The role of pharmacogenomics in antipsychotic-associated weight gain will be discussed in a later section.

Mood Stabilizers

Despite the common clinical practice of monitoring plasma concentration and an inadequate response rate of <50% in lithium-treated patients, no pharmacogenomic studies exist on lithium pharmacokinetics. Published studies have mainly focused on the genes involved in the signaling and biochemical pathways involved in the mechanism of action of lithium, which will be described in a later section.

Genes Encoding Drug Transporters

The lack of data supporting a primary role for CYP gene polymorphisms in determining psychotropic drug response might be due to the presence of the drug efflux ATP-binding cassette (ABC, and formerly known as multidrug resistance [MDR]) superfamily of transporters residing at the blood–brain barrier (BBB). P-glycoprotein (P-gp) was the first-recognized and the most-studied ABC transporter, and together with other more recently discovered ABC transporters, such as multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP), plays a significant role in limiting the amount of drug crossing the BBB and reaching the cerebral circulation.

P-gp is encoded by the *ABCB1* gene (also known as the *MDR1* gene) in humans. Over the years, several polymorphisms have been identified in the promoter and exon regions of the *ABCB1* gene. The most studied single-nucleotide polymorphisms (SNPs) are the c.C1236T (rs1128503) polymorphism in exon 12, the c.G2677T (rs2032583) polymorphism in exon 21, and the c.C3435T (rs1045642) polymorphism in exon 26. In a randomized study of the effect of C3435T polymorphism in 54 nortriptyline-treated patients and 72 fluoxetine-treated patients, Roberts et al. found no difference in nortriptyline serum concentrations among the three genotypes (C/C, C/T, and T/T) but observed a higher incidence of postural hypotension for homozygous carriers of the T allele [63]. Fukui et al. [64] showed that the effect of the C3435T polymorphism on fluvoxamine pharmacokinetic is dose dependent, with the TT homozygote showing a significantly higher concentration-to-dose ratio than the CC homozygote only at the highest dose of 200 mg/ day. Therefore, the effect of P-gp polymorphism on drug concentrations could be similar to the dose-dependency effect shown with the *CYP2D6* polymorphism.

Although each of the aforementioned three *ABCB1* SNPs is associated with altered P-gp expression, larger-scale studies investigating their effect on antidepressant response have been conflicting [65–68]. This discrepancy might be due to the presence of strong linkage disequilibrium (LD) between several of these *ABCB1* polymorphisms, although conflicting results have also been reported for haplotype association studies [20,69]. In addition, some negative studies evaluated the association with C3435T polymorphism for too many drugs (9) in too few patients (n = 55) [70], which would pose a problem for statistical power. The choice of drug to be evaluated would also be important, as better remission rate was only demonstrated for patients carrying the C allele for the rs2032583 polymorphism *and* receiving a P-gp substrate (e.g., amitriptyline, citalopram, paroxetine, sertraline, or venlafaxine). Meanwhile, the response prediction associated with *ABCB1* polymorphism disappeared when data from all patients or from patients receiving non-P-gp substrates were analyzed [71,72]. Sarginson et al. confirmed the significance of

this substrate dependency for response association with paroxetine but not with mirtazapine, which is not a P-gp substrate [72]. In the large prospective randomized International Study to Predict Optimized Treatment in Depression, the investigators also reported association between response (remission and side effects) and *ABCB1* SNP association in 576 patients who completed an 8-week regimen of escitalopram, sertraline, or venlafaxine for their major depressive disorder.

Breitenstein et al. recently evaluated the clinical application of *ABCB1* genotyping in 58 depressed inpatient participants of the Munich Antidepressant Response Signature (MARS) trial. In this "head-to-head" comparison of treatment outcomes between pharmacogenomics-guided algorithm versus standard of care, the *ABCB1* gene test results (rs2032583 and rs2235015 SNPs) were incorporated into the treatment-decision process. The investigators reported that pharmacogenomics-guided patients had higher remission rates ($P = .005$) with less severe symptoms ($P = .0195$) upon discharge, compared to patients receiving usual care [73]. Needless to say, this pilot, yet encouraging, result needs confirmation. Unfortunately, the *ABCB1* gene variants were not assessed in some of the recent cost-utilization studies (see later section on Clinical Applications).

POLYMORPHISMS IN PROTEINS THAT MEDIATE DRUG RESPONSE

In addition to polymorphisms in the drug-metabolizing enzymes and the transporters, recent work has revealed that genes encoding drug targets such as receptors, ion channels, and intracellular-signaling proteins also play a significant role in determining drug efficacy and safety in patients. Multiple targets for the psychotropics exist for the neurotransmitter systems, including those that affect synthesis, degradation, or uptake of neurotransmitters, as well as their binding to pre- and postsynaptic receptors; and the cascade of downstream signal-transduction proteins within the synapse. Dysregulation of individual or combination of these targets can play a significant role in the etiology of psychotic diseases (Fig. 7.1). Abundant pharmacogenomic data on target polymorphisms exist for the psychotropics, in particular the antidepressants and the antipsychotics.

Antidepressants

Serotonin Transporter

With the primary role of serotonin (5-HT) in regulating emotions and mood, the serotonin transporter (SERT or 5-HTT) and also known as solute carrier family 6 [neurotransmitter transporter, serotonin], member 4 (SLC6A4), which function to transport serotonin within the synapse back to the presynaptic neurons, is one of the main therapeutic targets for SSRIs and also serotonin norepinephrine reuptake inhibitors (SNRIs). The SERT is encoded by the *SLC6A4* gene with several functional polymorphisms that have been extensively investigated and identified to impart variable therapeutic response in patients with different *SLC6A4* genotypes (Table 7.2). Specifically, a functional polymorphism in the promoter region (rs4795541, serotonin transporter-linked promoter-region polymorphism, or 5HTTLPR) of the *SLC6A4* gene results in the insertion/deletion of a 44-base pair repeat. The long (L) allele of the gene has higher transcriptional activity of the *SLC6A4* gene promoter and, hence, higher 5-HTT basal expression and serotonin uptake than the short (S) allele [74].

In vivo neuroimaging studies reported the contradictory effects of 5HTTLPR on brain 5-HTT availability [75–77], However, given the ability of the SSRIs to downregulate the SERT function, investigators hypothesized that SSRI efficacy could be affected by 5HTTLPR polymorphism. Since then, many studies have shown an association between homozygosity for the S allele and inferior response to the SSRIs, in contrast to

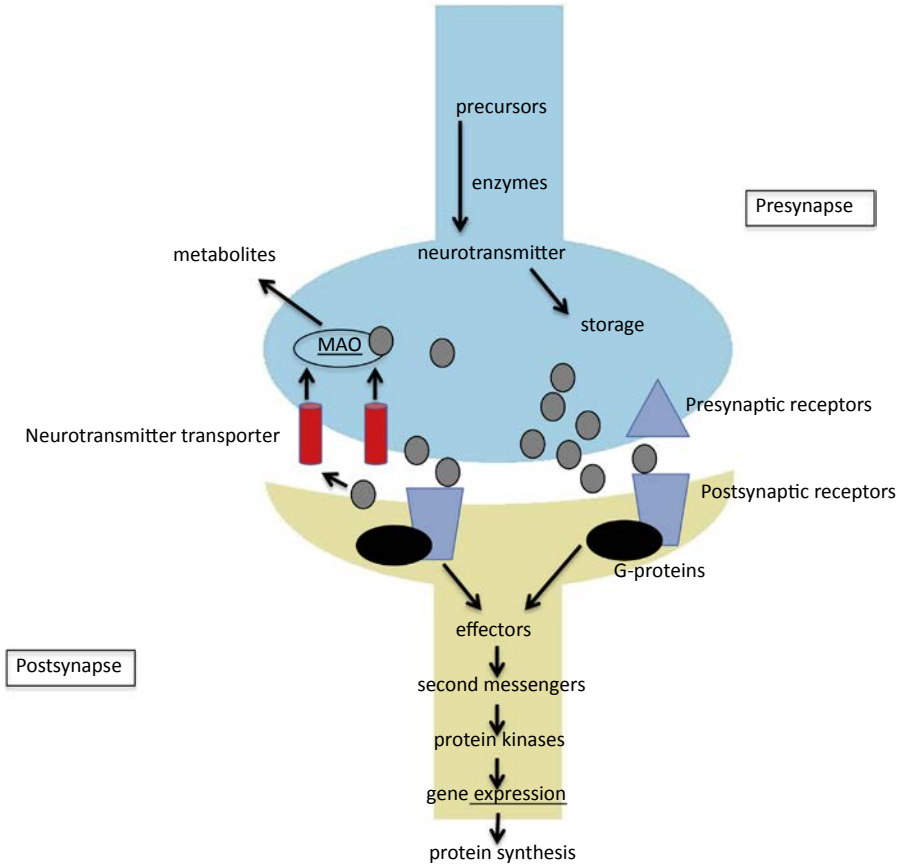

FIGURE 7.1 Schematic representation of psychotropic target proteins. *MAO*, monoamine oxidase.

homo- or heterozygosity for the L allele of the gene, which predicts beneficial outcome with SSRI treatment. Using positron emission tomography (PET) to evaluate the influence of genetic factors on 5-HT_{1A} receptor expression in a living human brain, David et al. observed that the S allele was associated with a reduction in availability of the postsynaptic 5-HT_{1A} receptors in man [78]. This might provide a possible biological basis of the decreased response to SSRIs in carriers of the S allele.

Not only was the therapeutic outcome reported different among patients with different *SLC6A4* genotypes, elderly patients with the *L/L* genotype treated with paroxetine or sertraline had a more rapid response, as early as after 1-week of treatment, than those with the *L/S* and *S/S* genotypes [79,80]. In addition, the lack of similar change in the onset of response in elderly patients treated with nortriptyline suggests that the difference in response is relevant only to antidepressants with a selective effect on serotonin [80]. Based on these findings, a case could be made for a preferential use of SSRI in patients with the *L/L* and *L/S* genotypes versus a TCA or a noradrenergic agent in patients with the *S/S* genotype. In addition, in patients with the *S/S* genotype, augmentation strategy of combining fluvoxamine and pindolol (being a 5-HT_{1A} antagonist as well and accelerating the antidepressant effects of SSRIs) has been shown to reduce the difference in response between carriers of the S and

TABLE 7.2 Summary of Selected Pharmacogenomic Studies With Major Genes Involved in the Serotonergic System

Genes	SNP	Major Findings	References
SLC6A4	5-HTTLPR, rs4795541	Homozygous or heterozygous carriers of 5-HTTLPR L allele showed better and/or faster response to SSRIs, especially in Caucasians Homozygous carriers of S allele showed less response, especially in Caucasians Homozygous carriers of S allele showed better response in patients of Asian descent Addition of pindolol to fluvoxamine improved treatment response in homozygous carriers of S allele	[79] [80] [81] [85] [86] [87,96] [96] [115]
	5-HTTLPR, rs25531	L_G variant (5 HTTLPR L allele with rs25531 allele) functionally similar to 5 HTTLPR S allele No association between treatment outcome and 5HTTLPR alleles or haplotypes. However, lower incidence of adverse effects were reported with the L_A allele of rs25531 Homozygosity for S allele and G alleles of HTR1A gene associated with nonresponse	[92] [91]
	STin2, 12 repeat units	No association with treatment outcome	[94]
	5-HTTLPR, rs25531 STin2	Association between remission and a haplotype consisting of S allele of 5-HTTLPR, L_A allele of rs25531, and 12-repeat allele of STin2	[95]
HTR2A	rs6311 and rs6313	*G* allele and *GG* genotype of rs6311 associated with better response, primarily in patients with Asian descent *C* allele and *CC* genotype of rs6313 associated with better response, primarily in Caucasians No association with response in Non-Hispanic Whites, or African or African American patients	[99,100] [101] [96]

the L allele, resulting in comparable treatment outcomes in all three genotypes [81]. Based on a decision-analytic model of pretreatment genetic testing for *SLC6A4* genotypes, Smits et al. concluded that the testing might result in a greater number of patients achieving remission earlier in the course of the treatment [82].

A meta-analysis of the literature supports a modest association between *SLC6A4* L allele and SSRI efficacy primarily in Caucasians but not in Asians [83]. Whether the more heterogeneous results within Asian populations could partially account for the lack of association between 5HTTLPR polymorphism and SSRI response reported in another meta-analysis that included more studies (n = 28) and patients (n = 5,408) is not known, because the investigators did not analyze the result separately in Caucasians and Asians [84]. This is especially important because opposite yet comparable associations (S allele conferring good therapeutic response) have been reported in Korean and Japanese populations, possibly at least partially related to either ethnic-related difference in the 5HTTLPR S allele frequency, being higher in Asians (74%–80%) than in Caucasians (40%) [85–87], interaction with other functional gene variants, or gene–environment interaction. In addition, a study showed that 5HTTLPR is not a simple insertion/deletion of a 44-base pair repeat, but a complex and highly polymorphic structure consisting of 14 kinds of alleles in different populations, including the Japanese and Caucasian, with variable distribution frequency [88].

The highly polymorphic nature of the *SLC6A4* gene is illustrated with the discovery of rs25531, an SNP located just upstream of the 5HTTLPR, from genetic analysis of the STAR*D sample. A

functional A>G variation in the L allele (but not S allele) of 5HTTLPR, known as the L_G allele, reduces mRNA expression of the *SLC6A4* to a level comparable to that of the S allele, and therefore changes the functional significance of the L allele of 5HTTLPR. On the other hand, the L_A allele increases *SLC6A4* mRNA expression, resulting in a "higher function" phenotype [89,90]. Therefore, by changing the expression of the L allele, this previously unrecognized L_G allele would further modulate the SSRI response predictive value of the *SLC6A4 L/S* and *L/L* genotypes. In essence, within SLC6A4, there would be two promoter polymorphisms and three alleles of functional importance: the high-expression L_A allele, and the low-expression S and L_G alleles. Because the S and L_G alleles are very comparable in SERT expression, the possible genotypes based on this L_A and L_G difference would be no L_A allele (either *S/S*, *S/*L_G, *or* L_G/L_G), one L_A allele (*S/*L_A or L_G/L_A), and two L_A alleles (L_A/L_A). Among the haplotypes constructed, only L_A/L_A is associated with high *SLC6A4* transcription [89]. This is consistent with the findings of carriers of L_A allele having favorable response to SSRI compared to carriers of the L_G allele [91].

In a second study of the STAR*D samples, Hu et al. reported an association between the L_A allele and citalopram adverse effects in all 1,655 subjects (Caucasians, Africans or African Americans, and mixed race). Lower adverse effects were associated with L_A/L_A genotype ($P = .004$) and L_A allele ($P < .001$) in all subjects, and a lesser association in a subset of 1,131 Caucasian subjects ($P = .03$ and $P = .007$, respectively). The adverse-effect association was also evident for the entire study population even when the L_A and L_G alleles were combined in the analysis. On the other hand, association for the Caucasian subject was present only with differentiation of the L allele into L_A and L_G alleles. There was no association between treatment outcome and 5HTTLPR alleles or genotypes in the Caucasian subjects [92].

In addition to 5HTTLPR, additional polymorphisms of the *SERT* gene have been identified with potential roles in modulating the response to SSRIs. Ogilvie et al. discovered a 17-base pair, variable number of tandem repeats (VNTR) polymorphism within intron 2 (STin2) of *SLC6A4*, resulting in three alleles containing 9, 10, and 12 copies of the VNTR element [93]. However, similar to 5HTTLPR polymorphism, both positive [85] and negative [94] associations have been reported. What is interesting is that, despite the lack of association between 5HTTLPR alleles or haplotypes and citalopram response [92], re-analysis of the same dataset from the STAR*D study revealed an association between remission and a haplotype that consists of 5HTTLPR, rs25531, and STin2 (haplotype S-L_A-12) [95].

Therefore, even for *SLC6A4*, a candidate gene with an obvious relevance to the therapeutic effect of antidepressants, especially the SSRIs, it is clear that predicting response in a patient solely with any one SNP would likely yield misleading and conflicting results. Together with the usual heterogeneous study limitations (ethnicity, outcome assessment, study design, sample characteristics, and sample size), significant work remains for appropriate pharmacogenomic study findings related to 5HTTLPR polymorphism to be translated from the bench to the bedside. In this respect, confirmatory findings in a naturalistic setting was reported by Staeker et al., who recently conducted a naturalistic study to evaluate the association between polymorphisms in serotonergic pathways (*SLC6A4*/rs25531, VNTR, and a 5-*HTR2A* intron 2 SNP [see later section on 5-HT_{2A} receptor]) and response (as assessed by Clinical Global Impression [CGI] Scale) and side effects (as assessed by the Dosage Record and Treatment Emergent Symptoms [DOTES] Scale) in psychiatric inpatients. They found significant associations between *SLC6A4*/rs25531 S/L_G alleles and response to SSRI treatment in patients ($P = .037$, CGI ≤ 2, 0% versus 19%, and $P = .0005$, DOTES cluster c, 0.76 vs. 0.19). In addition, there was significant association between *SLC6A4* VNTR 12/12 with adverse effects ($P = .0001$, side

effect rates 51% versus 19%). They also found significant association between the 5-*HTR2A* intron 2 SNP and side effects (to be described in later section) [96]. Additional recent investigations to lay the groundwork for broader-scale implementation have started to appear in the literature, and will be discussed in the later Clinical Application section.

5-Hydroxytryptamine Receptors

5-HYDROXYTRYPTAMINE 2A (5-HT_{2A}) RECEPTOR

The postsynaptic 5-HT_{2A} receptor represents another serotonin-related target for psychotropics. Antidepressants, typical, and atypical antipsychotics all act as antagonists toward and downregulate the receptor [97], reportedly overexpressed in patients with depression [98]. In humans, the polymorphic 5-hydroxytryptamine receptor 2A (*HTR2A*) gene encodes the 5-HT_{2A} receptor, and several polymorphisms had been investigated, including a c.-1438 A/G (rs6311) promoter polymorphism, and two coding region polymorphisms: the silent c.T102C (rs6313) polymorphism in exon 1, and the c.C1354T (rs6314) polymorphism resulting in a p.His452Tyr amino acid substitution. Two of these (rs6311 and rs6313) are in LD and had been associated with antidepressant response (Table 7.3) [99–101]. Although a specific allele, e.g., the C variant of the C102T polymorphism [101] and the G allele of the −1438 A/G polymorphism [99,100] were reported to be associated with antidepressant response, the findings are conflicting and not supported by the large-scale association study of 68 candidate genes in the STAR*D sample.

In the STAR*D study, a single synonymous variant of *HTR2A*, IVS2 A/G (rs7997012) within intron 2, emerged as the only SNP with sufficient predictive value for response to citalopram in a Caucasian population. Homozygous carriers of the A allele have better response (18% reduction in absolute risk of treatment failure) than homozygous carriers of the G allele. In addition, analysis of the genetic data showed that Africans or African Americans had a higher frequency of the "non-responding" allele [102], which might partially account for the findings of poorer response among citalopram-treated African or African American patients in the clinical STAR*D study [103]. Lucae et al. provided the first replicate confirmation of the role of rs7997012 shown in the genetic STAR*D study. In evaluating 637 German Caucasian patients with a major depressive episode, the SNP rs7997012 was significantly associated with remission of depression after 5-weeks treatment with a variety of antidepressants. However, the association (A allele conferred impaired treatment response) was inverse to that of the genetic STAR*D study [104]. Ethnic differences in patient samples (Caucasians versus a more heterogeneous population comprising Caucasians, Africans or African Americans, and mixed races in the genetic STAR*D study) and time of evaluation of treatment response (after 5-week treatment versus at study exit, regardless of length of duration since study entry for the genetic STAR*D study) can complicate interpretation of results. In addition, smaller sample size (Table 7.2) in the study of Lucae et al. could confound the result, limit the comparability between study results, and require additional studies to ascertain the direction of the association.

A more recent meta-analysis found significant association of rs6313 and rs7997012 SNPs with good treatment response to SSRIs in Caucasians but not in Asians [105], which may reflect the two SNPs being more common in Caucasians (about 54% and 36% for rs6313 and rs7997012, respectively) than in Asians (about 49% and 22% for rs6313 and rs7997012, respectively). As mentioned earlier, Staeker et al. studied the impact of serotonergic polymorphisms at the transporter and receptor level on response to SSRI. Although they did not report an impact of the 5-*HTR2A* intron 2 SNP on response, a significant association was found between *A/A* genotype of rs7997012 SNP and side effects ($P = .020$, side effect rates 43% versus 11%). The investigators

TABLE 7.3 Summary of Selected Pharmacogenomics Studies With Major Genes Involved in the Dopaminergic and Serotonergic System for Antipsychotic Response and Toxicity

Genes	SNP	Antipsychotics	Main Findings	References
COMT	c.472 G>A V158M rs4680	Clozapine, olanzapine	Homozygous carriers of Met allele have increased clinical response	[193,194]
DRD1	−48 A>G (rs4532), rs5326, rs265975	Haloperidol, chlorpromazine, sulpiride, flupenthixol, zuclopenthixol	CGC haplotype of the three SNPs associated with tardive dyskinesia (TD) risk	[206]
	rs4532	First-Generation Antipsychotic (FGA) and Second-Generation Antipsychotic (SGA)	No association with TD	[207,208]
DRD2	−141C del/ins, rs1799732	Chlorpromazine, bromperidol, nemonapride, risperidone	Del allele associated with less response	[154–156,159]
	Taq1A, rs1800497, also associated with *ANKK1* gene	Haloperidol, nemonapride, risperidone	A1 allele, A1/A1 genotype, Ins-A2/Del-A1 diplotype associated with better response	[165,166,168,169]
		Risperidone, chlorpromazine	No association with response	[159,170]
		Antipsychotics	A2 carriers at risk for TD	[204]
		Haloperidol, perphenazine, levomepromazine, fluphenazine, chlorpromazine, thioridazine, zuclopenthixol	A1 carriers associated with EPS	[202]
		Bromperidol, nemonapride	No association of A1 allele with EPS	[203]
		Nemonapride, olanzapine quetiapine, risperidone	A1 carriers associated with increased prolactin level	[209–211]
	Ser311Cys	Risperidone	Better response with Ser/Cys genotype	[171]
DRD3	Ser9Gly	Risperidone, chlorpromazine	Gly allele and Gly/Gly genotype associated with less response	[170,192]
		Antipsychotics	Gly allele associated with TD	[213–215]
DRD4	VNTR	Clozapine	No association with response	[178,179,184]
SLC6A4	44 bp del/ins	FGA	No association with response	[180]
HTR2A	102-T/C	Clozapine	T allele associated with better response	[182,184]
		Risperidone	Better response with CC genotype	[191]
		Antipsychotics	C allele associated with TD risk	[217]
	His425Tyr	Clozapine	Better response with His allele	[182,184]
		Antipsychotics	No association with TD	[217]
HTR2C	−759C/T (rs3813929)	Risperidone, chlorpromazine	Less response with C allele, C/C genotype	[170]
		Atypical antipsychotics	Weight gain associated with T allele	[218,219]

noted that all of the response and side effects associations were strong enough to be detectable in a naturalistic clinical setting [96].

5-HYDROXYTRYPTAMINE 1A (5-HT$_{1A}$) RECEPTOR

The 5-HT$_{1A}$ receptor is encoded by the 5-hydroxytryptamine receptor 1A (*HTR1A*) gene. Desensitization (or downregulation) of the somatodendritic 5-HT$_{1A}$ receptor by chronic SSRI treatment results in enhanced serotonergic neurotransmission [106,107]. In addition, antagonism of the 5-HT$_{1A}$ receptor has also been suggested to be associated with antidepressant effects [108,109]. Therefore, genetic variation of the *HTR1A* might change the functional properties of the 5-HT$_{1A}$ receptor, resulting in differences in antidepressant response.

Of the 10 polymorphisms identified in the *HTR1A* gene, the most investigated ones are c.-1019C/G (rs6295) located in the promoter region, p.Gly22Ser (rs1799920), and p.Ile28Val (rs1799921). The G allele of the rs6295 polymorphism has been associated with up-regulation of 5-HT$_{1A}$ receptor expression [110] and response prediction with antidepressant treatment [111]. In 118 patients treated with fluoxetine or nefazodone augmented with pindolol, or monotherapy with flibanserin (a 5-HT$_{1A}$ agonist), the homozygous *G/G* genotype was more prevalent in nonresponders than the homozygous *C/C* genotype ($P = .0497$ for the augmentation group and $P = .039$ for the monotherapy group) [111]. However, other investigators reported positive association being evident only for females [112] or in patients with specific depressive manifestation [113]. In a retrospective study, Levin et al. found no association between seven *HTR1A* polymorphisms, including rs6295, and SSRI response in 100 responders and 33 nonresponders [114]. As additional evidence that response to antidepressants likely is influenced by more than one gene, Arias et al. reported in 130 subjects treated with citalopram that homozygosity for both the G allele of the *HTR1A* polymorphism and S allele of the *SLC6A4* polymorphism predict nonresponse to SSRI treatment ($P = .009$) [115]. Differences in ethnic and allele distributions in study subjects could partially account for the conflicting results in replication studies. As an example, with very low frequencies of the Gly22Ser and Ile28Val polymorphisms in Japanese populations, the effect of the more common Gly272Asp polymorphism of the *HTR1A* on clinical response to fluvoxamine was studied in 65 depressed Japanese patients. Subjects with the Asp allele had a significantly higher % reduction in score of the 17-item Hamilton Rating Scale for Depression (HAMD-17) than homozygous carrier of the Gly allele at week 2 ($P = .009$), week 6 ($P = .036$) and at week 12 ($P = .031$) [116].

Antidepressant-associated side effects are well-known contributory factors to lower medication adherence, poor-health outcomes, and premature discontinuance of treatment. In a randomized placebo-controlled trial of 12-week treatment of escitalopram in patients 60 and older, Garfield et al. reported that side effects (increased sleep duration, dry mouth, diarrhea, and decreased sexual desire) are associated with genetic polymorphisms affecting *SLC6A4*, *HTR1A*, and *HTR2A*. Decreased sexual desire was experienced more in patients with high-expressing genotypes of the three serotonergic components, whereas higher incidence of dry mouth and diarrhea are associated more frequently with patients with the low-expression genotypes for *SLC6A4* polymorphism and low-transcription genotype for the *HTR1A* polymorphism, respectively. In contrast, there was no relationship between the three genetic polymorphisms and drug concentration [117].

Glutamate Receptor

With glutamate as the primary excitatory neurotransmitter in the brain, the glutamatergic system has also been investigated in pharmacogenomic studies of antidepressant response. Glutamate receptors selectively bind to glutamate to modulate excitatory neurotransmission, and increased glutamate levels have been observed in patients with depression [118]. Chronic use of

SSRIs such as citalopram was shown to attenuate glutaminergic transmission and reduce excitatory glutamate activity [119]. The STAR*D study has identified significant association between antidepressant response and a C/T SNP (rs1954787) residing in the first intron of the ionotropic kainite 4 gene (*GRIK4*) that encodes a kainic acid-type glutamate receptor. The C allele was associated with better outcome and suggested that the glutamate system could have a significant role in antidepressant response. In addition, homozygous carriers of both the A allele of *HTR2A* and the C allele of *GRIK4* were twice as likely to be associated with better response to citalopram than patients who did not carry either of these two outcome-related alleles [120]. The association of the C allele and *C/C* genotype with response has been confirmed in a meta-analysis [121]. In contrast, Perlis et al. reported they could not replicate the rs1954787 association in 250 Caucasian patients with nonpsychotic major depressive disorder and treated with daily regimens of duloxetine 60 mg/day for 6 weeks. In addition, to smaller sample size and difference in study population, one additional reason for the discrepancy could be related to the differential mechanisms of action of duloxetine (a serotonin–norepinephrine reuptake inhibitor) versus SSRIs (inhibiting selectively the reuptake of serotonin). It is also noteworthy that the investigators also reported their failure to replicate previously reported associations with rs25531, 5HTTLPR, and the 17-base pair VNTR polymorphism in intron 2 (STin2) for *SLC6A4*. Negative associations were also shown in the same study for four SNPs for *ABCB1*, six SNPs for four genes coding for phosphodiesterases, and a single SNP for *OPRM1* coding for the opioid receptor μ 1 [122].

Genome-Wide Association Studies

In contrast to candidate-gene studies involving, for example, the 5-HTTLPR, advances in sequencing technology have enabled the interrogation of many millions of SNPs within the entire genome and elucidation of molecular pathways involved in disease etiologies and drug actions through genome-wide association studies (GWASs). Unfortunately, the three major GWASs in patients with major depression, namely the Genome-Based Therapeutic Drugs for Depression (GENDEP) [123], MARS [124], and the STAR*D [125], have not identified any individual gene with convincing replication results in a sufficiently large sample size. In an effort to identify SNPs most likely associated with antidepressant response, investigators conducted several meta-analyses of GWASs, which partially overlap in dataset. The first meta-analysis included data from three large response cohorts in United Kingdom, Germany and the United States: the GENDEP, the MARS, and the STAR*D, respectively. Together, the three studies, which included 2,256 subjects of Northern European descent with major depressive disorder, were deemed to have statistical power to detect individual variants accounting for one to 2% of variation in antidepressant response. However, no individual variants were found to meet the genome-wide significance [66]. The second meta-analysis, Novel Methods Leading to New Medications in Depression and Schizophrenia (NEWMEDs), included additional cohort to that of the GENDEP, and also found no association with efficacy [126]. O'Dushlaine et al. conducted a meta-analysis of two GWASs: STAR*D cohort and another cohort drawn from electronic health records of a large health system (i2b2 cohort) that together comprised 1,263 Caucasians with major depressive disorder. Initial treatment responders were contrasted with those with treatment-resistant depression (TRD), defined as no symptomatic remission despite two antidepressant treatment trials. Copy number variants (deletions and duplications) were derived from 778 subjects (including 300 with TRD) in the i2b2 cohort and 485 subjects from the STAR*D cohort (including 152 with TRD). They reported a modest contribution of rare copy number variants to treatment-resistant phenotypes, both individually and in aggregate, but no associations survived genome-wide correction [127].

These meta-analysis results are not unexpected given the results of Tansey et al., in which data from two large major depression studies (NEWMEDs and STAR*D) in about 4,100 patients were analyzed. Using genome-wide complex trait analysis [128], the investigators reported additive effects of common genetic polymorphisms across the human genome accounting for about 42% of individual variation in antidepressant response [129]. Not only do the results confirm the highly polygenic basis of antidepressant response that involve many variants, but as importantly, none of the variants have large effects, despite collectively accounting for a substantial portion of the variation. It remains to be determined which pharmacogenomic markers for drug disposition and/or response could account for a large portion of the variability.

In summary, despite significant progress in antidepressant pharmacogenomic research over the years, the lack of consistent findings among all studies of different neurotransmitter receptors and transporters, including single candidate-gene association studies, GWASs, and meta-analyses, make it difficult to identify definitive association that can be used to predict antidepressant response in clinical setting. Differences in study design, disease phenotypes, patient population, response definition and assessment, and sample size all contribute to the conflicting results. In addition, it is also unclear how many pharmacogenomic studies measure medication adherence, which acts as a confounding variable that affects treatment outcome. Nevertheless, studies that employ pathway analysis of gene variants involved in fluoxetine pharmacodynamics have shown some potential utility in identifying important gene variants with significant contributions to treatment response with fluoxetine [130].

Potential Role of Other Molecular Pathways

Research over several decades suggest that increased monoaminergic neurotransmission is important for antidepressant action [131]. Although studies reviewed in previous sections mostly demonstrate the essential function of the serotonergic system (transporter and receptor) and the impact of their regulating genes for antidepressant response, Nicket et al. reported that both paroxetine and the serotonin reuptake *enhancer* tianeptine are effective antidepressants [132]. In addition, a meta-analysis also showed that the effects of monoamine depletion are conflicting, and depletion does not induce depression in healthy subjects [133]. Hence, additional biological pathways, including those identified recently [134–137], could possibly serve as biomarkers for treatment response. The following section illustrates how studies of molecular pathways associated with neuronal plasticity over the last few years provide insight of additional antidepressant targets.

The neuroplasticity hypothesis suggests that antidepressant action is partially related to proliferation of neuronal stem cells, and that the slow onset of antidepressant action is a result of neuroplasticity changes mediated by such proliferative effects in the hippocampus [138]. Hence, other investigators have proposed an entirely different approach to search for SSRI-response biomarkers, which is based on reports of genome-wide expression profiling in human lymphoblastoid cell lines (LCLs) previously demonstrated for anticancer drugs [139–141]. They first identified and demonstrated the existence of LCLs with variable SERT functional expression and hence high or low sensitivities to different SSRIs [142]. The investigators then screened 80 LCLs for growth inhibition by paroxetine. A 6.4-fold difference in expression between the two paroxetine-sensitivity phenotypes was demonstrated for the cell adhesion molecule with homology to L1 cell-adhesion molecule (L1CAM) gene (close homolog of L1 [CHL1]) encoding a neuronal cell-adhesion protein that is implicated in correct brain circuitry, and *CHL1* was identified as a tentative transcriptome biomarker of paroxetine. In addition to *CHL1*, 12 additional genes implicated

in brain function or psychiatric disorders also showed more than 1.5-fold difference in expression between the two phenotypic groups [143]. In a follow-up study, the effect of fluoxetine on cell proliferation and gene expression in LCLs derived from patients with documented treatment response outcome was investigated. The investigators identified multiple genes with different expression before and after ex vivo incubation with fluoxetine [144].

Although one can argue that the discovery of yet another set of genes for predicting SSRI response does not necessarily translate to definitive and clinically relevant biomarkers, comparison of gene-expression levels from patients with major depression could be further investigated to identify targets for antidepressant therapy. In a study of 58 patients selected from the MARS study, investigators showed an association between response (better remission with antidepressants) and basal expression of *CHL1* and another gene, integrin beta 3 (*ITGB3*). After 5 weeks of antidepressant treatment, homozygous carriers of the T allele of the *CHL1* SNP (rs1516338) had significantly better response than homozygous carriers of the C allele, further suggesting that CHL1 expression in patient-derived LCLs correlated with clinical outcome [145]. Another group of investigators analyzed genes associated with outcomes from the STAR*D GWAS and confirmed the potential roles of *CHL1* and *ITGB3* [146].

Antipsychotics

Dopamine Receptors

The catecholamine neurotransmitter dopamine controls a variety of central nervous system functions including cognition, emotion, endocrine system regulation, food intake, and locomotor activity. The five dopamine receptors are grouped into the D_1-like receptors (DRD1 and DRD5) generally associated with stimulatory functions, and the D_2-like receptors (DRD2, DRD3, and DRD4), which are more associated with inhibitory functions. All antipsychotic agents, especially the first-generation antipsychotics, are dopamine D_2 receptor (DRD2) blockers [147]. Functional brain-imaging studies suggest, and pooled analyses and meta-analyses confirmed, that a threshold level (60%–65%) of D_2 receptor binding by antipsychotic agents in the mesolimbic pathway is needed for sustained therapeutic effect, and excessive blockade (≥78%–80%) in the nigrostriatal pathway is associated with extrapyramidal side effects [148–151].

Of the five subtypes of dopamine receptors, D_2, D_3, and D_4 receptors are the most studied for pharmacogenetic evaluation of antipsychotic efficacy. Several polymorphisms of the D_2 receptor gene (*DRD2*) have been identified: the −141-C ins/del polymorphism (rs1799732) with deletion of a cytosine in the promoter region at position −141, the Taq1A polymorphism (rs1800497), and the p.Ser311Cys polymorphism (rs1801028) within the coding region. The del allele of the −141-C ins/del polymorphism is associated with not only lower expression of the D_2 receptor in vitro [152], but also higher striatal D_2 receptor density in vivo [153]. Studies that evaluated the functional effects of the polymorphism have yielded mixed results. Several investigations and meta-analysis have also shown that the del allele predicts less beneficial response from antipsychotics (Table 7.3). [154–159], Interestingly, a recent report showed that carriers of the del allele have higher rates of improvement in depressive symptoms severity during treatment with olanzapine, perazine, and ziprasidone [160]. In addition, even though the del allele was associated with lesser clinical improvement in risperidone-treated patients. [159], Wang et al. reported no association with *DRD2* polymorphisms in patients treated with paliperidone, the active metabolite of risperidone [161]. Thus, in addition, to replication challenges such as different study designs and outcome measurements, the issue of whether response association with the candidate-gene

approach is limited to an individual psychotropic drug versus applicable to a wide range of antipsychotic medications would need to be addressed as well.

The Taq1A polymorphism (rs1800497), now also associated with *ANKK1* gene [162], is located downstream of *DRD2* and has two variants: A1 and A2, with lower striatal D_2 receptor density reported in carriers of the A1 allele [163]. The A1 allele is in LD with two *DRD2* intronic variants (rs1076560 and rs2283265) that affect DRD2 splicing [164], The *A1/A1* genotype had been reported to be associated with better response (greater improvement in positive symptoms) to aripiprazole, haloperidol, nemonapride, and risperidone [165–168], whereas the *Ins-A2/Del-A1* diplotype was reported to be associated with better response to risperidone [169]. In contrast, lack of association have been reported, primarily in patients of Asian descent with first-episode schizophrenia [170] or drug-naïve schizophrenic patients [159]. These negative study results are in agreement with the lack of association reported in a meta-analysis [157].

The rs1801028 polymorphism represents a C>G SNP in exon 7 that changes the codon 311 from the more common Ser to the less common Cys, with the Cys311 variant associated with lower affinity for dopamine. In 123 Chinese patients treated with risperidone for up to 42 days, patients with the *Ser/Cys* genotype of *DRD2* polymorphism showed greater absolute score reduction and greater percent change in negative symptoms than patients with the *Ser/Ser* genotype. However, there were only 12 subjects with the *Ser/Cys* genotype and no patient had the homozygous *Cys/Cys* genotype [171]. Nevertheless, a meta-anlaysis by Hwang et al. also showed a trend for lesser response in carriers of the Ser allele [172]. In summary, although most studies of the *DRD2* polymorphisms have been associated with treatment outcome, the effect of individual polymorphism has not been consistent across different studies.

Dopamine binds to the D_2 receptor and inhibits its prolactin secretion, and the Taq1A genotype has been associated with hyperprolactinemia [173]. Fukuri et al. hypothesized that basal prolactin level accurately reflects DRD2 function, and investigated the association of the basal prolactin levels of 140 healthy Japanese subjects with *DRD2* "tagging" SNPs that covered the *DRD2* gene, as well as with the Taq1A, Ser311Cys, and −141C Ins/Del polymorphisms. Significant associations were found between two *DRD2* variants (rs7131056 and rs4648317) in intron 1 and serum prolactin levels, but only in the female subjects, which is consistent with the known gender difference in prolactin concentration [174]. These preliminary data suggest that the two new polymorphisms can be considered as candidate functional *DRD2* polymorphisms, and should be further investigated in future studies.

Antipsychotic agents also show affinity for the dopamine D_3 receptor (DRD3), with increased receptor expression after treatment [175]. The *DRD3* gene contains an SNP that results in a serine to glycine amino acid substitution (rs6280). The p.Ser9Gly polymorphism had been implicated with conflicting results showing lesser [172] versus greater [176] antipsychotic response in carriers of the Gly allele. Literature data also evaluated its association with development of tardive dyskinesia, which will be discussed in latter sections of this chapter.

The ten-fold higher affinity of the atypical antipsychotic agent clozapine for the D_4 receptor than for the D_2 and D_3 receptors results in a lower risk of inducing extrapyramidal side effects. The *DRD4* gene is highly polymorphic, with a tandem duplication of 120 base pairs (120-bp duplication) in its promoter region, resulting in reduced DRD4 expression in vitro and lower gene transcription. Despite the earlier report of this tandem-repeat polymorphism linked to clozapine efficacy with a better response in carriers of the long allele (240 base pair) [177], subsequent studies were not able to detect significant association [178,179].

Serotonergic System

Although no differences in response to typical antipsychotic agents were reported in 684 patients with different *SLC6A4* genotypes [180], the pharmacological action of the atypical antipsychotic agents partially involves the serotonergic system, with single-photon emission computed tomography evidence of high occupancy of the 5-HT_{2C} receptor by clozapine and risperidone [181], making it a logical candidate gene for evaluation of response association. Based on clozapine's high affinities for the 5-HT_{2A} and 5-HT_{2C} receptors, several polymorphisms of the *HTR2A* gene (c.-1438-G/A and c.102-T/C in the promoter region and p.His425Tyr in the coding region) and the *HTR2C* gene (c.-759-T/C [rs3813929] in the promoter region and p.Cys23Ser [rs6318] in the coding region) have been extensively investigated in the literature for response prediction. Meta-analyses of literature data reported association between 102-T/C and His425Tyr polymorphisms and response [182]; Although a significant association was found between the Ser allele of the Cys23Ser polymorphism of the *HTR2C* gene [183], subsequent studies were not able to replicate the results.

Recognizing the limitation of evaluating single SNPs in a single gene, Arranz et al. evaluated 19 genetic polymorphisms that affect the different pharmacological targets of clozapine. Based on association studies of these polymorphisms in 133 responders and 67 nonresponders, the investigators reported a combination of six different polymorphisms across different loci. These six (the −1438-G/A and 102-T/C polymorphisms that are in LD; the His425Tyr polymorphism of the *HTR2A* gene; the Cys23Ser and −330-GT/-244-CT polymorphisms of the HTR2C gene; the 5HTTLPR polymorphism of the *SLC6A4* gene; and the −1018-G/A polymorphism for the histamine-2 receptor) resulted in a 76.7% success in predicting response to clozapine. In addition, about 50% of the patients are homozygous carriers of the T allele of the 102-T/C polymorphism and the His allele of the His425Tyr polymorphism of the *HTR2A* gene, and good response was evident in 80% of this patient subgroup. Interestingly, despite the high affinity of clozapine for the D_4 receptor, no association was found with response [184]. Nevertheless, despite the appeal of this polymorphism-combination approach to more accurately predict clozapine response, the result was not replicated in another study [185]. To date, no studies replicate the primary findings of Arranz et al. [184].

The dopamine and serotonin receptors targeted by the antipsychotics are G-protein-coupled receptors (GPCRs) and signal to effector proteins through intracellular G-protein subunits. Regulators of G-protein signaling shorten the time period of neurotransmitter signaling through the GPCRs. The regulator of G-protein signaling 4 (RGS4) is one such regulator, and it regulates the activity of the GPCRs. The gene that encodes RGS4 had been identified as a vulnerability gene for schizophrenia [186,187], and variants of *RGS4* have been studied as predictors for antipsychotic treatment response. Conflicting reports of treatment response association with three SNPs (rs951439, rs2842030, rs2661319) of *RGS4* have been reported in three ethnic groups (patients of African descent, European descent, and Chinese descent) for different antipsychotics (perphenazine, ziprasidone, quetiapine, and risperidone) [188,189]. These data with *RGS4* polymorphisms underscore the importance of stratification of patient population by ethnicity in pharmacogenomic investigations, which is further evidenced by the lack of association reported in another study of 482 unrelated schizophrenia patients of South Indian origin [190]. It is noteworthy that the investigators of the Chinese study [189] also had reported in several different studies that polymorphisms affecting the D_2 receptor (Ser311Cys), D_3 receptor (Ser9Gly), and 5-HT2A receptor (102-T/C) predict treatment response to risperidone [171,191,192]. Whether a combination of

polymorphism approach similar to that for clozapine could result in better response prediction remains to be investigated.

Catechol-O-MethylTransferase

Dopamine level in the frontal lobe of the brain is essential for executive function. The catechol-O-methyltransferase (COMT) mediates the degradation of dopamine and terminates its action, especially in the frontal cortex. Although not studied as extensively, polymorphism in *COMT* encoding the enzyme may modulate antipsychotic effect. The Val108Met polymorphism (rs4680 with G to A transition at codon 158 of the membrane-bound form of COMT, which corresponds to codon 108 of the soluble form of the enzyme) results in 3–4-fold lower COMT activity in homozygous carrier of the Met allele when compared to those with the *Val/Val* genotype. Studies have shown carriers of the Met allele (with less dopamine degradation and, hence, more dopamine in the synapse) have improved cognitive function after treatment with clozapine and olanzapine [193,194]. A recent meta-analysis of studies with a total of 1,416 patients confirmed the association between the *Met/Met* genotype and antipsychotic efficacy [195]. Interestingly, although the independent effect of the DRD4 120-base pair duplication for predicting clozapine response [177] has not been duplicated [178,179], a more recent study by Rajagopal et al. demonstrated a gene–gene interaction between the *DRD4* and *COMT* for clozapine response in 93 patients. A carrier of the Met variant who also is a homozygous or heterozygous carrier of the *DRD4* 120-base pair allele showed better clinical response to clozapine than those without these alleles. A carrier of the Met allele and the *DRD4 240/240* genotype showed no additive clinical response, whereas poor response was associated with both the *DRD4 120/120* and *120/240* genotypes in the presence of the *COMT Val/Val* genotype [196]. Although the mechanism for the additive response interaction between the *COMT* Met and the *DRD4* 120 allele is not known and this result needs confirmation, this study highlights the need of evaluating interaction among target genes in pharmacogenomic research.

Additional Regulatory and Development Genes

In addition to the genes involved in the dopaminergic and serotonergic system, there are additional genes of interest that, although not as extensively studied as dopaminergic and serotonergic genes, could contribute to antipsychotic response, probably via their influence on neuronal function and neurotransmitter signaling. Although an extensive review of investigations of all these SNPs is outside the scope of this chapter, these include the glutamatergic system [197], specifically two SNPs in the glutamate-receptor delta 2 (GRID2) gene involved in glutamate signaling, as abnormal glutamatergic function could modulate dopaminergic function in psychosis [198], rs13025959 (E1647D) in *MYO7B* encoding myosin VIIB, which plays a role in brain development, and rs10380 (H622Y0 in *MTRR* encoding 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, which might play a role in determining antipsychotic response similar to that of methylene-tetrahydrofolate reductase (MTHFR), which is encoded by the *MTHFR* gene [199].

In summary, compared to the antidepressants, the research data for antipsychotic pharmacogenomic studies are very limited. Among the literature studies, some association studies with individual candidate genes encoding their respective targets showed positive findings with overall antipsychotic response prediction with genes involved in the dopaminergic system, and improved negative symptoms with genes involved in the serotonergic system. Currently, the *DRD2* -141-C Ins/Del and the Taq1A polymorphisms are included in some pharmacogenomic test panels. However, the data are far from convincing, and there are just about as many negative associations reported

in the literature. Although it is obvious that a combination of different genes would account for a greater portion of the response variance than individual genes, analysis of how genetic variants influence improvement in positive or negative symptoms as well as cognitive function would likely yield more useful insight than improvement in overall symptomatology.

As with antidepressants, GWASs over the years have identified additional SNPs, including rs17390445 on chromosome 4p15 from the CATIE study, to be associated with treatment response, even though the study itself was not designed as a pharmacogenomic study [200]. However, the SNP is located in an intergenic region with unknown functional significance of the associated variants. Additional SNPs in the ankyrin repeat and sterile alpha motif domain-containing protein 1B gene (ANKS1B) and in the contactin-associated protein-like 5 gene (CNTNAP5), which play a role in modulating neuronal cell proliferation and differentiation, as well as communication among neurons within the brain, have also been shown in the same study to approach genome-wide significance. However, how these borderline significant results could affect antipsychotic response remains unknown. Although GWAS results could have implications in identifying new molecular pathways and targets that warrant additional investigations, currently the practical utility of the SNP results from GWASs of the CATIE trial for practitioners is minimal.

Adverse Drug Reaction with Antipsychotics

Antipsychotic use is associated with a variety of adverse effects, with extrapyramidal symptoms (EPS) and weight gain being the most commonly reported and also the focus of much of the pharmacogenetic studies of psychotropic-induced adverse-drug reactions. Among the different EPS, tardive dyskinesia (TD) is a debilitating and irreversible movement disorder that develops in up to 30% of patients after long-term antipsychotic treatment. As indicated earlier, excessive blockade of D_2 receptor is associated with extrapyramidal side effects, although primarily a problem for the typical antipsychotics [149,201].

Both positive [202] and negative [203] associations with EPS had been reported in A1 carriers of the Taq1A polymorphism of the *DRD2* gene, whereas a meta-analysis found a risk-increasing effect for TD in carriers of the A2 allele [204]. Because an imbalance between D_1 and D_2 receptors had been suggested to result in TD [205], the conflicting results reported for association between *DRD2* polymorphism and EPS as well as the risk of TD [202–204] could be related to genetic variants in *DRD1* as well. In a recent study involving 220 Chinese patients with TD and 162 Chinese patients without TD treated with stable dosage regimens of typical antipsychotics for at least 6 months, the SNP rs4532 (also known as −48 A>G) in *DRD1* was significantly associated with TD risk in the schizophrenic patients. The positive association was also evident in haplotype analyses involving two additional SNPs: rs5326 and rs265975, specifically the haplotype CGC (rs5326-rs4532-rs265975) [206]. The study result contrasted with the negative association reported by two studies [207,208], which could be related to ethnic differences in allele frequency of rs4532 (18% frequency for the G allele in Chinese versus 39% in Caucasians [207]) and contribution of *DRD1* to TD, as well as the inclusion of patients treated with atypical antipsychotics in the two negative studies.

Dopamine binds to D_2 receptor and inhibits its prolactin secretion; therefore, use of antipsychotic agents results in increased prolactin level, although the effect is less with the atypical antipsychotics. Several studies showed that hyperprolactinemia is related to the Taq1A polymorphism, with the A1 allele associated with elevated prolactin level [209–211], as well as being drug specific, with the effect being more prominent with risperidone and olanzapine than with quetiapine [211]. However, no such

association was reported in a subsequent study of 47 younger patients with autism-spectrum disorders [212].

Brain-imaging studies also showed that haloperidol-treated patients with the *Gly/Gly* genotype for the Ser9Gly polymorphism of *DRD3* gene (rs6290) had greater fluorodeoxyglucose metabolism in the anterior striatum than patients who were either heterozygous or homozygous carrier of the Ser allele. The increased brain activity observed in the patients correlated with the presence of the most severe TD symptoms [213]. In a meta-analysis of data from 317 patients with TD and 463 patients without TD, patients with the Gly allele were found to experience a higher incidence of TD ($P = .04$). In addition, patients who were homozygous carrier for the Gly allele had higher abnormal involuntary movement scores than heterozygotes ($P = .006$) and homozygotes for the Ser allele ($P < .0001$). The effect of the Gly allele, though significant, was modest with an odds ratio of 1.33 [214]. Nevertheless, the role of the Ser9Gly polymorphism in TD was confirmed in another meta-analysis, which also suggested that the association was related to ethnicity, with a stronger association in non-Asians versus Asians [215]. In contrast, both the CATIE trial and a more-recent meta-analysis of 13 studies reported no association between *DRD3* rs6280 polymorphism and prevalence of TD [61,216]. Finally, a pooled analysis of 256 patients with TD and 379 patients without TD showed a positive association for the C allele of the 102-T/C polymorphism of *HTR2A*, especially in the elderly. This suggests that 5-HT receptors can also be involved in etiology of TD [217].

Although the atypical antipsychotic agents have lower propensity to produce extrapyramidal side effects, their use is associated with a higher incidence of weight gain than the typical antipsychotics. Given the deleterious effects of weight gain on the cardiovascular system as well as lipid and glucose metabolism, identification of potential markers for weight gain in at-risk patients treated with psychotropics would be beneficial. Among the various neurotransmitters involved in etiology of schizophrenia and/or mechanism of antipsychotic drug action, the involvement of the 5-HT_{2C} receptor is the most convincing with evidence converging on the –759C/T (rs3813929) polymorphism in the promoter region of the *HTR2C* gene as a predictor of risk of weight gain associated with atypical antipsychotic use [62,218–223], despite conflicting report of the functional significance of the C versus the T allele [224,225]. Nevertheless, most study results showed the C allele was significantly associated with weight gain. In contrast to studies that showed positive association of weight gain with the T allele, atypical antipsychotic treatment duration (less than 3 months) and ethnicity (European Americans and not African Americans or Asians) are variables that are found to be more prominent in studies with positive association of the C allele as a risk for weight gain.

More recently, research has also focused on the leptin–melanocortin system. The melanocortin 4 receptor (MC4R) is primarily located in the hypothalamus and mutations in the *MC4R* gene encoding MC4R are the most common genetic cause of obesity [226]. In a GWAS of pediatric patients, Malhotra and colleagues reported that SNPs in *MC4R* showed the strongest association after 12-weeks of second generation antipsychotic treatment in an initial discovery cohort of the 139 pediatric patients. Similar results were replicated for rs489693 in three additional cohorts comprising a total of 205 adult schizophrenic patients [227]. Subsequent replication studies showing lesser magnitude of association in autistic pediatric patients after 8-week risperidone treatment [228] and in adult patients after 4-weeks of second-generation antipsychotic treatment [229] suggests the association might be related to other factors related to the chronic nature of the illness and/or the duration of treatment.

Leptin is a peptide hormone secreted by the adipose tissue, with a proportional correlation

between the adipose-tissue amount and leptin level. Leptin activates secondary signals associated with food-intake inhibition and increased energy expenditure and high serum leptin level results in appetite suppression and energy storage. A functional −2548 A/G (rs7799039) polymorphism occurs in the promoter region of the gene coding the leptin protein (LEP), with the G allele implicated as the risk allele for weight gain [230,231], However, this SNP was not one of the four *LEP* SNPs identified in a more recent study [232]. Another SNP, a 223 Gln/Arg (rs1137101) polymorphism of the gene coding the leptin receptor (LEPR) have also been reported as risk predictors for weight gain [230,231]. Nevertheless, a report of negative association [222] makes it difficult to assess the clinical significance of these SNPs.

Mood Stabilizer

Response to Lithium

Even though therapeutic efficacy of lithium as a mood stabilizer has been shown to be associated with *SLC6A4* genotypes, with better outcome for patients with the *L/L* or *L/S* genotypes [233], most of the published pharmacogenomic studies of lithium primarily focused on the inositol turnover signaling pathway and the inhibition of glycogen synthase kinase 3-β (GSK3B). Patients with bipolar disorder are reported to have hyperactive signaling in the inositol turnover signaling pathway, and lithium use inhibits the activity of inositol polyphosphate-1-phosphatase (INPP1) and inositol monophosphotases (IMPA1 and IMPA2), resulting in reduced amount of free inositol available for signaling activity [234]. When comparing responders and nonresponders, an SNP (rs2067421) in the *INPPI* gene had been reported to be associated with lithium response [235], and Bremer et al. reported that the association is likely dependent on clinical subtype [236]. Benedetti et al. reported an association between *GSK3B* polymorphism and lithium response [237]. In the study by Bremer et al., the SNP (rs2199503) for *GSK3B* also was shown to be associated with lithium response in patients with posttraumatic stress disorder [236]. Failure to differentiate clinical comorbidity in past association studies might contribute to the conflicting results with *INPPI* and *GSK3B* polymorphisms in the literature. The potential role of *INPP1* and *GSK3B* polymorphisms has also been confirmed in a more recent study [238].

More recently, Hou and colleagues conducted a GWAS of lithium response in 2,563 patients worldwide and reported a single locus of four-linked SNPs on chromosome 21 detected genome-wide significance for response association. However, the same study did not report any association between lithium response and any of the previously reported SNPs [239]. Hopefully, the pending results from the multicenter prospective Pharmacogenomics of Bipolar Disorder (PGBD) study (ClinicalTrials.govNCT01272531) would provide additional insight and clarification on the genetic factors that influences clinical response to lithium [240].

Adverse Drug Reaction to Carbamazepine

One of the most useful applications of pharmacogenomics in psychiatry relates to the use of the anticonvulsant carbamazepine as a mood stabilizer. Despite its usefulness for patients with bipolar disorder, carbamazepine use is associated with severe adverse effects such as aplastic anemia and life-threatening cutaneous drug reactions such as Stevens–Johnson syndrome/toxic epidermal necrosis (SJS/TEN). The highly polymorphic Human Leukocyte Antigen Class 1 (HLA-1) genes encode proteins that bind and present antigens to immune cells. Abundant literature data support that the major histocompatibility complex *HLA-B*15:02* is a strong predictor of carbamazepine-induced Stevens–Johnson syndrome, primarily in patients with Asian descent [241]. The presence of *HLA-B*15:02* was documented in all 44 Taiwanese Chinese of Han descent with SJS/TEN.

Another study reported a positive association with *HLA-B*15:02* in 98% of 60 Han Chinese patients with the adverse drug reaction compared to 4% of patients who did not have the reaction [242,243]. A subsequent study confirmed the positive association in 94% of Han Chinese patients SJS/TEN compared to 9.5% of carbamazepine-tolerant patients, and 9% of healthy control subjects [244]. Similar associations have been reported for other Asian populations, despite variability in the frequency of *HLA-B*15:02* in those populations (Fig. 7.2) [245–249]. A black-box warning regarding this association in specific populations of susceptible individuals carrying the *HLA-B*15:02* allele was issued by the FDA in 2007, with a recommendation that regardless of their countries of origin, all patients of Asian descent should be screened for *HLA-B*15:02* prior to initiation of carbamazepine therapy, and alternative agent to be used in patients who are tested positive for the allele. However, it should be noted that (1) phenytoin also causes SJS/TEN [250] and is not a suitable alternative agent for carbamazepine in patients with the *HLA-B*15:02* variant, and (2) *HLA-B*15:02* is rare in both Japanese and Korean patients. Instead, other more common HLA alleles such as *HLA-B*15:11* and *HLA-B*31:01* are associated with carbamazepine-induced SJS/TEN in these two Asian populations

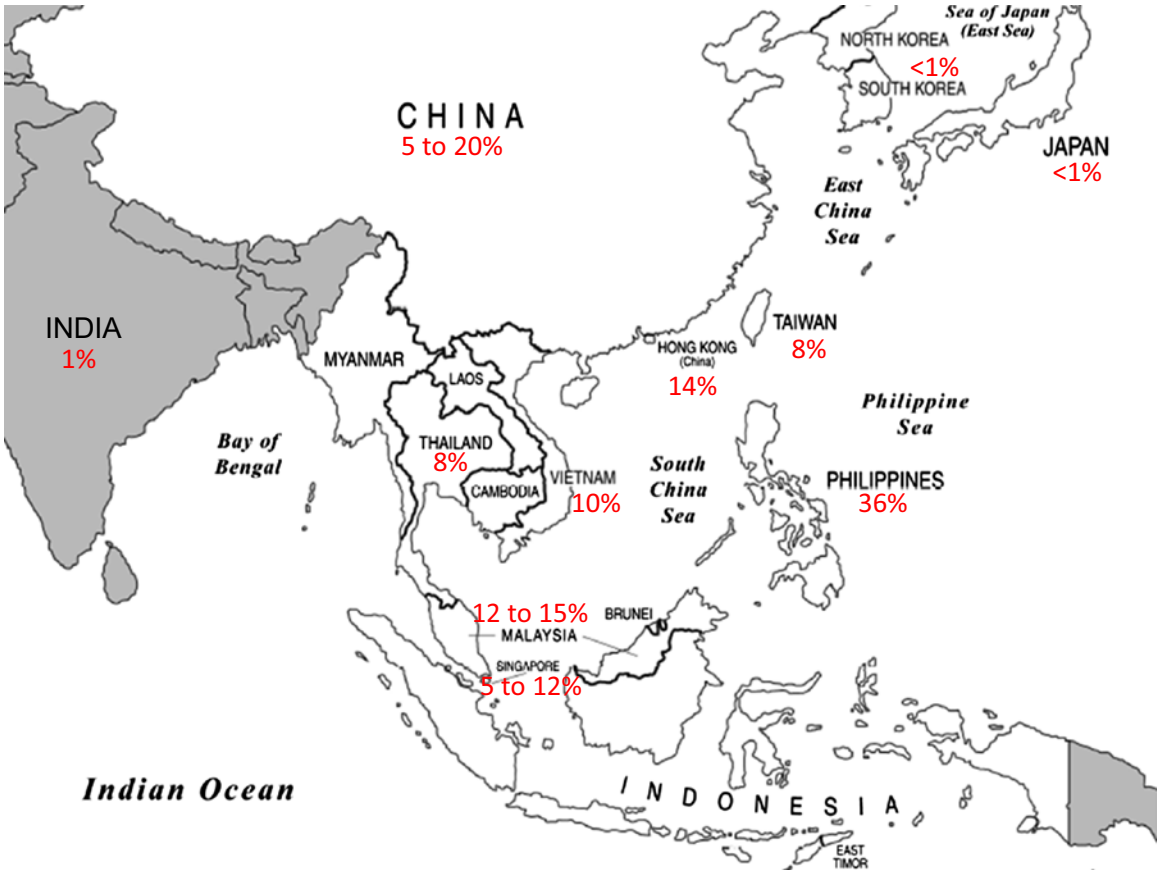

FIGURE 7.2 Ethnic differences in *HLA-B*15:02* in Asian populations.

TABLE 7.4 Challenges for Psychopharmacogenomic Evaluation and Implementation

Genomic studies mostly based on *post hoc analyses* of DNA samples collected from clinical trials that are not initially designed for pharmacogenomic evaluations
Heterogeneity of study populations with respect to
• Allele frequencies
• Ethnicity
• Patient-specific variables (gender, age, concurrent drug)
• Disease phenotypes
• Prior drug use
Study design with differences in
• Prospective versus retrospective versus naturalistic study
• Gene(s) investigated
• Selection of genetic biomarkers: single SNP versus haplotypes
• Treatment duration
• Response definition
• Response assessment
Inadequate sample size
Few prospective trials of pharmacogenomic-based clinical practice versus standard of care
Small incremental value in current quality and evidence-based driven clinical environment

[245,251,252] as well as in Caucasians [253,254]. A detailed clinical guideline for using HLA genotype in conjunction with carbamazepine or oxcarbazepine is included in the recently published 2017 update [255].

Despite documented substantial variability in psychotropic drug exposure among subjects with different genotypes of the cytochrome P450 (CYP) enzymes and suggested dosage regimens for carriers of the different CYP genotypes [6], there were only few studies that provided clear evidence of association with adverse effects, with even lesser-documented clinical validity based on psychotropic response prediction. Given the current literature data and the emerging role of neurotransmitter receptors and transporters in psychotropic response association, the utility of CYP genotyping in improving drug treatment could ultimately be in reducing side effects and improving medication adherence. Despite this utility, current literature suggests that the serotonergic system, in particular the –759C/T polymorphism affecting the 5-HT_{2C} receptor is much more promising in specifically predicting weight gain associated with the use of antipsychotics. Nevertheless, even with the abundance of research with neurotransmitter receptors and transporters, predicting psychotropic drug response remains a significant challenge (Table 7.4). Similar to CYP genotyping, the same limitations of lack of large-scale, prospective clinical trials, sample size, and ethnic variability need to be overcome. In addition, unlike drug metabolism, drug response is more likely to be mediated by multiple genes, and haplotype analyses would be critical in identifying appropriate association for prediction. Furthermore, disease progression could be impacted by environmental factors [256], which in turn, could impact treatment response and make interpretation of pharmacogenomic study data more difficult.

Application of Pharmacogenomics in Psychiatry

Drug development in psychiatry had made little progress over the last several decades. Although there have been better safety profiles for newer psychotropics, the CATIE study showed that the atypical antipsychotics represent only small improvement over the typical antipsychotics. Among all antidepressants, there were no real advantages of any newer SSRI over their older counterparts. Over the years, there have been many advances in

pharmacogenomics and expectations of what psychopharmacogenenomis could bring to psychiatric practice. Arguments for utilizing genetic information to maximize effectiveness of current drugs have been made by many investigators within the field of psychopharmacogenenomis. Nevertheless, there is still concern among many clinicians of the lack of clear evidence (based on large-scale randomized clinical trials) demonstrating when pharmacogenomic testing would be appropriate.

Clinical Validity and Utility of Psychopharmacogenomics

Genetic differences in psychotropic metabolism, most of which are mediated by the CYP enzyme systems, are well-established, and the frequency of drug-metabolizing enzyme polymorphisms also had been characterized in different ethnic groups. However, the limitation of single *CYP* gene screening is well-recognized [257], and clinical validity of such approach is only demonstrated for a few psychotropics that are significantly metabolized by one CYP isoenzyme. In addition, the small effect size for association between clinical outcomes and most of the *CYP* variants make the clinical significance somewhat questionable. The combinatorial pharmacogenomics approach combines different variant alleles to achieve more complete genomic information related to a drug, and has been advocated as a logical replacement for individual-gene testing [258,259]. Screening of different variant alleles for metabolizing enzymes, including common *CYP2C19* and *CYP2D6* alleles could be achieved with commercially available test panels [260], and is the most common application of pharmacogenomic advances in clinical practice when abnormal metabolic capacity is suspected to contribute to unexpected response [261]. However, even though multigene panels incorporating different genetic variants into a single assay is available [260], there is no standardization as to which pharmacokinetic and pharmacodynamic genetic variants are included in commercially available genetic test panels [262]. This lack of standardization is discussed further with respect to clinical implementation in Chapter 4.

Although current evidence demonstrates that most commercially available pharmacogenetic panels possess high analytic validity with good sensitivity and specificity in *CYP* genotype prediction (similar to the AmpliChip *CYP* genotyping test that was approved in 2004), demonstration of clinical benefit (clinical validity and utility) rests with the practitioners [261]. This not only leads to absence of specific dosing guidance from the regulatory agency for psychotropics, including atomoxetine, but also provides support against reimbursing *CYP* genotyping in psychopharmacotherapeutics. In addition, the availability of some of the gene analysis panels (primarily CYP1A2, CYP2C19, and CYP2D6), and a list of 26 psychotropic medications classified into different categories of recommendations that include "use as directed," "use with caution," and "use with caution with more frequent monitoring,"[263] but with little interpretation and/or guidance might be confusing to the consumers.

Not surprisingly, with very few well-designed clinical trials using patient-specific genotypes to demonstrate the clinical relevance of pharmacogenomic-guided dosing to optimize response rates and/or minimize adverse drug reactions, the utility of pharmacogenomics in clinical practice to influence prescribing pattern and patient outcome is almost nonexistent. Hall–Flavin et al. provided one of the few examples of potential utility and benefit of pharmacogenomic testing in the clinical environment. They first demonstrated in a prospective, proof-of-concept study that utilization of pharmacogenomic testing (*CYP1A2*, *CYP2C19*, and *CYP2D6* genotypes) in an outpatient setting resulted in significantly improved outcome in 44 patients (31.2% reduction in depression scores from baseline for pharmacogenomic-guided study participants compared to 7.2% in non-guided

participants (Quick Inventory of Depressive Symptomatology—Clinician Rated [QIDS-C16], $P = .002$) for different antidepressants and antipsychotics [263]. They then replicated the results in a follow-up prospective open-label study. Antidepressant response and remission rates in 227 patients were compared between genomic-guided prescribing (n = 114) with provision of pharmacogenomic report to clinician for their use, and usual care (n = 113) with no sharing of pharmacogenomicsinformation until completion of study. *CYP1A2*, *CYP2C19*, *CYP2D6*, *SLC6A4*, and *HTR2A* were the five genes available in the multigene test panel. After 8 weeks of therapy, patients receiving antidepressants based on genomic-guided interpretative reports provided to their prescribers had greater response (HAMD-17, $P = .03$; QIDS-C16, $P = .005$) and remission (QIDS-C16, $P = .03$) [264]. Despite the limitations of open-label design and lack of blinding of patients or clinicians that could be problematic with the well-known substantial placebo response to antidepressants, these two studies provide data (improved outcome) and perspective related to real-world application of pharmacogenomic testing. Such approach suggests an opportunity for incorporating pharmacogenomic data into clinical workflow for implementation in practice settings to guide treatment decision.

Cost-Effectiveness of Psychopharmacogenomics

In addition to clinical validity and utility, the issue of cost-saving remains uncertain. Chou and colleagues provided the earliest pilot utilization data in supporting potential cost-effectiveness of pharmacogenetic testing, consisting primarily of *CYP* genotyping at that time. The investigators genotyped 100 patients for *CYP2D6* and followed them over 1 year with assessment of adverse drug reactions, hospital stays, and total cost. They found three trends, including a higher incidence of side effects in patients with IM or PM phenotypes, a longer hospital stay for PMs, and an estimated higher annual cost of US$,4000 to $6,000 when treating patients with the extreme phenotypes (UMs and PMs) [47,265]. These results suggest that proper application of pharmacogenomic information could help reduce adverse drug events and better managing hospitalization duration, with resultant cost reduction. Subsequently, the cost-effectiveness for genetic testing with clozapine was evaluated [266]. Since then, more recent cost-effectiveness studies have also shown some encouraging data of reduced resource utilization and/or decreased average cost associated with pharmacogenomic testing. [34,258,267–272], including cost-effectiveness of pharmacogenomic testing in developed countries [271]. Implications of these study results will be explored further in Chapter 4.

Nevertheless, despite these real-world application results, additional studies with larger sample size are needed to validate the clinical utility of pharmacogenomic testing, including determining whether these multigene panels can shorten the remission time course, sustain duration of clinical remission, and reduce hospitalization and outpatient visits. In addition, such studies should expedite clinicians' decisions on medication choice or dosage adjustment with reasonable turnaround time for result reporting and interpretation [273], especially in patients from a diverse geographical locations and/or ancestral origins. In this regard, perhaps another approach to clinical psychopharmacogenomic investigations would be with a concentration-controlled trial to integrate relevant pharmacokinetic variants with important pharmacodynamic variants and complemented with PET evidence of drug-target occupancy, for example serotonin transporter occupancy for SSRIs [274,275]. Based on PET study, there is evidence of threshold 76%–85% serotonin-transporter occupancy for therapeutic response from different SSRI treatments [275–279]. In a 2001 study that investigated the

relationship between paroxetine concentration and serotonin-transporter occupancy, Meyer et al. showed the plateau occupancy of about 85% occurred when serum concentration of paroxetine exceeded 28 ng/mL [274]. Because paroxetine is metabolized by the polymorphic CYP2D6, the threshold drug concentration in the range of 28 ng/mL would not be achieved in some patients administered the standard dosage regimens, especially the UMs. One could argue that, without sufficient drug exposure at the target site, the relevance of any target polymorphism might be less. Data from this concentration-controlled approach might provide a more pragmatic design as an alternative to randomized controlled clinical trial, and hopefully an alternative perspective to the value of testing panels of genomic variants. For most practicing psychiatrists and clinicians, this may be more useful information than an endless list of potential and almost completely different sets of biomarkers of SSRI efficacy that have been identified by different GWASs [123–125,143].

Challenge Posed by Ethnic Variation in Allele Frequency

In assessing the clinical utility and cost-effectiveness of psychopharmacogenomic testing, the major challenges for drawing appropriate conclusions from drug-disposition and response investigations are undoubtedly related to differences in phenotypes (response definition, clinical presentation, treatment history), sample size (variable and mostly small), and different study designs (non-uniformed protocols and lack of standardization of data collection). In addition, significant variations in genetic background exist among various ethnic groups. Therefore, interpretation of psychopharmacogenomic findings in drug disposition and response among many of the study groups could be further complicated by regional differences in frequencies of known alleles and/or overinterpretation of data for a large region consisting of different racial or ethnic groups. Among people residing in the Pacific region, the frequency of *HLA-B*1502* risk allele for SJS is extremely high for subjects of Chinese heritage, but occur in less than 1% in Koreans and Japanese. Using ethnic variation in allele frequencies for genes encoding drug-metabolizing enzymes (CYP2C19 and CYP2D6) and targets (SLC6A4) that are relevant for antidepressant disposition and response, the following sections will highlight the importance of ethnicity definition and the implications of ancestry for psychopharmacogenomics research.

As described earlier in this chapter, the S-allele for 5-HTTLPR is associated with inferior response to SSRI therapy. However, this association appears to hold true primarily for Caucasian populations, whereas the opposite association (S allele conferring better therapeutic response) is observed in patients within Asian populations [83,87]. This may be partially related to ethnic-related differences in the frequency of the 5-HTTLPR L- and S allele, with the L allele as the predominant allele for Caucasians, whereas the S allele is the predominant variant for the Asian populations (Table 7.5).

The SNP rs25531, located just upstream of the 5-HTTLPR, was also shown to affect *SLC6A4* expression. The SNP results in expression level for the G allele that is comparable to that of the S allele for 5-HTTLPR, and much lower than that of the A allele for rs25531. Therefore, carriers of the L_G allele (G allele of rs25531) would be expected to respond less to SSRI compared to carriers of the L_A allele (A allele of rs25531). This has been shown by the study results of Dreimueller et al. The investigators reported a favorable therapeutic outcome in L_A allele carriers that was correlated with serum SSRI concentration ($P = .001$) but not in patients with the L_G allele ($P = .31$) [91]. Not surprisingly, significant ethnic variations in the triallelic and resulting genotype frequencies exist, as reported by Haberstick et al. The L_G allele for rs25531 is less frequent (<25%) than the L_A allele and the S allele for all studied populations from North America, Southeast Asia, and Africa. Among the different populations, the

TABLE 7.5 Ethnic Differences in Allele Frequencies (%) of 5HTTLPR and STin2 VNTR

Ethnicity	L Allele of 5HTTLPR	S Allele of 5HTTLPR	9-Repeat Allele of VNTR	10-Repeat Allele of VNTR	12-Repeat Allele of VNTR
Caucasians	60	40	1	47	52
African Americans	83	17	1	26	73
EAST ASIANS					
Chinese	26	74	<0.1	8	92
Japanese	20	80	<0.1	2	98
Koreans	23	77	<0.1	10	90
SOUTH ASIANS					
Chinese		64			
Indian		58			
Malay		61			
Hispanics	49				N/A

Pooled data from references S. Porcelli, C. Fabbri, A. Serretti, Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with antidepressant efficacy, European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology 22 (2012) 239–258, T. Niitsu, C. Fabbri, F. Bentini, A. Serretti, Pharmacogenetics in major depression: a comprehensive meta-analysis, Progress in Neuro-psychopharmacology and Biological Psychiatry 45 (2013) 183–194.

frequency of the L_G allele is lower (absence or near absence) for Hispanics, Caucasians, and Native Americans than in Asians and Africans or African Americans [280].

In addition to 5-HTTLPR, additional polymorphisms of the *SERT* gene have been identified with potential roles in modulating SSRI response. This include the extralong allele with high-frequency occurrence in Asians, African Americans, and non-Hispanic Whites reported by Haberstick et al. [280]; 14 novel allele variants in Japanese and Caucasians, all with variable distribution frequency [88]; and the 17-base pair, variable number of tandem repeats (VNTR) polymorphism within intron 2 of *SLC6A4* [93], with higher allelic frequency in Asians compared to Caucasians (Table 7.5) [281]. Therefore, it is clear that even for *SLC6A4*, response prediction would have limited success if evaluating only single SNPs in a given patient, which could be further impacted by the significant ethnic variation in frequency of important alleles, small number of study participants from various ethnic populations, and the use of self-identity for defining ethnicity in pharmacogenomic investigations.

Another issue for considering ethnicity as a variable in associating response with genetic variants is the ancestral origin of the subject cohort. Although it might be commonly perceived that Asian populations in regions of close proximity are relatively "homogeneous," that might not be necessarily true in reality. As an example, the South Indian populations as a group are genetically distinct from the North Indians and East Asians[282]. Similarly, early pharmacogenetic studies reported population differences in distribution of *CYP2C19* and *CYP2D6* variants and genotypes exist among Han, Bai, Wei, Zang, and Mongolian subpopulations in China [283,284]. More recently, Suarez–Kurtz et al. also reported ancestral influence on frequency distributions of different pharmacogenomic genes among three Brazilian populations with different ancestral roots [285].

In this regard, even though there is no unanimous accepted definition of ethnicity and race, and maybe even ancestry, it is important to recognize that "ethnicity" and the usually interchangeable term "race" are not biological terms. Rather, they are sociological terms describing groups of people with common heritage and sharing similarities in culture, beliefs, values, and possibly language as well as cuisine. Ancestry, on the other hand, is a biological term in population genetics, and represents the origin or genetic line of descent of one individual or family. Although outside the scope of this chapter, it is generally accepted that genetic diversity has been "modified" since the first human colonization of Europe more than 40,000 years ago, resulting in admixed populations around the world with variable extent of genetic diversity from African, European, and Native American ancestries. This undoubtedly has an influence on allele frequencies of any gene of interest among global populations, as discussed earlier with the *SLCA64* gene.

One can even further argue that the complicated issues associated with admixed populations and self-identified ethnicity discussed in aforementioned sections are the reasons that data extrapolation from one ethnic/racial group to another should be minimized. Nevertheless, while awaiting future pharmacogenomic studies enrolling large numbers of cohorts from under-represented ethnic minority groups or development of dosing guidelines/algorithms specific to individual ethnic groups or admixed populations, ethnicity-related genetic information may still be useful for practitioners when no genotyping result is available. A good example is carbamazepine. Regardless of the exact reason why *HLA-A*3101* is of high prevalence in Caucasians versus *HLA-B*1502* is of high frequency in Asians, the fact remains that in the absence of genotype information, one could make a rational therapeutic decision and advocate use of other antiepileptic drugs instead of carbamazepine or phenytoin in patients of Chinese heritage.

In summary, the "increased' genetic diversity represented by admixed populations [286] in pharmacogenomic studies presents further challenges to assessment of association between genetic variants and pharmacological responses in studies not properly stratified by ethnic groups. The challenge of interpreting different, sometimes even contrasting, allele frequencies reported from multiple studies of gene variants is further compounded by subject cohorts with self-identified ethnicity. Although financial restraints and "ease-of-use" are legitimate reasons allowing self-identified ethnicity to categorize study subgroups, the absence of allele(s) important for response assessment from pharmacogenomic studies or test panels would complicate interpretation of study results, as illustrated with the case of warfarin and highlighted in Chapter 6.

Clinical Application in Selective Patients

From the perspective of *individualized* therapy, *individual* difference in drug response is attributable to his or her specific genotype for the gene variant of interest. Hence, in this regard, ancestral origin and/or ethnicity (regardless of whether it is well defined or self-identified) of the patient is not necessarily a good predictor of pharmacological response. This is illustrated with the example of James Watson, who, despite being self-identified as a Caucasian, is a homozygous carrier of the *CYP2D6*10* allele [287]. Therefore, despite the rare occurrence of this **10* variant in the overall Caucasian population, Dr. Watson would be expected to metabolize CYP2D6 substrates at a rate similar to that of most Asians, which is slower than most Caucasians. The following sections present how psychopharmacogenomics information could be used in optimizing therapy for individual patients.

Most of the literature focuses on assessing potential improved efficacy and/or reduced toxicity with pharmacogenomic testing, and very few studies evaluate the potential utility

for genetic testing to guide appropriate use of alternative drug therapy. Although there are suggestions that homozygous carriers of S allele of *SLC6A4*, especially Caucasians, would be least likely to benefit from SSRI, few literature data document the clinical outcome, let alone cost-effectiveness, of switching to antidepressants other than SSRIs. Rather than waiting for affirmative studies or consensus guidelines, which might not happen for years to come, perhaps one value of pharmacogenomic testing in clinical psychiatric practice is to help determine the basis for an individual patient's lack of response and/or exhibition of unusual adverse reactions to drug regimens. Leahy described an 18-years-old patient with intermittent explosive disorder who had failed multiple medication regimens, including fluoxetine and escitalopram (produced side effects of restlessness and diarrhea in the patient), as well as risperidone, aripiprazole, and ziprasidone (produced side effects of irritability and weight gain). The patient consented to pharmacogenomic testing, which revealed that he is a heterozygous carrier of the S allele of *SLC6A4* and the risk allele of *DRD2* rs1799732, as well as a homozygous carrier of the C allele of the *5HT2C* rs3813929. This genetic profile provides a biological basis for his poor response to SSRIs and dopamine-2 receptor antagonists, as well as his history of intolerable weight gain associated with the use of the atypical antipsychotic agents. Just as importantly, the pharmacogenomic analysis suggested that the patient is likely not a candidate for drug that targets the SLC6A4, DRD2, or 5HT2C. Based on this information, a trial of lithium was initiated for the patient and titrated to achieve a target concentration of 1 mEq/L, which resulted in decreased outbursts and disappearance of extreme rage. Over a 3-month period after starting lithium, the patient only exhibited two brief anger episodes, both of which were of much less severity and much shorter duration compared to those before initiation of lithium therapy [288].

Another example of using genetic profile to identify appropriate alternate therapy involves variants of *MTHFR*, which encodes methylenetetrahydrofolate (MTHFR). MTHFR is an important enzyme involved in the pathway that produces methylfolate and the mood-regulating monoamine neurotransmitters. The C677T is a *MTHFR* variant associated with decreased MTHFR activity and methylfolate level, ultimately resulting in impaired synthesis of neurotransmitter, increased risk of depression, and reduced response to antidepressants [289,290]. The use of L-methylfolate after identification of the C677T variant was reported in a 69-years-old Caucasian male patient with major depressive disorder. The patient failed duloxetine therapy and partially responded to venlafaxine. Pharmacogenomic analysis revealed the patient as a carrier of the S allele of *SLC6A4*, the C677T variant of *MTHFR*, as well as a homozygous carrier of the Val allele of *COMT* and the C allele of the −759C/T polymorphism. The patient's genetic profile provides an insight into his therapeutic responses. Duloxetine has higher selectivity for serotonin and norepinephrine transporters. The Val allele is associated with higher COMT activity and partially explains his apathy, poor concentration, and lack of motivation. After evaluating his genetic profile, the clinician initiated a trial of 15 mg of L-methylfolate, which resulted in a complete remission of symptoms [291].

Therefore, a potential *practice* model for patient care, especially in primary care settings, could involve using the electronic health record to identify patients who can potentially benefit from pharmacogenomic testing. Clinical pharmacists can then perform comprehensive medication review and strategies for best-candidate genes. Results from testing can then be used to guide any necessary medication changes in patients with suboptimal control of symptomatology, with further patient evaluations using standardized clinical ratings and additional medication

monitoring. Further contribution to advancing the field can involve collecting standardized data from the healthcare professionals regarding the impact of the testing for the providers and the patients. The value from such an approach would be an emphasis on how pharmacogenomic testing results in appropriate drug selection with improvement in outcome and reduction in associated healthcare cost in an individual patient, rather than whether pharmacogenomic testing should be part of the standard of psychiatric care.

Incorporation in Drug Development

Chapter 3 provides several examples of how pharmacogenomic data can be incorporated in drug development. The following section will illustrate another example of pharmacogenomics application.

Vilazodone, approved by the FDA in January 2011, is the first of a new class of antidepressant (the indolealkylamines) with dual action of serotonin reuptake inhibition and partial agonist activity at the 5-HT_{1A} receptor [292]. The initial development of vilazodone in Phase II was discontinued because response was not significantly better than placebo, even though studies incorporating an active comparator also showed failure of comparators demonstrating superiority over placebo [293]. Subsequent development included a clinical trial with patient stratification according to a combination of genetic biomarkers most likely to be associated with therapeutic response to vilazodone but not to other antidepressants [293].

A report described the association of haplotypes of biomarkers involved in neurotransmitter signaling and vilazodone metabolism with clinical response, although the identity of the biomarkers was not revealed. The result indicated that 75.5% of 49 vilazodone-treated patients who also possess one specific biomarker ($M1^+$) responded to therapy (defined as a decrease of at least 50% from the baseline Montgomery–Asberg Depression Rating Scale [MADRS] score after 8-weeks of treatment), whereas only 35.2% of 108 "marker-negative" patients ($M1^-$) treated with vilazodone. Remission (defined as final MADRS score of less than 10) was achieved in 44.9% of "marker-positive" patients and 20.4% of "marker-negative" patients. 57.1% of 14 vilazodone-treated patients with another biomarker ($M2^+$) were reported to have nausea and vomiting compared to 15.5% of patients without the same biomarker [294]. Despite the small number of patients, the study represents an example of early use of biomarkers in drug development. Vilazodone studies listed on ClinicalTrials.gov include one that investigates genetic markers associated with response in major depressive disease. When published, results from this trial and those from ongoing replication studies will provide insight as to whether these biomarkers allow clinicians to predict which patients might respond more fully to vilazodone and who would experience adverse side effects. If confirmed, the unique dual pharmacological action of vilazodone and availability of clinically relevant biomarkers could provide significant contribution to individualized clinical treatment.

CONCLUSIONS

Psychopharmacogenomic research over the last decade or so has attempted to associate treatment response with neuronal circuits upstream and regulatory genes downstream [134–137,144–146]. Despite many findings within the field of psychopharmacogenomics, only a few of the results are ready for translation into clinical practice. Although *CYP* genotyping was previously recommended for incorporation into the therapeutic decision-making process, the current evidence-based approach significantly limits its application in clinical practice. Compared to other therapeutic areas such as cardiovascular disease and

cancer, promising research findings to predict drug response in psychiatric illnesses is still in its infancy. Multiple genetic biomarkers have been identified by either candidate-gene approach or GWAS, and evaluated in clinical studies involving different designs and various ethnic populations. To date, lack of consistent results among the clinical studies does not point to definitive associations for most biomarkers. However, that should not preclude the rational use of psychopharmacogenomic test panels to guide choice of therapy for patients in clinical practice, especially for those who could not respond to, or are intolerant of, evidence-based first-line therapies.

Given the currently available psychotropics and the lack of novel compounds in the foreseeable future, pharmacogenomics hold significant promise in optimizing drug therapy for the mentally ill populations. Further advances in the field would require indepth understanding of mental-disease etiology, developing clear definitions of response phenotypes and outcome measurements, and refining current molecular approaches. Pharmacogenomic results can nevertheless be incorporated into a decision-making model to enable a genetically informed and data-driven approach to optimize therapy for individual patients.

QUESTIONS FOR DISCUSSION

1. How do ethnic variabilities in allele frequencies affect interpretation of study results in psychopharmacogenomics?
2. Are there significant roles for *ABCB1* polymorphism in psychotropic disposition and response?
3. What is the significance of the STAR*D study with respect to 5HTTLPR polymorphism?
4. What are some of the factors that slow translation of pharmacogenomic findings into practice for psychopharmacology, in contrast to other therapeutic areas such as oncology and cardiovascular diseases?

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CHAPTER

8

Pharmacogenomic Considerations in the Treatment of HIV Infection

Surulivelrajan Mallayasamy[1,2], *Scott R. Penzak*[3]

[1]Post-Doctoral Research Associate, Department of Pharmacotherapy, University of North Texas System College of Pharmacy, Fort Worth, TX, United States; [2]Department of Pharmacy Practice, MCOPS, Manipal University, Manipal, India; [3]Department of Pharmacotherapy, University of North Texas System College of Pharmacy, Fort Worth, TX, United States

OUTLINE

INTRODUCTION

As of 2016, approximately 37 million people were infected with the human immunodeficiency virus (HIV), with two million new infections reported each year [1]. Once uniformly fatal, HIV has largely become a chronic manageable illness due to the advent and widespread use of potent combination antiretroviral therapy (cART). Indeed, AIDS-related deaths have dramatically fallen from their peak number in 2005 and millions of new cases are prevented due to the use of cART [2].

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00008-5

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Currently, there are 24 available antiretroviral (ARV) medications from six classes. These include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), entry inhibitors (EIs), and integrase strand inhibitors (INSTIs) [3]. Combination products and pharmacokinetic enhancers such as ritonavir and cobicistat are also available. The goal of cART, which must be administered throughout the course of a patient's life, is virologic suppression and maintenance, or reconstitution of immunologic function.

In simplest terms, the goal of antiretroviral therapy (ART) is to maximize therapeutic benefits while minimizing adverse events. Understanding variation in efficacy and toxicity of antiretroviral (ARV) drugs is evolving over time. Individualization of ARV therapy requires a holistic understanding of the drug, virus, and patient in terms of demography and pharmacogenetics. Testing for *HLA-B*57:01* and its association with abacavir hypersensitivity has been a success story and a notable example of pharmacogenetics-guided individualization of therapy [4]. Success of abacavir pharmacogenetic testing in routine clinical practice offers valuable insight for future implementation of pharmacogenetics as a tool to optimize antiretroviral pharmacotherapy (Table 8.1).

A number of factors, including drug interactions, age, gender, ethnicity, comorbidities such as hepatic or renal failure, pregnancy, and genetic differences can result in interpatient variability in ARV drug response [5]. Indeed, differences in genes that encode for drug targets, receptors, metabolizing enzymes, and drug transporters can contribute to variations in ARV efficacy and toxicity. Knowledge of interindividual pharmacogenetic differences has the potential to assist clinicians in individualizing ART to maximize therapeutic benefits. This chapter will discuss those ARV medications for which pharmacogenetic data are available.

NUCLEOSIDE AND NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS

Abacavir

Abacavir is an NRTI indicated for the treatment of HIV infection when used in conjunction with other ARVs [6]. It is commercially available as a single-dose formulation or in fixed-dose combinations with other ARVs that include lamivudine, zidovudine, and dolutegravir.

Although abacavir is generally safe, hypersensitivity reactions (HSRs) have been reported after initiation of treatment. These reactions occur in 5%–8% of patients treated with abacavir and typically manifest after 2–6 weeks of treatment [7,8]. The abacavir HSR is characterized by signs and symptoms in two or more of the following categories: fever; rash; gastrointestinal symptoms such nausea, vomiting, and diarrhea; constitutional symptoms such as myalgia, fatigue, and achiness; or respiratory symptoms such as dyspnea, cough, and pharyngitis. Upon developing the abacavir HSR, it is imperative that the drug be discontinued. Continuing abacavir in this setting can result in worsening of symptoms and rechallenge is contraindicated as it can result in severe, potentially fatal reactions [9,10]. Because symptoms of the abacavir HSR are nonspecific, they might be confused with other common conditions or infections. This issue was problematic in controlled double-blind trials leading to false positive diagnoses of the abacavir HSR in patients not receiving abacavir [11].

Shortly into the new millennium, an association was reported between the abacavir-induced HSR and the presence of the major histocompatibility complex (MHC) class I allele HLA-B*57:01 [12,13]. Since then, a number of additional studies have explored this association and observed similar findings [11,14,15]. The prevalence of the HLA-B allele varies among ethnicities. It is more commonly seen in Caucasians (6%–10%) compared

TABLE 8.1 Summary of Pharmacogenomics of Antiretroviral Medications

Drug	Genes	Effect	References
NUCLEOTIDE AND NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS			
Abacavir	*HLA-B*57:01*	Hypersensitivity reaction	[11,12,13]
Tenofovir	*ABCC4* 3463 A>G, *ABCC4* 4131T>G	Higher intracellular TFV concentrations	[31,34]
	ABCC2 24T	Higher urinary excretion	[35]
	ABCC10 SNPs (rs9349256 G>A, and rs2125739 C>T)	Renal tubular dysfunction	[36]
Zidovudine	*UGT2B7**1C	Higher AZT clearance	[42]
	ABCC4 G3724A	Higher intracellular AZT-TP concentrations	[43]
Lamivudine	*ABCC4* T4131G	Higher lamivudine-TP concentrations	[48]
NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS			
Efavirenz	*CYP2B6* 516TT, *CYP2B6* 516GT	Higher efavirenz concentration and CNS side effects	[53]
	CYP2B6 983TC		[53]
	CYP2B6 15582CT (rs4803419)		
Nevirapine	*CYP2B6* 516GT and TG	Lower nevirapine concentrations	[61]
	CYP2B6 516TT	Higher nevirapine concentrations	[62]
	ABCC10 (rs2125739)	Lower nevirapine concentrations	[63]
	ABCB1 3435CT	Less likelihood of hepatotoxicity	[64]
	*HLA-DRB1*01:01*, *HLA-DRB1*01:02*, *HLA-B*35*, and *HLA-Cw*04*	Adverse skin events	[65]
	*HLA-Cw*08*	Hepatic adverse events	
Etravirine	*CYP2C19*2*, *CYP2C9*3*	Reduced clearance of etravirine	[66]
PROTEASE INHIBITORS			
Atazanavir	*CYP3A5*1*	Higher clearance	[73]
	ABCB13435C→T homozygous	Severe hyperbilirubinemia	[74]
	PXR T63396T	Lower atazanavir concentrations	[76]
	UGT1A1(*28/*28 or *28/*37)	Jaundice	[37]
Lopinavir	*SLCO1B1*4*	Higher lopinavir clearance	[78]
	CYP3A and *ABCC2*	Lower lopinavir clearance	
	*SLCO1B1*521T→C	Higher plasma lopinavir concentrations	[79]
INTEGRASE STRAND TRANSFER INHIBITORS			
Raltegravir	*UGT1A1*28/*28*, *ABCG2* 421 CA/AA, *ABCB1* 4036 AG/GG	Higher raltegravir concentrations	[84]
Dolutegravir	*UGT1A1*(*28/*28,*28/*37,*1/*6,*1/*28, *1/*37,*28/*36, and *36/*37)	Lower dolutegravir clearance	[91]

to East Asian (1%–3%) or African populations (1%–2%). In fact, this allele is completely absent in certain ethnic groups such as Japanese individuals [16]. HLA-B allele status determines the susceptibility to hypersensitivity reactions but does not alter the pharmacokinetics or pharmacodynamics of abacavir in any of the studied ethnic groups.

In a large prospective multicenter study, PREDICT-1, the predictive power of HLA-B*57:01 (rs2395029) screening was tested by Mallal et al. [11] Out of 1956 HIV-infected patients from 19 countries, the prevalence of HLA-B*57:01 was 5.6%. The positive predictive power of HLA-B*57:01 was 47.9% (i.e., out of 100 people who are HLA-B*57:01 positive, 48 will develop the abacavir HSR). The negative predictive power was 100%, indicating that no subjects developed the abacavir HSR who were not HLA-B*57:01 positive [11]. As such, when a patient tests negative for HLA-B*57:01, they can safely be administered abacavir, as there is no chance they will develop the abacavir HSR. In those patients who test positive for HLA-B*57:01, abacavir should be withheld. To this end, HLA-B*57:01 screening has become the standard of care for all abacavir-naïve individuals before initiating therapy [17].

In 2008, the United States Food and Drug Administration (FDA) approved a change in the abacavir package insert that recommended HLA-B*57:01 screening for all patients prior to abacavir initiation, regardless of race or ethnicity [18]. Similar recommendations were made by the Department of Health and Human Services (DHHS) Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents, the European Medicines Agency, and the Clinical Pharmacogenetics Implementation Consortium (CPIC). CPIC guidelines recommend that HLA-B*57:01 screening be performed in all abacavir-naïve individuals before initiating therapy. Noncarriers of this polymorphism can be initiated with abacavir therapy in recommended doses. Carriers of HLA-B*57:01 should not be prescribed abacavir except under exceptional circumstances when potential benefits outweigh the risks [16,19]. Medical records of HLA-B*57:01-positive patients should clearly indicate that the patient is allergic to abacavir to avoid future administration of the drug.

There are a number of reasons why HLA-B*57:01 screening has been successful. First, HIV care providers comprise a relatively small group of clinicians who are largely accessible through publications and conference presentations. This is not typically the case for larger groups of clinicians such as those treating patients with diabetes or cardiovascular disorders. Second, most HIV clinicians are already familiar with the use of genetic tests to interpret viral resistance panels; therefore, they may be more amenable to incorporating an additional genetic screening test into clinical practice. Third, the HLA-B*57:01 screening test is simple to interpret: if it is positive, abacavir should not be administered. In addition, because HLA testing for abacavir HSR does not involve complex dose adjustments or knowledge of multiple polymorphisms, its implementation is straightforward and easily adopted [20]. Although it depends on specific policies and plans, most third-party payers will cover HLA-B*57:01 screening for HIV-infected patients who may be starting abacavir therapy.

A study was carried out to assess the economic efficiency of prospective HLA-B*57:01 screening in ARV-naïve patients, using a 60-da decision tree model. Prospective HLA-B*5701 testing cost an additional US$17 per patient, and prevented 537 HSRs per 10,000 patients. Based on these results, the authors recommended that abacavir screening is economically viable and should become the standard of care [21]. Additional studies in the United States and other countries have also concluded that HLA-B*5701 screening is cost effective and should be performed as a part of standard care [14,22,23]. HLA-B*57:01 testing for the abacavir HSR represents an accessible, cost-effective example of successful implementation of pharmacogenetic testing in clinical practice.

Because HLA-B*5701 screening has a 100% negative predictive potential and is economically feasible (considering the high cost of treating a single case of abacavir HSR), it represents a widely accepted example of successful pharmacogenetic testing in clinical practice.

Tenofovir

Tenofovir disproxil fumarate (TDF) and tenofovir alafenamide are prodrugs of the NRTI tenofovir. Tenofovir is combined with other ARVs to treat patients with HIV. It is also used for pre- and post-HIV prophylaxis (PrEP and PEP, respectively) [24]. Tenofovir has a long intracellular half-life and is largely excreted unchanged in the urine by glomerular filtration and tubular secretion [25]. Although tenofovir is generally safe, it has been associated with increases in serum creatinine and modest reductions in creatinine clearance [24].

In a systematic review and metaanalysis, it has been reported that TDF-containing regimens significantly increased the risk of acute renal failure in a small number of patients (risk difference, 0.7%, 95% confidence interval [CI], 0.2–1.2). There was a trend toward greater degree of TDF-associated renal function loss in ARV-experienced patients compared to ART-naïve patients (Mean decrease in glomerular filtration rate (GFR), −2.50 vs. −5.15 mL/min; difference in TDF-associated renal function loss, 2.92 mL/min; 95% CI, 6.02 to −0.18 mL/min) [26]. Rarely, Fanconi Syndrome, which is characterized by loss of electrolytes, amino acids, glucose, and reduction in creatinine clearance, has been reported in patients receiving tenofovir (<0.1% incidence) [27]. Renal toxicity is observed more commonly when tenofovir is combined with other nephrotoxic agents or HIV-protease inhibitors. Long-term consequences such as bone demineralization due to calcium and phosphate wasting is a concern with tenofovir therapy [28].

Tenofovir is transported (20%–30%) into renal epithelial cells via organic anionic transporters (OAT) OAT1 (solute carrier [*SLC*]*22A6*) and OAT3 (SLC*22A8*). It is then secreted into the tubular lumen through multidrug-resistant proteins (MRPs). MRP2 and MRP4, encoded by *ABCC2* and *ABCC4*, respectively, were associated with renal toxicity secondary to tenofovir in several reports [29–33]. In vitro studies suggest that tenofovir is secreted by MRP4. The reported association between MRP2 and tenofovir-induced renal tubulopathy is unclear [28]. One theory is that an unidentified factor secreted by MRP2 potentiates tenofovir-induced tubulopathy. An alternative proposal is that the ABCC2 haplotype may be in linkage disequilibrium with polymorphic genes that encode an unidentified factor that might play a role in tenofovir-induced tubulopathy [28].

Kiser et al. investigated the relationship between intracellular tenofovir concentrations and [34]genetic polymorphisms in ABCC2 and ABCC4. ABCC4 3463 A>G was significantly associated with 35% higher intracellular concentrations of tenofovir compared to the ABCC4 wild-type gene ($P = .04$) [31]. In another study by the same group of investigators, the relationship between single-nucleotide polymorphisms (SNPs) that encode for renal proximal tubule efflux transporters and tenofovir pharmacokinetics was assessed. Carriers of the ABCC4 3463G variant had renal clearance values 15% lower, and area under the concentration-versus-time curve (AUC) values 32% higher compared to wild-type patients ($P = .05$). In addition, urinary excretion of tenofovir was 19% higher in ABCC2 24T carriers compared to wild type ($P = .04$) [35]. In a recent study conducted in a Thai patient population, patients carrying the ABCC4 4131T>G variation (genotype TG or GG) had on average, 30% higher plasma tenofovir concentrations compared to patients carrying the TT genotype ($P = .072$), although this did not reach statistical significance. When a middose concentration of tenofovir >160 ng/mL was used as a cutoff for risk of renal toxicity, all patients with the ABCC4 4131T>G variation

(genotype TG or GG) had concentrations above this cutoff value and were potentially at higher risk for developing renal toxicity [34].

Pushpakom et al. [36] assessed the influence of ATP-binding cassette subfamily C member 10 (*ABCC10)* (which encodes for MRP7) genetic variants on kidney tubular dysfunction using ABCC10-transfected human embryonic kidney (HEK)293 cells and cluster of differentiation (CD)4+ cells of monocyte derived macrophages. Results from this in vitro study revealed that tenofovir is a substrate for MRP7, and two ABCC10 SNPs (rs9349256 G>A, and rs2125739 C>T) and their haplotypes were significantly associated with tubular dysfunction ($P < .05$). In addition, rs9349256 was associated with microglobinuria and urine phosphorus wasting ($P = .04$ and .02, respectively). Results from this study suggest that ABCC10 genetic variants may contribute to renal tubular toxicity with tenofovir. Further study in humans is necessary to confirm or refute these preclinical findings [36].

An observational study conducted in a cohort of 500 patients receiving tenofovir assessed the influence of genes previously reported to be associated with tenofovir renal toxicity; these included ABCC2 (rs2273697; G>A) and ABCC4 (rs899494 C>T). Neither of these genetic variants were significantly associated with rates of tenofovir discontinuation. Of note, the final cyclooxygenase analysis identified low body weight (<60 or 60–69 kg) as a risk for tenofovir discontinuation [37].

Tenofovir-induced tubulopathy and its long-term impact on bone health are concerns for individuals receiving this drug for PrEP, PEP, Hepatitis B, or as a part of cART. Although ABCC4 3463 A>G has been significantly associated with renal tubular toxicity in patients receiving tenofovir, development of this condition is generally slow in onset and can be monitored for by periodically assessing measures of renal function such as serum creatinine, blood urea nitrogen, electrolytes, and urine protein. The tenofovir manufacturer states that in patients with creatinine clearances (CrCl) 30–49 mL/min, tenofovir should be dosed at 300 mg every 48 h; in patients with CrCl 10–29 mL/min, tenofovir should be dosed at 300 mg every 72 or 96 h. In patients undergoing hemodialysis, tenofovir should be dosed 300 mg once weekly or after a total of 12 h of dialysis [38]. Currently, data are insufficient to support pharmacogenetic testing (i.e., ABCC4) to identify patients at risk for renal tubular toxicity with tenofovir. Until more data become available, clinicians are advised to carefully monitor renal function in patients receiving tenofovir and adjust tenofovir dosing if indicated.

Zidovudine

Zidovudine, also known as azidothymidine (AZT), was the first antiviral to be approved for the treatment of HIV. Although no longer a first-line agent, zidovudine is still used in combination with other ARVs for the treatment of HIV [39]. Zidovudine is an NRTI that undergoes intracellular phosphorylation reactions to eventually yield zidovudine-triphosphate (zidovudine-TP), which is the active moiety of the drug [40]. Zidovudine is largely renally eliminated as parent compound and inactive metabolites; it also undergoes glucuronidation primarily by uridine diphosphate glucuronosyltransferase (*UGT)2B7* [41]. Individuals with the UGT2B7 polymorphism UGT2B7*1C had a mean zidovudine clearance value that was 196% higher compared to individuals who did not possess the variant [42]. In a pharmacogenetic study of zidovudine, Anderson et al. observed elevated intracellular concentrations of zidovudine-TP in carriers of the *ABCC4* G3724A variant. There was a 49% increase in zidovudine-TP intracellular concentrations in individuals with at least one variant allele (AG or AA) compared to wild-type (GG) individuals ($P = .03$) [43]. Nonetheless, data are limited and there is still not a well-defined relationship between zidovudine-TP concentrations and efficacy or toxicity. As a result, pharmacogenetic testing for zidovudine is not currently indicated and any future role is highly unlikely.

Lamivudine

Lamivudine is an NRTI that is used in combination with other ARV agents for the treatment of HIV infection. Lamivudine undergoes rapid oral absorption and is largely excreted in the urine (approximately 70%) as unchanged drug [44]. Lamivudine is a substrate for three types of organic cation transporters (OCT), OCT1 (SLC22A1), OCT2 (SLC22A2), and OCT3 (SLC22A3); it is also transported by breast cancer resistance protein (BCRP) (ATP-binding cassette subfamily G member 2 [*ABCG2*]). Lamivudine-triphosphate (lamivudine-TP) is transported by MRP4 (ABCC4) [42]. Genetic polymorphisms in the genes encoding for these transporters could potentially impact the disposition and pharmacodynamics of lamivudine and/or lamivudine-TP [45–47]. Carriers of the ABCC4 T4131G variant allele had 20% higher lamivudine-TP concentrations compared to wild-type individuals ($P = .004$) [48]. When healthy volunteers were administered 100 mg of lamivudine, the observed difference in lamivudine AUC for various *ABCG2* genotypes was not significant ($P = .85$) [49].

Lamivudine pharmacogenetic data are minimal and do not predict a future role for pharmacogenetic testing with this drug in the future. Moreover, lamivudine has a wide safety margin and dosage adjustments made secondary to pharmacogenetic testing would be unlikely to yield clinically relevant benefits.

NONNUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Efavirenz

Efavirenz is an NNRTI with a long terminal elimination half-life of 36–100 h. It is used in combination with other classes of drugs such as NRTIs. The usual recommended adult dose of efavirenz is 600 mg once daily [50]. Plasma concentrations of efavirenz vary widely among individuals and higher plasma concentrations (>4 μg/mL) are associated with central nervous system (CNS) side effects. CNS disturbances with efavirenz include dizziness, and vivid dreams including nightmares, insomnia, and, less frequently, hallucinations. These symptoms are usually mild to moderate in severity, and tend to progressively subside over weeks. Nonetheless, approximately 20% of patients discontinue efavirenz due to persistent adverse CNS effects [51,52].

Efavirenz is metabolized predominantly by cytochrome P450 family 2 subfamily B member 6 (*CYP2B6*) and cytochrome P450 family 3 subfamily A member 4 (*CYP3A4*), and its plasma concentrations are impacted by CYP2B6 genetic polymorphisms, specifically rs3745274 (c.516 G>T) and rs28399499 (c.983 T>C) [53]. Median efavirenz concentrations in individuals with the CYP2B6 516TT genotype, which has been associated with decreased CYP2B6 catalytic activity compared to the wild type, were at least five times higher than both the CYP2B6 516GT and 516GG genotype groups (heterozygous and homozygous wild type, respectively). The median efavirenz concentration for CYP2B6 516GT genotype was 1.2 times higher than that for the 516GG genotype. Out of CYP2B6 polymorphisms, CYP2B6 516GT is the most common (21%–38% allele frequency) [54]. This allele is more common in Sub-Saharan Africans (35%–42%) compared to Caucasians (23%–27%) and Asians (15%–18%), which may explain higher efavirenz plasma concentrations in people of African origin [51]. Kwara et al. reported that median efavirenz concentrations in CYP2B6516TT patients were at least five times higher than those with CYP2B6516GT ($P < .001$) and CYP2B6516GG ($P < .001$) genotypes. Other CYP2B6 polymorphisms, which may be associated with increased efavirenz exposure, are CYP2B6 983TC and CYP2B6 15,582CT (rs4803419) [54]. Swart et al. reported the impact of genotype on the plasma levels of efavirenz in 301 South African HIV patients.

Results of a multivariate regression analysis in their study showed that, CYP2B6516 *G>T* and *983T>C* SNPs were the two most significant predictors of efavirenz plasma concentration above 4 μg/mL ($P < .006$), which has previously been associated with efavirenz-associated CNS toxicity [52]. The *NR1I2239-1089T>C* SNP played a minor role ($P = .011$) in explaining variability in efavirenz plasma concentrations [55].

ENCORE1 was a noninferiority trial in HIV-1-infected antiretroviral-naïve adults in 38 clinical sites across 13 countries. ENCORE1 subjects were randomized to receive tenofovir and emtricitabine with either a reduced daily dose (400 mg) or a standard dose (600 mg) of efavirenz. This trial showed that efavirenz 400 mg was noninferior to 600 mg in terms of viral growth suppression and $CD4^+$ cell counts, although CNS adverse effects were lower in the efavirenz 400 mg group. HIV-RNA < 200 copies/mL at week 48 of treatment was achieved in 94% of 321 subjects in the efavirenz 400 mg group compared to 92% of 309 subjects in the efavirenz 600 mg group (difference: 1.85; 95% CI: −2.1 to 5.79). $CD4^+$ cell counts were significantly higher in the efavirenz 400 mg group compared to the efavirenz 600 mg group (mean difference: 28 cells/μL; 95% CI: 8 to 48, $P = .01$). Adverse events in the efavirenz 400 mg group were reported in 37% of subjects compared to 47% of subjects in the efavirenz 600 mg group (difference: −10.5; 95% CI: −18.2 to −2.8, $P = .08$) [56].

Ribaudo et al. assessed the relation between CYP2B6 genotypes and plasma efavirenz concentrations after treatment discontinuation in 152 subjects. Plasma efavirenz concentrations were predicted to exceed the estimated protein binding–adjusted 95% inhibitory concentration (IC_{95}) for wild-type HIV virus (46.7 ng/mL) for a median of 5.8 days (interquartile range [IQR]: 4.4–8.3 days), 7.0 days (IQR: 5.0–8.0 days), and 14 days (IQR: 11.1–21.1 days) in CYP2B6 516GG (homozygous wild type), GT (heterozygous mutant) and TT (homozygous mutant) genotypes, respectively ($P < .001$). This is potentially problematic in CYP2B6 516TT individuals who simultaneously discontinue an efavirenz-containing cART regimen. If coadministered medications (NRTIs and/or PIs) with short half-lives are discontinued at the same time as efavirenz, efavirenz plasma concentrations (>46.7 ng/mL) would be expected to persist after the other medications are eliminated; thereby resulting in virtual monotherapy with efavirenz. Such a scenario could place patients at risk for the development of HIV-resistance mutations and virologic failure. The authors recommended that NRTIs and PIs with shorter half-lives be continued for a period of time after discontinuation of efavirenz in carriers of CYP2B6 516TT. This study highlights the potential role for CYP2B6 genotyping when efavirenz-containing cART regimens are discontinued [53].

In a retrospective study involving 191 Spanish patients receiving efavirenz 600 mg daily, doses were reduced in 31(16%) subjects. In subjects with the CYP2B6 516TT genotype, the efavirenz dose was reduced to 200 mg. The dose reduction resulted in decreased CNS adverse effects, effective virological control, and an average cost savings of 43,539 Euros per year [57]. Shackman et al. studied the cost-effectiveness of CYP2B6 genotyping in guiding efavirenz dosing in ART-naïve patients in the United States; the investigators used the widely published Cost-Effectiveness of Preventing AIDS complications (CEPAC) microsimulation model. In this model, genotyping strategy was compared to the current standard of care in a simulated cohort of patients receiving efavirenz-based ART as their initial regimen. This simulation study showed that CYP2B6 genotyping reduced lifetime treatment cost and marginally increased quality-of-life years (QUALYs) compared to standard care. This held true even if lowering efavirenz doses resulted in suboptimal control of HIV replication. Differences in QUALYs between the groups were negligible, suggesting that genotyping is a preferable option that results in a cost-effectiveness threshold of $100,000/QALY [58].

Next to abacavir, efavirenz has the strongest data supporting the use of pharmacogenetic testing to optimize therapy. CYP2B6 genotyping appears to be a cost-effective approach to improving efficacy—especially during efavirenz discontinuation—while reducing CNS-mediated adverse effects.

Nevirapine

Nevirapine is an NNRTI indicated for use in combination with other ARV agents for the treatment of HIV-1 infection [59]. Nevirapine is metabolized by CYP2B6 into 3- and 8-hydroxynevirapine, and CYP3A4 into 2- and 12-hydroxynevirapine [60].

In a study by Schipani and coworkers, pharmacogenetic data was integrated into a population pharmacokinetic (PopPK) model for optimizing nevirapine dosing. The PopPK model was developed with 406 nevirapine concentrations from 275 patients receiving nevirapine for 4 weeks or more. Inclusion of CYP2B6 genetic data improved the model fit and the change in objective function value (OFV) was −27.8, which was significant ($P < .001$). The 516TT genotype was associated with a 37% reduction in nevirapine clearance compared to wild type. The 516GT genotype was associated with a 15% decrease in clearance compared to patients expressing wild-type CYP2B6. The impact of 983T>C was also significant ($\Delta OFV = -9.4$, i.e., $P < .005$), with heterozygotes for this allele having a 40% lower clearance compared to wild type [61]. A study by Penzak et al. reported the impact of genetic polymorphisms of CYP2B6 G516T on nevirapine plasma trough concentrations in 23 HIV-infected patients from Uganda. genotypes at position 516 were expressed by 57%, 26%, and 17% of patients, respectively. The median nevirapine concentration for carriers of the variant allele (TT) was 7607 ng/mL compared to 4181 and 5559 ng/mL for individuals carrying the GG and GT alleles, respectively [62]. Of note, nevirapine trough concentrations were above the [62]target level for therapeutic efficacy (3000 ng/mL) in all three CYP2B6 genotype groups.

An in vitro study showed that the ABCC10 (which encodes for MRP7) expressing HEK cell lines C17 and C18 had significantly lower nevirapine accumulation compared with parental HEK 293 cells that did not express ABCC10. This nevirapine transport process was reversed by the MRP7 inhibitor cepharanthine, thereby confirming the role of MRP7 as a nevirapine transporter. These data were corroborated in a clinical pharmacokinetic and pharmacogenetic analysis in 163 HIV-infected patients of the German Competence Network for HIV/AIDS. Patients who were homozygous for the ABCC10 variant C allele of rs2125739 showed significantly lower nevirapine plasma concentrations compared to those with the heterozygous genotype (4212 vs. 5931 ng/mL; $P = .004$), respectively [63].

A case control study investigated the relationship between ATP-binding cassette subfamily B member 1*(ABCB1),* CYP2B6, and CYP3A4 genotypes and hepatotoxicity with nevirapine or efavirenz containing regimens. Of 201 subjects receiving nevirapine therapy, 14 experienced severe hepatotoxicity. Univariate analysis showed that ABCB1 (formerly MDR1) 3435 C→T polymorphism was associated with reduced likelihood of hepatotoxicity (OR, 0.25; 95% CI, 0.09–0.76). Independently the following genetic polymorphisms did not significantly impact the likelihood of hepatotoxicity: CYP2B6 1459CrT, CYB2B6 516GrT, and CYP3A4 -392ArG ($P > .1$). Multifactorial dimensionality reduction analysis showed an interaction between ABCB1 3435C→T and CYP2B6 1459C→T, which predicted hepatotoxicity status correctly 74% of the time ($P < 0$ 0.001). Similarly, hepatotoxicity risk was predicted by an interaction between ABCB1 C3435T and hepatitis B surface antigen (HBsAg) positivity with 82% accuracy ($P < .001$). The favorable association between ABCB1 3435T and reduced likelihood of hepatotoxicity was lost in the presence of HBsAg positivity [64].

Nevirapine has also been reported to cause immune-mediated skin and liver toxicity in

subjects with higher CD4+ cell counts. Human leukocyte antigen (HLA)-*DRB1∗01:01* and higher CD4+ T-cell percentages predicted rash-associated liver events in white patients, whereas HLA-DRB*1*01:02* predicted liver events among Black Africans. In patients from Sardinia and Japan HLA-*Cw*08* was associated with liver events. Studies from Thailand implicated HLA-B**35:05* and HLA-*Cw*04:01* with isolated skin events. HLA-B**35* and HLA-*Cw*04* were associated with skin events in a large cohort study that included subjects of African, Asian, and European descent. Unlike HLA-B*57:01 testing for abacavir HSR, HLA genetic polymorphism testing for nevirapine had a low negative prediction value for these adverse reactions, thus making these tests impractical in clinical practice [65].

Neither CYP2B6 nor HLA testing is feasible for widespread clinical implementation. Moreover, CYP2B6 genotyping, although associated with nevirapine exposure is not expected to inform nevirapine dosing or improve the safety profile of the drug. Currently, CD4+ counts are measured to determine the risk of hepatic events prior to nevirapine initiation. Women with CD4+ counts >250 cells/mm^3 should not receive nevirapine, as they were shown to have a 12-fold higher risk of symptomatic hepatic events compared to women with CD4+ counts <250 cells/mm^3. Similarly, an increased risk of hepatic adverse events was found in men with CD4+ counts >400 cells/mm^3 [64]. Baseline and frequent monitoring of CD4+ cell counts can help to identify patients at potential risk for hepatic toxicity and guide prescribing decisions.

Etravirine

Etravirine is an NNRTI that is used to treat ARV-experienced HIV-infected patients who harbor resistance mutations to other NNRTIs. It is dosed at 200 mg twice daily [66]. Etravirine is primarily metabolized by CYP3A, CYP2C9, and CYP2C19 [67]. A study was conducted to assess the impact of genetic polymorphisms on the key enzymes involved in etravirine metabolism. Compared to the wild type (CYP2C19*1), carriers of CYP2C19*2 showed a 23% reduction in etravirine clearance ($P = .003$), which explained 5% of the variability in clearance. Similarly, carriers of CYP2C9*3, showed a 21% (95% CI: –6.8–48.3%) reduction in clearance, but the effect was not significant. Pharmacogenetic testing with etravirine is not supported based on the limited data that are currently available.

PROTEASE INHIBITORS

Atazanavir

Atazanavir is indicated in combination with other antiretroviral agents for the treatment of HIV infection [68]. Although no longer recommended for first-line treatment, atazanavir is still a widely used protease inhibitor with long-term efficacy data, low pill burden, and an acceptable tolerability profile. Atazanavir is typically administered as a 300-mg dose boosted with a pharmacokinetic enhancer such as ritonavir or cobicistat. Depending upon a patient's ARV-treatment status (naïve vs. experienced) and ability to tolerate ritonavir, atazanavir may also be given as a single 400-mg dose without a pharmacokinetic enhancer, or in combination with cobicistat. Plasma atazanavir concentrations are markedly higher with boosted regimens compared to 400-mg unboosted regimens [68–70]. Atazanavir is metabolized by CYP3A and is also an inhibitor of CYP3A and UGT1A1, the enzyme responsible for conjugating bilirubin in the liver. As an inhibitor of UGT1A1, atazanavir produces grade 3 hyperbilirubinaemia (>2.5 × upper limit of normal [ULN]) in 40% of treated subjects and grade 4 (>5 × ULN) in 4%–8% of HIV-infected patients taking the drug. Plasma indirect (unconjugated) bilirubin increases from baseline in virtually every patient who takes atazanavir [71]. Although bilirubin increases with atazanavir can result in a jaundiced appearance in some patients, this effect is largely cosmetic in nature [72].

In a study to assess the population pharmacokinetics and pharmacogenetics of ritonavir-boosted atazanavir, 272 atazanavir concentrations from 35 patients were analyzed. Subjects with at least one copy of the (functional) CYP3A5*1 allele (n = 12) had 42% higher atazanavir oral clearance compared to those who did not have at least one CYP3A5*1 allele ($P < .01$) [73]. The influence of ABCB1 polymorphisms were also assessed for their influence of atazanavir disposition. ABCB1 genotypes were found to correlate with the risk of developing severe hyperbilirubinemia in a study of 74 HIV-infected patients. The risk was 24% in subjects with ABCB1 wild-type alleles, but close to zero in patients who were homozygous for the 3435C→T polymorphism. It was proposed that subjects carrying these polymorphic alleles had reduced P-glycoprotein (P-gp)-mediated cellular efflux, thereby leading to elevated intracellular, and lower plasma-drug concentrations [74]. A reduction in plasma concentrations would then explain reduced UGT1A1 inhibition by atazanavir and a lower risk of atazanavir-induced hyperbilirubinemia.

Pharmacogenetic-guided dosing of unboosted atazanavir was assessed for its impact on plasma atazanavir concentrations. Eighty subjects were randomized to receive standard-dose atazanavir (400 mg once daily) or pharmacogenetic-guided atazanavir (400 mg once daily or 200 mg twice daily). Genetic polymorphisms in pregnane X receptor (*PXR*), ABCB1, and SLCO1B1 were assessed. PXR is a nuclear receptor that regulates the expression of several genes involved in drug metabolism and transport. ABCB1and SLC01B encode for P-gp and organic-anion-transporting polypeptide (OATP) 1B1, respectively. Patients were classified into two groups based on their genetic profiles. The groups were labeled as "most favorable" and "least favorable." The "most favorable" group received atazanavir 400 mg once daily and the "least favorable" group received 200 mg twice daily. The geometric mean trough concentration after weeks 4–12 of atazanavir treatment was significantly higher ($P < .001$) in the pharmacogenetic-guided treatment arm (253 ng/mL [150–542]) compared to the standard-dose treatment arm (111 ng/mL [64–190]) [75]. These results are interesting, and suggest that certain patients may benefit from receiving atazanavir 200 mg twice daily versus 400 mg once daily. However, identifying such patients would require pharmacogenetic testing for atazanavir, which is not widely available. Moreover, it would be easier to give patients a boosted atazanavir regimen, which does not require pharmacogenetic testing and yields atazanavir trough concentrations in excess of unboosted regimens (including 200 mg twice daily).

Siccardi et al. analyzed 3 pregnane X receptor (*PXR*) SNPs in relation to unboosted atazanavir trough concentrations in two cohorts. The 3 PXR SNPs were 44,477T→C, 63,396C→T and 69,789A→G. In cohort A, which included 47 white subjects, median C_{trough} was lower for T63396T individuals compared to the other two groups (C63396T or C63396C) (34 ng/mL [IQR, 25–63 ng/mL] vs. 152 ng/mL IQR, 47–388 ng/mL; $P = .001$). The PXR T63396T genotype was associated with atazanavir trough concentrations below the recommended threshold for efficacy in atazanavir-naïve patients (150 ng/mL) with an odds ratio of 18 (95% CI, 2.1–153.9; $P = .008$) [76].

Lubomirov assessed a number of UGT1A1 genetic polymorphisms to assess their impact on premature discontinuation of first-line ARV therapy containing ritonavir-boosted atazanavir. Thirty of 121 patients receiving ritonavir-boosted atazanavir discontinued treatment during the first year of treatment. Homozygosity for reduced function alleles (*28/*28 or *28/*37) was associated with treatment discontinuation risk (adjusted hazard ratio [HR_2] = 9.13, 95% CI: 0.77–5.03) [37]. These data suggest that knowledge of UGT1A1 polymorphisms may inform atazanavir prescribing decisions. Nonetheless, in patients who do not develop noticeable (or cosmetically unacceptable) jaundice, the risk of atazanavir discontinuation is minimal regardless of UGT1A1

genotype. For individuals carrying two copies of reduced function alleles (i.e., homozygous mutants), risk of discontinuation is higher. In heterozygous or homozygous wild-type subjects, risk of atazanavir discontinuation due to hyperbilirubinemia is lower [71]. When UGT1A1 pharmacogenetic information is available prior to atazanavir initiation, patients should be warned about the risks of hyperbilirubinemia according to their genotype.

A simulation study was conducted to determine the cost-effectiveness of UGT1A1 pharmacogenetic testing when choosing a protease inhibitor-containing regimen. The simulation revealed that UGT1A1 testing was not cost-effective in most of the tested scenarios, except when patients were lost to follow-up due to hyperbilirubinemia. In other words, the cost of testing outweighed any potential benefits [77]. Unless further studies confirm the cost effectiveness of UGT1A1 testing to address atazanavir-associated hyperbilirubinemia, it is unlikely to have a role in routine clinical practice.

Lopinavir

Lopinavir undergoes extensive presystemic metabolism by CYP3A. As such, it is co-formulated with the CYP3A inhibitor and pharmacokinetic booster, ritonavir [78]. In a study to assess genetic polymorphisms in the genes that encode for enzymes and transporters involved in lopinavir disposition, the roles of SLCO1B1, ABCB2, and CYP3A were explored. SLCO1B1 encodes for the uptake transporter OATP1B1 and ABCC2 and CYP3A encode for MRP2 and CYP3A, respectively. Overall, only 5% of variation in clearance could be explained by genetic variants; however, in a small subset of patients, these genetic variants had significant impact. Individuals homozygous for SLCO1B1*4 (3%) had a mean lopinavir clearance of 12.6 L/h compared with 5.5 L/h in the reference population ($P < .01$). Patients with multiple variants (13%) of SLCO1B1, CYP3A, and ABCC2 had a mean lopinavir clearance of 3.7 L/h which was significantly lower compared to the reference population ($P < .01$) [78].

The role of SLCO1B1388A→G, 463C→A and 521T→C genetic variants on the disposition of lopinavir were studied. A trend toward increasing concentrations of lopinavir from TT to TC to CC genotypes was observed. It has been proposed that the 521T→C polymorphism is associated with reduced OATP1B1 activity in vivo, resulting in decreased uptake into hepatocytes and higher plasma concentrations of substrates, including lopinavir. There is uncertainty surrounding this interpretation, because there is extensive overlap in plasma lopinavir concentrations across the three 521T→C genotypes, and one of the variants (521CC) was only present in 5% of patients [79].

Genetic polymorphisms appear to explain lopinavir pharmacokinetics in a small subset of the population, but extensive data from large studies are lacking. This, plus the fact that the Department of Health and Human Services (DHHS) no longer recommends lopinavir/ritonavir as a first-line ARV agent to treat ARV-naïve patients [80], strongly suggests that there is not a role for lopinavir/ritonavir pharmacogenetic testing in the future.

INTEGRASE STRAND TRANSFER INHIBITORS

Raltegravir

Raltegravir is the first-in-class integrase strand transfer inhibitor (INSTI), an HIV-1 specific enzyme that is required for viral replication [81]. It is metabolized primarily by UGT1A1 with UGT1A3 and UGT1A9 playing minor roles [82]. Raltegravir is not a substrate of CYP enzymes, but is transported by P-gp and BCRP [83]. Combined data from a cohort study and two clinical trials were analyzed to determine the influence of SNPs in UGT1A, UGT2B, and nuclear receptors

on raltegravir plasma concentrations. In total, 544 raltegravir plasma concentrations from 145 HIV-infected patients and 19 healthy volunteers were analyzed. Higher plasma raltegravir concentrations were observed in one subject who was homozygous for UGT1A9*3; this association reached study-wide significance ($P = .0004$), whereas none of the other SNPs reached study-wide significance [84].

In a study to investigate the role of UGT1A1*28/*28 on plasma raltegravir concentrations, 30 subjects with UGT1A1*28/*28 (homozygous mutant) genotypes and 27 matched subjects with UGT1A1*1/*1 (homozygous wild-type) genotypes were enrolled. UGT1A1*28 homozygotes had a mean AUC from time zero to infinity ($AUC_{0-\infty}$) that was approximately 40% higher than their comparators (geometric mean ratio [GMR]: 1.41; 90% CI, 0.96–2.09). The magnitude of this increase was modest and the CI included 1.0; therefore, the results are not deemed statistically or clinically significant [85].

Tsuchiya et al. investigated the impact of ABCB1 (P-gp) and ABCG2 (BCRP) polymorphisms on peak (2–4 h post-dose) and trough (predose) plasma concentrations of raltegravir in Japanese patients. Thirty-one trough and 41 peak concentrations were collected in 20 patients. All patients were receiving raltegravir 400 mg twice daily as part of cART. Raltegravir trough concentrations were not impacted by any of the ABCB1 or ABCG2 polymorphisms. Conversely, significantly higher mean peak raltegravir concentrations were observed in carriers of ABCB1 4036 AG/GG versus AA (3466 ± 3174 ng/mL vs. 1628 ± 1878 ng/mL; $P = .03$). None of the other ABCB1 polymorphisms (1236 C>T, 2677 G>T/A, or 3435 C>T) significantly impacted raltegravir peak concentrations. Plasma concentrations of raltegravir were also noted to be higher in carriers of ABCG2 421 CA/AA versus ABCG2 421CC (3576 ± 3488 vs. 1702 ± 1572 ng/mL; $P = .03$) [86]. Increased peak raltegravir concentrations were postulated to be due to reduced intestinal expression of P-gp and BCRP in individuals with ABCB1 4036 AG/GG and ABCG2 421 CA/AA genotypes (heterozygous and homozygous variant alleles, respectively). Because raltegravir peak concentrations have not been shown to be associated with efficacy or a particular toxicity, the clinical relevance of these findings appears minimal.

Dolutegravir

Dolutegravir is an integrase strand transfer inhibitor (INSTI) indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection [87]. dolutegravir has a terminal elimination half-life of 12 h and can be given as a part of a once-daily single-dose regimen without pharmacokinetic boosting [88]. Dolutegravir is predominantly metabolized by UGT1A1, with CYP3A4 playing a minor role [89].

To assess the impact of UGT1A1 polymorphisms on the disposition of dolutegravir, pooled data from nine Phase I and II clinical studies was assessed in 89 subjects receiving dolutegravir 50 mg daily. Subjects were categorized as having low (*28/*28 and *28/*37), reduced (*1/*6, *1/*28, *1/*37, *28/*36, and *36/*37) or normal (*1/*1 and *1/*36) UGT1A1 activity. In subjects harboring low-activity UGT1A1 polymorphisms, dolutegravir oral clearance was 32% less compared to subjects with normal UGT1A1 function (GMR: 0.68; 92% CI: 0.54–0.86). When grouped together, subjects with low and reduced UGT1A1 activity had a geometric mean oral clearance that was 23.5% lower compared to subjects with normal UGT1A1 function (GMR: 0.77 [92% CI: 0.66–0.89]) [90]. Despite the observed increase in dolutegravir exposure in patients with low and reduced UGT1A1 activity, the increase was modest and not clinically significant based on accumulated safety data [91]. Therefore, dolutegravir dose adjustments are not necessary based on UGT1A1 polymorphisms.

CONCLUSION

Completion of the human genome project at the turn of the 21st century represented a significant achievement that was made possible via collective international efforts. At that time, it was predicted that in 20 years pharmacogenetic testing would be embraced as the standard of practice for managing a number of diseases [92]. However, nearly two decades later, pharmacogenetic testing is only employed routinely for a small number of medications. This is likely due to the presence of barriers that limit the translation of pharmacogenetic knowledge into clinical practice. These barriers include test-related barriers, knowledge barriers, evidence barriers, and ethical, legal, and social implications [93,94]. In addition, polymorphic genes that have been studied in the setting of HIV are associated with mild–moderate pharmacokinetic changes that do not warrant a change in therapy or dosing. Recently, several genome-wide association studies (GWAS) were conducted to search for novel genetic factors and pathways involved in HIV infection, replication, pathogenesis, and treatment [95]. Most of these studies have centered on virologic response and disease progression. A shift from GWAS to whole-genome sequencing (WGS), along with continued classical candidate gene approaches is poised to allow for the identification of novel genetic variations that impact antiretroviral drug response.

Successful implementation of HLA-B*57:01 testing offers valuable information that can be applied to other medications. HLA-B*57:01 screening was backed by a prospective, double-blind randomized clinical trial (PREDICT-1) that clearly demonstrated the benefit of genetic testing. The clinical interpretation of this test is straightforward, thus making it easy for clinicians to implement into routine practice. Furthermore, the cost-effectiveness of abacavir pharmacogenetic testing also assisted in its widespread implementation.

Another ARV agent for which pharmacogenetic testing may have a future role is efavirenz. Pharmacogenetic testing with efavirenz may improve safety and tolerability of this drug by allowing certain patients to receive doses below the recommended 600 mg. This approach has been shown to potentially result in cost savings. Barriers to the widespread acceptance of pharmacogenetic testing with efavirenz include availability of low-cost testing, turnaround time of tests, lack of education or knowledge of clinicians, and questions surrounding reimbursement.

Much information has been learned regarding pharmacogenetic testing with ARV medications. Despite improvements in ARV treatment over the past 35 years, challenges remain when attempting to optimize therapy in HIV-infected patients. Many antiretroviral agents are metabolized by CYP enzymes, which are susceptible to modulation by coadministered medications. As such, the phenomenon of phenoconversion may result in genotypic extensive metabolizers being converted to phenotypic-poor metabolizers. A similar phenomenon may occur with P-gp-transported medications. As such, the impact of phenoconversion secondary to drug interactions must be taken into account along with pharmacogenetics in HIV-infected patients receiving cART. Indeed, pharmacogenetic testing ± therapeutic drug monitoring of ARV therapy may prove helpful in managing unique populations such as children, pregnant females, those with organ dysfunction, and those on multiple interacting medications.

Inherent barriers to pharmacogenetic testing with ARV medications remain. However, previous success of genetic testing with abacavir, and the need to continually improve the efficacy and safety of ARV medications, indicate that this is a ripe area for continued research. Education of clinicians and development of cost-effective testing are also key factors that must be addressed as part of any pharmacogenetic testing program.

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CHAPTER

9

The Role of Pharmacogenomics in Diabetes

Y. W. Francis Lam[1,2], *Ravindranath Duggirala*[3], *Christopher P. Jenkinson*[3], *Rector Arya*[3]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]College of Pharmacy, University of Texas at Austin, Austin, TX, United States; [3]South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Edinburg, TX, United States

OUTLINE

OBJECTIVES

1. Identify key candidate genes and polymorphisms that influence the disposition, response, and adverse effects of the sulfonylureas, metformin, and thiazolidinediones.
2. Discuss the utility of genome-wide association studies in identifying genes and polymorphisms associated with the diabetes risk, pathophysiology, and response to antidiabetic medications.

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00009-7

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3. Discuss the challenges and opportunities associated with the potential translation of pharmacogenomic information to the clinical management of diabetes.

DIABETES OVERVIEW

Diabetes mellitus has emerged as one of the most alarming public health epidemics in the 21st century, with an estimated 552 million people worldwide having diabetes by 2030, and 398 million people will be considered at high risk for developing the disease [1]. Type 2 diabetes (T2D) is the most prevalent form of diabetes in adults, accounting for 90%–95% of cases worldwide [1]. It is characterized by a relative deficiency in pancreatic β-cell insulin secretion and diminished tissue responsiveness to the normal action of insulin (i.e., insulin resistance) [2]. In contrast, type 1 diabetes is observed primarily in children and young adults and is characterized by an absolute deficiency in insulin secretion with minimal insulin resistance [2]. Both type 1 diabetes and T2D are associated with periods of chronic hyperglycemia, which contribute to micro- and macrovascular complications such as retinopathy, nephropathy, neuropathy, cardiovascular disease, peripheral vascular disease, and stroke [2]. Given these deleterious clinical consequences, there is a critical need to identify optimal treatment strategies to achieve and maintain good glycemic control in this patient population.

Prior to 1995, sulfonylureas and insulin were the only pharmacologic agents available to treat diabetes. Since that time, the field has witnessed a dramatic increase in the number of antidiabetic medications. Insulin remains the mainstay of therapy for type 1 diabetes. In contrast, numerous treatment modalities are available for T2D including: sulfonylureas, meglitinides, biguanides, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, α-glucosidase inhibitors, bile acid sequestrants, dopamine receptor agonists, incretin mimetics, amylin mimetics, and insulin. This large treatment arsenal has dramatically improved patient care. However, it is also associated with significant challenges, namely the selection of the right drug for the right patient. Clinical diabetes guidelines have helped in this regard by providing a tiered approach to drug selection (e.g., tier 1 represents well-validated core therapies that are clinically effective and cost effective) [3]. Yet even with clinical guidelines, it is difficult to predict which patients will derive the best efficacy or be predisposed to toxicity for a given antidiabetic medication [4]. As such, the potential of pharmacogenomics to aid in the selection of antidiabetic drug therapy for an individual patient has garnered considerable attention among clinicians.

LESSONS FROM DIABETES-SUSCEPTIBILITY GENES

T2D is a heterogeneous disorder characterized by defects in insulin secretion and/or insulin action. This common, yet complex, disease is influenced by genetic and environmental factors and their interactions. As disease susceptibility in patients with diabetes is multifactorial, and disease progression is related to continuous decline in β-cell function and inadequate response or failure to pharmacologic management, there could be a link between diabetes risk and genetic variants affecting drug disposition and/or response.

There have been continued efforts to localize and characterize T2D-susceptibility genes using genome-wide linkage and, in the last decade, genome-wide association study (GWAS) approaches, in addition to the traditional candidate-gene approach. Although genome-wide linkage studies have identified various T2D-susceptibility loci [5–9], subsequent gene discovery successes from such efforts have been limited. In contrast, the GWAS method has become a powerful method, which has transformed the genetic landscapes of T2D and its related traits, with unprecedented successes in localization of multiple novel T2D-susceptibility loci (more than 100 loci) and loci for T2D-related glycemic

traits (more than 75 loci) [9–21]. The GWAS approach using large samples, large research consortia, meta-analysis, and tran-sethnic meta-analysis is being continued to reaffirm previous findings or to find new T2D-association signals in ethnically diverse populations (e.g., African Americans and Mexicans), because most of the GWASs have been conducted in populations of European ancestry, followed by Asian populations [17,19,22–26]. However, GWAS-identified common variants explain only a minority of the overall genetic risk for T2D (i.e., ~20%) [27] indicating that a large proportion of heritability is still unexplained.

Therefore, focus has been on the potential role of rare variants in common complex diseases, which are likely to have larger effect sizes with potential functional consequences and could contribute to missing heritability [28–32]. Recent advances in next-generation sequencing technologies have made it possible to obtain complete information on rare, low-frequency, and common variants across the whole exome or genome or a targeted region, and new information on low-frequency or rare variants including coding variants that are associated with T2D and its related traits is emerging [23,33–37]. However, the findings from these studies, including those that used a more comprehensive set of sequencing studies representing multiethnic populations (e.g., Type 2 Diabetes Genetic Exploration by Next-Generation Sequencing in Ethnic Samples (T2D-GENES) Consortium and exome sequence data) failed to support a major role for low-frequency variants in susceptibility to T2D [36]. In addition, no evidence was found in favor of any rare variants of large effect associated with T2D or its related traits, using data from large Mexican-American families and whole-genome sequence data, from a recent study conducted as part of the T2D-GENES Consortium research activities [38]. Thus, based on the currently available information, low-frequency or rare variants appear to have limited roles in susceptibility to T2D, and rare-variant association studies would require relatively much larger sample sizes [22], larger samples of extended pedigrees, or different study designs that further enrich for such variants [38].

A large of number of the T2D-susceptibility loci identified by common-variant association studies have been associated with insulin secretion highlighting potential pancreatic β-cell dysfunction. Meanwhile, other studies involving glycemic traits (e.g., fasting glucose and insulin) in non-diabetic populations have found loci with relevance to insulin resistance [12,15,20,27,39,40]. Because the overlap between the association signals of T2D and glycemic traits such as fasting glucose and insulin is partial, a diverse set of physiological mechanisms are thought to be associated with these traits [12,20,41,42]. These GWAS-association signals have, so far, yielded few causal-gene identifications and the functional relevance of the implicated genetic variants have yet to be established. A few T2D exceptions include non-synonymous variants and intronic variants with potential regulatory relationships pursued through follow-up studies, related to such genes as *GCKR*, *TCF7L2*, *SLC30A8*, *KCNJ11*, and *KCNQ1* [12,19,24,35,40,41]. However, most GWAS-identified variants including T2D fall in noncoding regions of the genome, highlighting their potential role in gene regulation [41,43–46].

To bridge the gap between genetic associations and disease-promoting molecular mechanisms, there have been enhanced/accelerated efforts in recent years to identify the potential molecular and biological mechanisms corresponding to the noncoding common-variant T2D-association signals using both experimental and bioinformatic approaches [13,22,47–49]. To examine potential causal-variant regulatory effects, there have been efforts to utilize the knowledge on pancreatic islet genome regulatory mechanisms [46,47,50–53] and the publicly available databases such as Encyclopedia of DNA Elements (ENCODE), Roadmap Epigenomics Project, RegulomeDB, and Genotype-Tissue Expression (GTEx) projects involving information from numerous human tissues for integrated functional annotations of noncoding variants [43,54–61]. To enhance our

knowledge on biological mechanisms and translational potentials corresponding to specific genetic findings, together with the basic clinical phenotypes, a range of deep physiological phenotypes and omic-metrics, such as genomics, transcriptomics, and metabolomics, can be examined in an integrated fashion to understand the molecular mechanisms underlying susceptibility to T2D [13,22,62,63]. The ultimate goal of the genetic studies is to translate the genetic findings into clinically relevant information for improved treatment or cure for T2D or its related diseases. Despite the aforementioned advances and challenges, there has been high enthusiasm for translating genetic findings into clinical practice [64–66], and the T2D-pharmacogenomic studies, current and future, will involve the use of the novel information on causal genes/variants at T2D-susceptibility loci to aid in the molecular classification of T2D, and, subsequently, antidiabetic drug selection [66–69].

In summary, at least 100 genetic variants have been associated with an increased risk of T2D [17,21,39,70]. Although the magnitude of risk associated with each genetic marker tends to be modest, these loci provide key insights into the molecular mechanisms of the disease [71,72]. For example, most genetic loci identified thus far have been genes involved in insulin secretion [71,72]. Thus, the future of T2D pharmacogenomics will likely involve the use of risk alleles to aid in the molecular classification of T2D and, subsequently, antidiabetic drug selection [73]. This will be described further with sulfonylureas and metformin in later sections of this chapter.

TYPE 2 DIABETES PHARMACOGENOMICS

It is well recognized that interindividual variability exists in the disposition (i.e., pharmacokinetics), response (i.e., pharmacodynamics), and adverse effects of medications used to treat T2D. As such, pharmacogenomics is viewed as a promising tool to elucidate pharmacologic response variability among patients. The potential use of an individual's genetic information to tailor antidiabetic drug therapy is, therefore, not a new concept. However, compared to other chronic diseases, T2D pharmacogenomics is in its infancy. To date, the candidate-gene approach has been the primary means to assess genetic determinants of antidiabetic drug disposition and response and has focused on the following areas: (1) antidiabetic drug clinical pharmacology, i.e., drug-metabolizing enzymes, drug transporters, drug targets, and effector pathways; and (2) genomic markers underlying T2D pathophysiology (i.e., disease-risk alleles) [73]. As is the case with pharmacogenomic investigations in other therapeutic areas, the candidate-gene approach has posed some challenges given that T2D is a polygenic disease, and the biological pathways underlying its pathophysiology are numerous and complex. To overcome these challenges, GWAS have recently been applied to T2D pharmacogenomics to discover novel genes and polymorphisms that underlie diabetes pathophysiology and drug response [73]. Regardless of the approach, replications of any reported associations should include cohorts of different ethnicity, as the frequencies of genetic variants are different in populations around the world.

The following sections will review major antidiabetic drug classes—sulfonylureas, biguanides, and thiazolidinediones—for which there exists a moderate amount of pharmacogenomic research. Within this framework, the most clinically relevant findings from candidate gene studies and/or GWAS are highlighted for each drug class (Table 9.1). The challenges and opportunities associated with the potential translation of pharmacogenomic information to the clinical management of diabetes are also discussed.

SULFONYLUREAS

Sulfonylureas have been a major component of T2D pharmacotherapy for over 50 years. Although the sulfonylureas are effective

TABLE 9.1 Selected Examples of Major Pharmacogenes of Interest in Type 2 Diabetes

Drug Class	Gene and Allelic Variant	Protein	Reported Impact on Therapeutic Outcome
SULFONYLUREAS (SU)			
	CYP2C9 (rs1799853, rs1057910)	Cytochrome P-450 2C9	Greater reduction in HbA_{1C} More adverse drug reactions
	KCNJ11 (rs5219)	Kir6.2 subunit of the K_{ATP} channel	Greater response
	ABCC8 (rs757110)	Sulfonylurea receptor-1 subunit of the K_{ATP} channel	Greater response
	TCF7L2 (rs7903146)	WNT-signaling pathway	Diabetes-risk allele T allele associated with SU failure
METFORMIN			
	SLC22A1 (rs72552763)	Organic cation transporter-1	No association with HbA_{1C} reduction
	SLC22A2 (rs316019)	Organic cation transporter-2	No association with HbA_{1C} reduction
	SLC47A1 (rs2289669)	Multidrug and toxin extrusion transporter-1	Greater reduction in HbA_{1C}
	SLC47A2 (rs12943590)	Multidrug and toxin extrusion transporter-2	Greater HbA_{1C} response
	ATM (rs11212617)	Ataxia-telangiectasia mutated gene	Greater HbA_{1C} response
THIAZOLIDINEDIONES			
	CYP2C8 (rs11572080, rs10509681)	Cytochrome P-450 2C8	No association with HbA_{1C} reduction
	PPARG (rs1801282)	Peroxisome proliferator-activated receptor-γ	Diabetes-risk allele No association with glycemic control
	NFATC2, *AQP2*, *SLC12A1*	Putative genes involved in the pathophysiology of thiazolidinedione-induced edema	Potential association with adverse drug reactions

antidiabetic agents, interindividual variability exists in their pharmacokinetics, pharmacodynamics, and adverse effects. It is estimated that 10%–20% of patients have less than a 20 mg/dL decrease in fasting plasma glucose following initiation of sulfonylurea therapy, which is referred to as primary sulfonylurea failure [74]. In contrast, other patients have an adequate early response to sulfonylurea therapy, but then later fail treatment. This represents secondary sulfonylurea failure and is estimated to occur at a rate of 5%–7% per year [74]. Sulfonylureas also have a higher failure rate when given as monotherapy as compared to other antidiabetic agents such as metformin and thiazolidinediones [75]. Although some patients experience sulfonylurea failure, other patients appear to have increased sensitivity to the hypoglycemic effects of sulfonylureas. The United Kingdom Prospective Diabetes Study (UKPDS) found that mild hypoglycemia occurred in 31% of patients during the first year of glibenclamide (also known as glyburide) therapy, and the incidence of severe hypoglycemia was 1% per year [76]. Given the therapeutic challenges associated with sulfonylurea therapy, there has been great interest in determining the contribution of

polymorphisms in drug metabolism, drug target, and diabetes-risk genes to interindividual variability in sulfonylurea disposition, response, and adverse effects [77].

Drug Metabolism

Most sulfonylureas are primarily metabolized by the cytochrome P450 (CYP) 2C9 enzyme in the liver. Therefore, *CYP2C9* is a logical candidate gene to interrogate in relation to sulfonylurea clinical pharmacology. Studies have shown that the *CYP2C9*3* (rs1057910 Ile359Leu, I359L), and to a lesser extent *CYP2C9*2* (rs1799853, Arg144Cys, R144C), polymorphisms are associated with decreased oral clearance and increased plasma exposure of tolbutamide, glyburide, glipizide, and glimepiride [78]. For example, glyburide oral clearance in *CYP2C9*3* homozygotes was less than half that of wild-type *CYP2C9*1* homozygotes [79]. However, most early studies were conducted in healthy volunteers; therefore, the clinical consequences of *CYP2C9* polymorphisms in patients with T2D were unknown until recently. Patient-focused studies have since begun to shed more light on this topic. A population-based study of 1,073 type 2 diabetics from the Genetics of Diabetes Audit and Research in Tayside Scotland (Go-DARTS) cohort treated with sulfonylurea monotherapy (primarily gliclazide) found that individuals with the *CYP2C9*2/*2*, **2/*3*, or **3/*3* genotypes, representing 6% of study subjects, were 3.4-times more likely to achieve a hemoglobin A_{1C} (HbA_{1C}) less than 7% compared with *CYP2C9* wild-type homozygotes [80]. Other studies have shown lower sulfonylurea dose requirements in carriers of the *CYP2C9*3* allele compared with wild-type homozygotes [81,82]. For example, in one study the daily dose of tolbutamide increased 279 mg from the first to 10th prescription in wild-type homozygotes, but only increased 12 mg in *CYP2C9*3* carriers [81]. Similar findings exist for glimepiride, in which a trend was observed for a lower dose in *CYP2C9*3* carriers (0.61 mg) versus wild-type homozygotes (1.01 mg) [82].

In terms of adverse effects, the *CYP2C9*3* allele has been associated with an increased risk of hypoglycemia in patients treated with sulfonylureas [83,84]. For example, a small study found that individuals with *CYP2C9*3/*3* or **2/*3* genotypes had 5.2-times the odds of a severe sulfonylurea-associated hypoglycemic event than those without these genotypes [83]. However, these results could not be replicated in a larger cohort of sulfonylurea-treated patients [85]. Possible reasons for the inconsistent reports in the literature include different definitions and assessments of hypoglycemia, clinical demographics of the sulfonylurea users (e.g., age, metabolic control), and possibly variability in other genes and proteins involved in the complex and multifactorial process of hypoglycemia. As an example, Klen et al. reported that *CYP2C9* genotype might affect the risk of sulfonylurea-associated hypoglycemia in elderly patients but not in the overall diabetic populations. Again, the sample size of the study is small [86].

Currently, the clinical utility of *CYP2C9* genotyping for the prediction of sulfonylurea dose, response, or adverse effects is unclear. A multitude of clinical and genetic factors influence the glycemic response to sulfonylurea therapy. As such, the most practical application of *CYP2C9* genotyping would likely be to identify patients with a predisposition to sulfonylurea-induced hypoglycemia [87]. However, additional prospective studies are needed to more comprehensively define the role of *CYP2C9* genotyping in this area. Importantly, a consensus definition of hypoglycemia will need to be formulated and applied consistently to avoid potential discrepancies between patient studies.

Drug Targets

The complexity of glycemic response to antidiabetic drug therapy has prompted researchers to move beyond drug metabolism in the quest to identify genetic predictors of drug response. The pancreatic ATP-sensitive potassium (K_{ATP})

channel is important for glucose-stimulated secretion of insulin from pancreatic β-cells. Under normal physiological condition, the K_{ATP} channel opens in response to decrease in plasma glucose, and results in suppression of insulin release. On the other hand, the K_{ATP} channel closes in response to increased metabolism, resulting in insulin secretion. The K_{ATP} channel is composed of extracellular sulfonylurea receptor-1 (SUR1) subunit and intracellular potassium inwardly rectifier 6.2 (Kir6.2) subunit, which are encoded by *ABCC8* and *KCNJ11*, respectively. The *ABCC8* and *KCNJ11* are the two primary drug target candidate genes that have been studied in relation to sulfonylurea clinical pharmacology.

Activating mutations in *KCNJ11* or *ABCC8* cause K_{ATP} channels to remain in the open state, thereby promoting hyperpolarization of the pancreatic β-cell membrane and impairing insulin release [88,89]. It was hypothesized that defects in the Kir6.2 and/or SUR1 subunits, because of genetic polymorphisms, may alter pancreatic β cell physiology, insulin secretion, and response to antidiabetic medications. Mechanistically, the sulfonylureas bind to the K_{ATP} channel proteins, resulting in channel closure, which results in pancreatic β-cell membrane depolarization and subsequent insulin release from the pancreatic β cell [89–91], and have been shown to be particularly effective in patients with *KCNJ11* or *ABCC8* activating mutations [92].

The most widely studied polymorphisms in *KCNJ11* and *ABCC8* are Glu23Lys (E23K, rs5219) and Ser1369Ala (S1369A, rs757110), respectively. Only a few studies have evaluated the impact of *KCNJ11* Glu23Lys and/or *ABCC8* Ser1369Ala polymorphisms on sulfonylurea efficacy in patients with T2D. Studies have shown that carriers of the K allele of rs5219 showed better response than patients with the *E/E* genotype [93,94]. On the other hand, other studies have shown either a negative association (K allele is associated with sulfonylurea failure) or no association [95–97]. In the largest study to date, Chinese patients with the *ABCC8 Ala/Ala* genotype had a 2.2 times greater odds of responding to gliclazide treatment than patients with the *Ser/Ser* genotype, over an 8-week period [98]. The study results were consistent with another study of smaller size [99]. Although these results are interesting and in line with in vitro findings, it is not known whether the results are specific to gliclazide or whether they could be generalized to other sulfonylureas.

In addition, the *KCNJ11* Glu23Lys and *ABCC8* Ser1369Ala polymorphisms are in strong linkage disequilibrium (LD); therefore, most individuals who carry a *KCNJ11* Lys23 allele will also carry an *ABCC8* Ala1369 allele [100]. Recombinant human K_{ATP} channels with the Lys23/Ala1369 risk haplotype demonstrated a 3.5-times increased sensitivity to gliclazide compared with wild-type K_{ATP} channels [101]. Subsequently, Ala1369 was determined to be the causative allele associated with increased sulfonylurea sensitivity in the Lys23/Ala1369 haplotype [101]. Another study also showed that recombinant human K_{ATP} channels containing the Lys23/Ala1369 haplotype were sensitive to gliclazide inhibition, whereas K_{ATP} channels containing the Glu23/Ser1369 haplotype were sensitive to tolbutamide, chlorpropamide, and glimepiride inhibition [102]. These data suggest that sulfonylurea chemical structure, e.g., a ring-fused pyrrole moiety on gliclazide, may influence the pharmacogenetic effects mediated by *KCNJ11* and *ABCC8* polymorphisms. Therefore, the choice of sulfonylurea will undoubtedly be an important factor to consider in future pharmacogenetic studies.

Of note, the *KCNJ11* Glu23Lys polymorphism has emerged as a T2D-risk allele in various cohorts [95,100,103–105]. On the other hand, *ABCC8* Ser1369Ala genotyping is not yet ready for translation to the clinic. Additional prospective studies are needed and should consider, in addition, to the choice of sulfonylurea, the following factors in their study designs: longer treatment durations (i.e., to assess genetic factors governing sulfonylurea failure after long-term treatment); clinically relevant glycemic

endpoints (e.g., fasting plasma glucose, HbA_{1C}); and assessment of other racial and ethnic groups.

Diabetes-Risk Alleles

In terms of sulfonylurea pharmacogenomics, T2D-risk genes that influence processes such as insulin secretion, glucose homeostasis, or pancreatic β-cell function, among others, could potentially contribute to variability in sulfonylurea response between patients. In this regard, the most intensively studied diabetes-risk gene has been transcription factor 7-like 2 (*TCF7L2*). *TCF7L2* is transcription factor involved in the Wingless/Integrated WNT-signaling pathway. The WNT-signaling pathway is involved in glucose homeostasis, lipid metabolism, proliferation and function of pancreatic β cells, and the production of glucagon-like peptide 1 [106]. Importantly, *TCF7L2* was associated with T2D in the first GWAS evaluating novel risk loci for T2D [107]. Since that time, the *TCF7L2* rs7903146 C>T polymorphism has been associated with impaired insulin secretion both in vitro and in vivo [108–110]. A few clinical studies have shown the *TCF7L2* rs7903146-variant T allele to be associated with an increased risk of sulfonylurea failure in patients with T2D [111–114]. For example, in the GoDARTS cohort of more than 900 Scottish subjects, individuals homozygous for the variant T allele had a 1.73 increased odds for sulfonylurea failure (HbA_{1C} >7% after 3–12 months of therapy) as compared with wild-type homozygotes [111]. In another study of 189 patients with German descent, the rs7903146 T allele occurred more frequently in patients who failed sulfonylurea treatment (HbA_{1C} >7% after 3–12 months of therapy) versus the control group (36% versus 26%; odds ratio, 1.57) [112]. Finally, Javorsky et al. reported an 80% greater HbA_{1C} reduction in 51 homozygous carriers of the wild-type C allele compared to nine patients with the *T/T* genotype. Interestingly, the response difference seems to be drug specific with gliclazide but not in cohorts treated with glibenclamide, glimepiride, and glipizide. Hence, as is the case with target polymorphism, the choice of sulfonylurea seems to be an important factor [114].

In summary, the literature data suggest a role for the *TCF7L2* polymorphism in mediating the response to sulfonylurea or at least with gliclazide. However, it is important to note that the reported studies have varied in design, sample size, treatment duration, and definition of sulfonylurea failure. Currently, the clinical utility of *TCF7L2* genotyping to identify sulfonylurea nonresponders is uncertain given that it appears that the observed effect is small and would not merit a stand-alone test. Nonetheless, additional studies are needed to determine if *TCF7L2*, in combination with other pharmacogenes, could improve the antidiabetic drug selection process and clinical outcomes. Besides *TCF7L2*, other disease-related genes such as insulin receptor substrate-1 (*IRS1*), nitric oxide synthase 1 adaptor protein (*NOS1AP*), and *CDKAL1* have been implicated in altered sulfonylurea response [115–117]. Prudente et al. confirmed the earlier finding of the importance of the Arg972 polymorphism in *IRS1*, as carriers of *IRS1* G792R demonstrated as much as 30% failure rate with sulfonylurea treatment [118]. However, in comparison to *TCF7L2*, most of these findings have not yet undergone replication in additional patient cohorts.

These examples have largely focused on single polymorphisms in known T2D-risk genes. However, findings from GWAS have allowed for a more comprehensive approach to assess the impact of diabetes-risk alleles on drug response. This paradigm is best exemplified by a study which hypothesized that a panel of 20 T2D-risk alleles would influence sulfonylurea response [119]. Most of the risk alleles included in this panel were putative mediators of insulin secretion and had been repeatedly associated with T2D risk in GWAS. The study found that patients who carried more than 17 diabetes-risk alleles had a 1.7-fold decreased likelihood of achieving a stable sulfonylurea dose, suggesting a decreased response to sulfonylurea therapy [119]. One of

the major limitations of this study was that it did not interrogate continuous endpoints of glycemic status (e.g., fasting plasma glucose). However, this study nicely illustrates the proof of concept that genetic underpinnings of T2D may mediate differential response to antidiabetic therapy [119].

METFORMIN

The biguanide antidiabetic agent metformin is the most commonly prescribed oral antidiabetic agent, and is recommended as tier 1, step 1 therapy according to consensus guidelines [3]. Despite its extensive use, metformin's mechanism of action and drug target(s) has largely remained a mystery since its introduction [120]. However, it is now generally accepted that metformin activates adenosine monophosphate-activated protein kinase (AMPK) in the liver [120]. Metformin's major pharmacodynamic actions are to suppress hepatic glucose production, increase glucose uptake, improve insulin sensitivity in peripheral tissues, decrease fatty acid synthesis, and decrease intestinal glucose absorption [121]. Significant interindividual variability exists in metformin response. Clinical studies have demonstrated that approximately 36% of patients fail to achieve a fasting plasma glucose of <140 mg/dL with metformin alone [75]. Furthermore, it is estimated that up to 50% of patients fail to achieve an HbA_{1C} <7% after 1 year of metformin monotherapy [111]. There has been considerable research geared toward identifying the genetic predictors of metformin disposition and response. Although most research has primarily focused on drug transporter candidate genes, recent studies have taken a GWAS approach to broadly interrogate the pharmacogenomics of metformin response.

Drug Transporters

At physiological pH, metformin exists as a cation that relies more on facilitated transport than passive diffusion down a concentration gradient across a cell membrane. Hence, its disposition is largely governed by several drug transporters that include the organic cation transporter-1 (OCT1) that mediates hepatic uptake; the organic cation transporter-2 (OCT2) that mediates renal excretion via active tubular secretion; as well as the multidrug and toxin extrusion transporters, MATE1 and MATE2. MATE1 and MATE2 are H^+/organic cation antiporters located on the brush border of the renal epithelium and the canalicular membrane of hepatocytes, respectively [120], and mediate the transport and excretion of metformin into the urine and bile [122].

Candidate gene studies in healthy volunteers and patients with T2D have investigated the impact of polymorphisms within these drug transporter genes on the pharmacokinetics and pharmacodynamics of metformin [120,123]. Early studies reported that reduced-function *SLC22A1* polymorphisms altered metformin pharmacokinetics and modulate its cellular and clinical response in healthy volunteers, most likely a result of decreased hepatic uptake of metformin [124,125]. In another study of 159 diabetic patients from the South Danish Diabetes Study, the almost 80-fold variation in trough plasma metformin concentrations (54–4,133 ng/mL) were shown to correlate with the number of reduced function allele Met420del (rs72552763) of *SLC22A1* ($P = .001$) [126]. However, in a follow-up corrigendum (Published in Pharmacogenetics and Genomics 2015; 25:48–50), the investigators reported a re-analysis of the correlation between *SLC22A1* genotype and HbA_{1C} outcome and confirmed no association between HbA_{1C} reduction and the number of reduced function *SLC22A1* alleles. Other studies in patients with T2D have also shown inconsistent results with respect to the impact of reduced-function *SLC22A1* alleles on metformin pharmacodynamics [127–129]. The recent study by Sam et al. confirmed a lack of effect of *SLC22A1* genotype on both metformin

pharmacokinetics and pharmacodynamics in 28 severely obese children with insulin resistance. After 6-months of metformin therapy, there are no differences in HbA_{1C}, fasting glucose, or insulin concentrations, and insulin resistance between patients with no variant and heterozygous carrier of *SLC22A1*-variant allele [130]. As such, it does not appear that *SLC22A1* polymorphisms consistently explain a sufficient degree of metformin response variability to be useful in clinical practice.

Polymorphisms in *SLC22A2*, particularly c.808G>T (rs316019) have been studied for their relationship with metformin renal clearance. This is important given that renal clearance of metformin is proposed to have a strong underlying genetic component [131,132]. Healthy volunteer studies have shown that individuals with the *SLC22A2* c.808 *T/T* genotype have marked reductions in metformin renal clearance compared with individuals who possess the *G/T* or *G/G* genotypes [120]. Based on these data, one might expect an increased response to metformin with the *T/T* genotype. However, in the same study that showed an impact of the variant on metformin pharmacokinetics, no association was found between *SLC22A2* polymorphism and HbA_{1C} reduction after metformin treatment, although there was a gene-gene interaction effect demonstrated between those encoding OCT2 and MATE1 [132]. Hence, the role of this polymorphism on metformin response in patients with T2D remains to be determined.

MATE1 and MATE2 are encoded by *SLC47A1* and *SLC47A2*, respectively. In a population-based cohort study, the *SLC47A1* rs2289669 G>A polymorphism was significantly associated with the reduction in HbA_{1C} following metformin therapy in 116 patients, implying that this polymorphic allele is associated with decreased transporter function [133]. Specifically, the decrease in HbA_{1C} was 0.3% greater per copy of the A allele, which may be a clinically important decrease in patients with T2D [133]. A 2-fold reduction in HbA_{1C} and higher plasma concentrations after 1 year metformin therapy in 220 patients were reported by He et al. [134] This result was consistent with that from the Diabetes Prevention Program (DPP) in which the *SLC47A1* rs8065082 C>T polymorphism, which is in tight LD with rs2289699, was associated with metformin response. Specifically, carrier of the minor allele benefit from metformin compared to homozygous carriers of the major allele [135].

In terms of *SLC47A2*, a gain-of-function promoter polymorphism (rs12943590 G>A) was associated with a weaker glycemic response to metformin in patients with T2D [136]. Specifically, Caucasian patients who were homozygous for the A allele had a smaller relative change in HbA_{1C} than G allele carriers (−0.03 vs. −0.15) [136]. These results have been confirmed in additional studies that demonstrated the effect of genetic variants of the MATE transporters [132,137,138]. Altogether, it appears that MATE transporters may be important determinants of metformin disposition and response. However, additional studies are needed to confirm previous associations and elucidate the pharmacologic alterations resulting from these genetic polymorphisms. In addition, recent data suggest that polymorphisms in genes encoding OCT and MATE may interact to influence metformin's pharmacodynamic effects [132,139]. Hence, future studies will need to consider the interaction between different drug transporters, and variation within these transporters, on metformin clinical pharmacology, as contribution of any single gene is unlikely to account significantly for the 34% heritability in metformin response reported in a genome-wide complex trait analysis [140].

Drug Targets

Compared to drug transporters, less is known about the effect of drug target or effector pathway gene polymorphisms on metformin pharmacokinetics, pharmacodynamics, and adverse effects. This is largely a result of

metformin's complex mechanism of action. Therefore, large-scale candidate gene studies and/or GWAS are essential components of future metformin pharmacogenomic research. Metformin works, in part, by activating AMPK, which is a master regulator of cell and body energy homeostasis and glucose uptake in skeletal muscle [120]. A large-scale candidate gene study of the DPP trial showed nominally significant, but interesting, associations with metformin response for genes such as serine-threonine kinase 11 (*STK11*, which catalyzes the activation of AMPK) and catalytic subunits of AMPK (e.g., *PRKAA1*, *PRKAA2*, and *PRKAB2*) [135]. More recently, the GWAS approach has been applied to metformin pharmacogenomics [141]. Importantly, this represents the first GWAS conducted for any antidiabetic medication. The 2011 GWAS identified a significant association between a polymorphism located in a locus containing the ataxia-telangiectasia mutated (*ATM*) and metformin response in patients with T2D [141]. ATM is a DNA repair gene that has also been implicated in insulin signaling pathways, β-cell dysfunction, and AMPK activation [141]. Following in vitro experiments, the study further concluded that *ATM* acts upstream of AMPK and is required for metformin action. Specifically, the minor allele (C-allele) of rs11212617 near *ATM* was reported to be associated with better metformin response in the initial discovery cohort of 1,024 subjects and a combined discovery and replication cohorts of 3,920 subjects. Homozygous carriers of the C-allele were found to have a 3.3-fold higher response rate (HbA_{1C} of ≤7% within 18 months of metformin monotherapy) [141]. The significance of the *ATM* polymorphism, which only explained 2.5% of the variance in metformin response, was confirmed in some but not all subsequent replication studies [142,143]. The conflicting results could be related to the small effect from the polymorphism with additional gene variants to be discovered or there are additional metformin targets other than AMPK activation [144–146]. This example illustrates the utility of GWAS for discovering previously unknown mediators of antidiabetic drug pharmacology.

Diabetes-Risk Alleles

As discussed in the sulfonylurea section, an emerging area of pharmacogenomic research is the extent to which T2D-risk alleles influence response to antidiabetic therapy. Few studies have been conducted in this area with respect to metformin therapy. However, researchers involved with the DPP trial devised a genetic risk score that comprised 34 T2D-risk alleles [147]. The risk alleles were selected based on published reports of their individual association with T2D at a genome-wide significance level ($p < 5 \times 10^{-8}$) [147]. No interaction between the genetic risk score and metformin treatment was observed in the study. Nonetheless, this "genetic risk score" approach will likely be used more often in the future as T2D-risk alleles continue to emerge through GWAS. In sum, no genetic markers have been identified thus far that explain a sufficiently high percentage of variability in metformin response. However, compared with other antidiabetic agents, metformin pharmacogenomic research has made substantial progress in adopting a GWAS approach to identify novel genetic sources of metformin disposition and response variability in patients with T2D [73].

THIAZOLIDINEDIONES

Thiazolidinediones are agonists for the peroxisome proliferator-activated receptor-γ (PPAR- γ) in the cell nucleus. As nuclear receptor agonists, thiazolidinediones regulate the transcription of numerous genes involved in fatty acid uptake and storage, glucose homeostasis, insulin sensitivity, and adipocyte differentiation [148]. The thiazolidinediones, also known as glitazones, are commonly referred

to as "insulin sensitizers" due to their ability to increase insulin sensitivity in muscle, liver, and fat. Although thiazolidinediones are useful in T2D patients who exhibit a moderate-to-high degree of insulin resistance, clinical guidelines classify these agents as Tier 2 (i.e., less well-validated) therapies [3], partially related to safety concerns, which have resulted in the withdrawal of rosiglitazone (increased cardiovascular risk) and troglitazone (hepatitis risk) from the market [71,149]. The only thiazolidinedione that is widely available at this time is pioglitazone, but its use has also been reported with increased risk of bladder cancer [150].

Interindividual variability in thiazolidinedione response and adverse effects has been demonstrated in clinical studies. Approximately 25% of patients with T2D fail to achieve a greater than 10% decrease in fasting plasma glucose following pioglitazone therapy [151]. Along the same lines, 30% of patients at high risk for T2D do not show an improvement in insulin sensitivity following thiazolidinedione treatment [152]. Edema and congestive heart failure are among the more troubling adverse effects associated with thiazolidinedione therapy [153,154]. In fact, edema occurs relatively frequently, with reported rates of 2%–28% for pioglitazone [155]. Because of this, the use of thiazolidinedione in patients with heart failure is discouraged, especially for those with moderate to severe heart-failure symptoms [156]. Clinical studies have sought to identify genetic determinants of thiazolidinedione pharmacokinetics, pharmacodynamics, and adverse effects [157]. However, compared to the pharmacogenetic progress of sulfonylureas and metformin, thiazolidinedione pharmacogenetic studies have lagged behind in their approach, mostly focusing on only a limited number of candidate genes and polymorphisms.

Drug Metabolism

The thiazolidinediones are primarily metabolized by CYP2C8, and to a lesser extent by CYP2C9 (rosiglitazone) and CYP3A4 (pioglitazone) [158,159]. Although thiazolidinediones have a wide therapeutic index, alterations in plasma exposure may influence glycemic control, insulin sensitization, and the risk of concentration-dependent adverse effects (e.g., edema and weight gain). The polymorphism most often studied in relation to thiazolidinedione metabolism is *CYP2C8*3* (rs11572080 Arg139Lys, R139K; rs10509681 Lys399Arg, L399K). Healthy volunteer studies have shown that *CYP2C8*3* carriers have greater oral clearance and lower plasma exposure of rosiglitazone and pioglitazone than *CYP2C8*1* homozygotes [160–162]. Thus, *CYP2C8*3* appears to function as a high-activity allele for thiazolidinedione metabolism, resulting in a 25%–35% decrease in plasma exposure. However, conflicting effect on pharmacodynamics response was reported, with no association reported [160] versus smaller decrease in HbA_{1C} in carriers of *CYP2C8*3* compared to noncarriers [163]. Hence, it remains to be determined whether *CYP2C8*-mediated differences in thiazolidinedione plasma exposure translate into differences in glycemic control or insulin sensitization in patients with T2D [157].

Drug Targets

Given that thiazolidinediones are PPAR-γ agonists, PPAR-γ (*PPARG*) is the most logical drug target candidate gene for this drug class. Pro12Ala (rs1801282 P12A) is the most frequently studied polymorphism in *PPARG*, and the Ala12 allele has been associated with an approximate 20% reduction in the risk of T2D [164]. Most pharmacogenetic studies have shown no association between the *PPARG* p.Pro12Ala polymorphism and glycemic response or insulin sensitization following thiazolidinedione therapy [1,151,152]. Thus, *PPARG* Pro12Ala genotyping does not have a role in optimizing thiazolidinedione management. Beyond *PPARG*, many other drug target and effector pathway genes have been interrogated for their relationship with

thiazolidinedione response including, but not limited to, adiponectin, lipoprotein lipase, lipin-1, perilipin, PPAR-γ coactivator-1, resistin, uncoupling protein 2, β_3-adrenergic receptor, tumor necrosis factor-α, and voltage-gated potassium channel-1 [157,165]. Unfortunately, most of these studies had significant limitations such as lack of replication cohorts, lack of study in other ethnic groups besides Asians, small sample size, and failure to consider the complexity of thiazolidinedione response. To ultimately move thiazolidinedione pharmacogenomics to the clinic, it is imperative that a more comprehensive discovery approach, such as GWAS, be undertaken in large thiazolidinedione-treated patient cohorts. This is especially important given the diverse genes and proteins known to mediate thiazolidinedione clinical pharmacology. In addition, little is known about the relationship between T2D-risk alleles and thiazolidinedione response. In GWAS, some insulin-resistance genes have shown a signal for increased T2D risk [166]. Thus, it can be hypothesized that patients with a T2D subtype driven primarily by insulin resistance may derive a greater benefit from thiazolidinediones than other antidiabetic therapies (e.g., sulfonylureas).

Another potential application of pharmacogenomics to thiazolidinedione therapy may be in the prediction of adverse effects, especially edema. Some recent work in this area has yielded promising results. A genetic substudy of the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) trial interrogated over 30,000 polymorphisms among Europeans receiving rosiglitazone [167]. One polymorphism in the nuclear factor of activated T-cells cytoplasmic calcineurin-dependent 2 gene (*NFATC2*) was significantly associated with rosiglitazone-induced edema, yielding an odds ratio of 1.9 [167]. Another study in Chinese patients found significant associations between thiazolidinedione-induced edema and polymorphisms in the aquaporin 2 (*AQP2*) and the bumetanide-sensitive Na-K-2Cl cotransporter (*SLC12A1*) genes [155]. These researchers went on to develop a prediction model, which included age, sex, *AQP2*, and *SLC12A1* polymorphisms, to estimate the risk of thiazolidinedione-induced edema in T2D patients [155]. Replication of these genetic findings and assessment of the clinical utility of this prediction model will need to be conducted in other populations. However, these findings demonstrate how genetic and nongenetic factors may be integrated into a clinically applicable model to aid in the prediction of adverse effects. Along these lines, these types of algorithms may be useful in selecting pharmacologic strategies for the prevention of T2D. Recently, pioglitazone, as compared with placebo, was associated with a dramatic 72% reduction in the risk of converting from impaired glucose tolerance to T2D [168]. However, pioglitazone was also associated with a significant increase in the incidence of edema and weight gain. Perhaps in the future, algorithms containing clinical and genetic factors may be used to tailor pharmacologic prevention strategies in patients at high risk for T2D to attenuate disease onset without inducing adverse effects.

OTHER ANTIDIABETIC DRUGS

The number of pharmacogenomic studies for other antidiabetic drugs (e.g., incretin mimetics, DPP-4 inhibitors) is relatively few. The nonsulfonylurea meglitinides, repaglinide and nateglinide, stimulate insulin secretion in pancreatic β cells and are classified as "other therapy" by consensus guidelines [3]. Polymorphisms in genes encoding drug metabolizing enzymes (i.e., *CYP2C8*, *CYP2C9*), drug transporters (e.g., *SLCO1B1*), and drug targets (e.g., *KCNJ11*, *ABCC8*) have been implicated in altered meglitinide disposition and/or response [165]. However, the meglitinides have limited use in clinical practice. Therefore, the clinical utility of pharmacogenomics is not likely to be pursued for these agents.

The incretin mimetics, for example, exenatide and liraglutide, or the DPP-4 inhibitors such as sitagliptin, saxagliptin, and linagliptin are relatively new classes of antidiabetic agents. Exenatide and liraglutide have substantially changed the landscape of T2D pharmacotherapy by placing more emphasis on the key role of gastrointestinal incretin hormones in T2D pathophysiology [169]. In addition, the incretin-enhancing DPP-4 inhibitors have gained in popularity due to their reasonable efficacy and excellent tolerability profile [170]. Interindividual variability exists in the pharmacodynamic effects of most of these newer agents. For example, a meta-analysis of the efficacy of DPP-4 inhibitor in patients with T2D showed that approximately 60% of patients failed to achieve an HbA_{1C} <7% with this therapy [171]. Along the same lines, another meta-analysis showed that 35 and 55% of patients failed to achieve an HbA_{1C} <7% following liraglutide and exenatide therapy, respectively [172]. Similar to sulfonylureas, treatment response to DPP-4 inhibitors has also been reported to be associated with *TCF7L2* polymorphism. In a recent study, Zimdahl et al. reported that carriers of the C allele of rs7903146 had a lower reduction of HbA_{1C} compared to patients with the *C/C* genotype [173].

Taken together, a substantial gap exists in knowledge regarding genetic and clinical predictors of response to these newer antidiabetic agents. Future research will need to consider how pharmacogenomics, along with the molecular mechanisms underlying T2D phenotypes, can be used to optimally guide the use of these newer antidiabetic therapies.

CHALLENGES AND OPPORTUNITIES OF PHARMACOGENOMICS IN DIABETES

Diabetes pharmacogenomics is in its early stages, and there are no examples of genetically guided diabetes treatment algorithms that are currently being used in practice for polygenic forms of diabetes such as T2D. To move the field forward and foster the translation of genetic information to the clinical setting, several challenges and opportunities will need to be considered by the medical and scientific communities.

A major challenge facing the field is optimal study design. To date, most studies have been retrospective in nature, had small-to-moderate sample sizes, investigated only a limited number of genes and polymorphisms, lacked statistical adjustment for multiple comparisons, and varied in study design, subject cohort (comorbidity, concurrent medications, and medication adherence), and outcome measure (e.g., disease progression). These factors have likely contributed to the lack of replication of pharmacogenomic findings between cohorts. Ideally, future studies should be conducted in large cohorts of patients with well-defined phenotypes, with adequate power to detect prespecified outcome differences, include a comprehensive approach for gene interrogation, apply appropriate statistical adjustments for multiple comparisons, and include both clinical and genetic factors. Accomplishing these goals, particularly in large diabetic cohorts, will not be an easy task. However, these obstacles may be overcome through collaborations among individuals in academia, federal and private grant agencies (e.g., National Institutes of Health), community settings, and the pharmaceutical industry [73].

Another challenge for the field is the variety of antidiabetic drug-response definitions that have been used in clinical studies. The sulfonylureas are a good example of this situation, in which several definitions exist for primary and secondary sulfonylurea failure. More uniform definitions of diabetic drug response and adverse effects will need to be applied to pharmacogenomic research to gain consistency between studies [174]. Along these lines, physiologically relevant endpoints (e.g., insulin sensitivity, hepatic glucose output) will need to be selected to define drug response, and attention

paid to the methodology used to measure these endpoints [174,175]. Another area that has been lacking in diabetes pharmacogenomic research is the assessment of the interplay between environmental (e.g., life style) and genetic factors in mediating diabetes risk and drug-response phenotypes. These interactions are especially important when conducting studies in different ethnic groups, as metabolic pathophysiology (e.g., insulin resistance and obesity) can differ substantially between ethnicities. For example, individuals of Asian heritage may have metabolic risk factors despite only modest increases in waist circumference or body mass index [176]. Lastly, future studies will need to carefully quantify the predictive ability and cost-effectiveness of precision medicine strategies [70,177]. For example, a cost–utility analysis of genetic testing for neonatal diabetes showed that genetic testing improved quality of life and produced a total cost savings of $12,528 at 10 years [177].

Despite these challenges, a substantial number of exciting opportunities exist for diabetes pharmacogenomics. The technological advances made possible through GWAS represent the most promising opportunity in the field. Although few diabetes pharmacogenomic GWAS have been published to date, it is certain that this approach will be used more often in the future. For example, genetic samples were collected as part of the Action to Control Cardiovascular Risk Factors in Diabetes (ACCORD) trial, and will likely be used for genome-wide assessments of drug response. In a complex disease such as T2D, GWAS has the potential to enhance our understanding of the disease and its treatment by: (1) providing a comprehensive approach to identify polymorphisms that govern drug response and adverse effects, (2) providing novel insights into the mechanism of action of antidiabetic pharmacotherapy, (3) providing information on the molecular basis of diabetes subtypes, and (4) identifying novel metabolic targets for drug development [4,70,73]. To date, many GWASs have been conducted in European cohorts. However, in the future, it will be important to conduct GWAS in non-European cohorts to identify ethnicity-specific T2D-risk alleles that were not identified in these European studies [178]. To be used in clinical practice, genomic markers will need to account for a sufficient amount of variability in drug response to be considered for future testing. Additionally, other technological strategies, such as next-generation sequencing, will need to be undertaken to identify less common variants that mediate differential drug-response phenotypes between individuals.

Once promising markers are identified, a major opportunity for the field will be to develop prospective studies to determine how genetic information may be used to select or optimize T2D pharmacotherapy, and whether this strategy results in better outcomes than the traditional approach. Prospective studies will most certainly evaluate the impact of genetics on glycemic endpoints. However, a more important question will be whether genetically guided drug therapy and glycemic control significantly decrease the incidence of microvascular and macrovascular complications associated with the disease. Lastly, another innovative opportunity for the field will be to use genetic information to predict diabetes risk, promote behavioral modifications, and devise individualized diabetes-prevention strategies [179]. In the future, it can be envisioned that individuals with a high T2D genetic-risk score may be subjected to earlier and more aggressive life-style or pharmacologic interventions to mitigate their risk of developing the disease [179,180].

QUESTIONS FOR DISCUSSION

1. Discuss the relevant polymorphisms that affect antidiabetic pharmacotherapy.
2. Describe how GWAS could offer additional insight into antidiabetic pharmacotherapy.

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CHAPTER

10

A Look to the Future: Pharmacogenomics and Data Technologies of Today and Tomorrow

Erick R. Scott[1,2], *Richard L. Wallsten*[2]

[1]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [2]Clinical Data Science, Sema4, Stamford, CT, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00010-3

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INTRODUCTION

The ability to measure chemical and physiologic states in tandem with good experimental design has enabled the discovery and characterization of a plethora of gene–drug interactions. Recent advances in methods to measure organic molecules and phenotypes, describe clinical states, and reason across federated data offer an increasingly precise set of technologies for pharmacogenomics discovery and clinical translation.

The preceding chapters have described principles, methods, and examples of gene–drug interaction discovery. This chapter explores how the rapid development of biomedical and computational technologies will influence the creation and analysis of pharmacogenomics knowledge. The first several sections will describe advances in the measurement of biological molecules and physiologic states. The following sections will describe emerging computational tools that facilitate data sharing and pharmacogenomic association discovery. The final sections of this chapter will forecast how specific technologies may be leveraged to create an efficient process of discovery and translation.

MEASURING GENES

The technical goal of genome and gene sequencing is the reconstruction of the maternal and paternal chromosome sequences (chromosome haplotypes). Knowledge of the full linear sequence of the gene and surrounding regions allows greater precision in the association of DNA variation with drug outcomes, in addition to a greater likelihood of identifying the causal variant(s).

The accuracy of haplotype reconstruction from sequencing data is a function of sequencing depth, error rate, and read length. Due to repetitive sequences in the genome, haplotype reconstruction requires data that can either span the repeat or connect unique sequence fragments flanking the repetitive region. Although short-read technology offered by Illumina yields excellent genotyping accuracy, long-range haplotype reconstruction can only be achieved with expensive and/or cumbersome methods [1]. Long-read DNA-sequencing technology that can generate read lengths greater than 7000 nucleotides provide improved ascertainment of copy-number variation [2,3] and haplotype phasing [4]. However, accurately sequencing human-DNA fragments long enough to determine phased haplotypes for all pharmacogenomic-relevant loci remains a technical challenge [5].

Application of short-read alignment and variant-calling algorithms to long-read sequencing data produce poor variant-calling accuracy and/or dramatic increases in compute time [5,6]. Algorithms tuned to accommodate per-base error rates of 10–15% and read lengths of thousands of bases have successfully called variants with greater than 95% precision and recall [7]. Linear haplotypes greater than one million bases can be constructed with switch error rates less than 0.01 [7,8]. A list of state-of-the-art algorithms for long-read sequencing data can be found in Table 10.1; as these algorithms are under continuous development, their evolving precision and recall statistics are purposefully not included.

Alternative strategies for assembling phased haplotypes include leveraging heterozygous variants in RNA-sequencing (RNA-seq) data [9,10], identity by descent (IBD) data (utilizes data from an individual's parents) [11], and haplotype reference phasing algorithms (identification of the most likely series of heterozygous variants given a reference panel of previously phased haplotypes) [12–15]. Haplotype phasing can also be accomplished using chromatin ligation and/or fragment barcoding (enables phasing via knowledge of chromosome proximity where variants on the same linear chromosome are more likely to be linked compared with variants on a separate chromosome) [1,16]. These methods present advantages and drawbacks with respect to short- and long-range switch errors and, therefore, are differentially appropriate for short and long pharmacogenomic genes (Table 10.2).

TABLE 10.1 State-of-the-Art Algorithms for Long-Read Sequencing Alignment, Variant Calling, and Haplotype Reconstruction

Algorithm	Use Case	References
Minimap2	Alignment	[5]
CoNvex Gap-cost alignMents for Long Reads (NGMLR)	Alignment	[6]
Canu	Assembly	[92]
Clairvoyante	Variant calling	[7]
DeepVariant	Variant calling	[93]
Nanopolish	Variant calling	[95]
Sniffles	Structural variant calling	[6]
HapCut2	Phased haplotype assembly	[8]
FastHare	Phased haplotype assembly	[94]

TABLE 10.2 Long-Range and Short-Range Phasing Accuracy of Experimental and Computational Methods

	Long-Range Accuracy	Short-Range Accuracy
Short reads	–	+
Long reads	++	+++
RNA-seq	++	++
Identity By Descent (IBD)	+++	+++
Statistical phasing	+	++
Chromatin-capture	+++	++

Excellent: +++, poor: –.

The cytochrome P450 2D6 (*CYP2D6*) locus exemplifies the challenges and importance of accurately reconstructing a pharmacogenomic haplotype and considering additional genetic assays. The *CYP2D6* locus contains a high density of polymorphic allelic variation, long repetitive elements, and two pseudogenes; more than 145 unique star (*) allele *CYP2D6* haplotypes have been described [17]. The *CYP2D6* locus contains both high-and low-complexity regions, presenting technical challenges for both genotyping and short-read sequencing. The freely available Astrolabe algorithm [18] was developed to address the challenge of detecting polymorphisms and reporting copy-number variation in the context of mapping short-read sequence data to the *CYP2D6* locus. Simulations of read mapping to the *CYP2D6* locus have demonstrated the need for read lengths exceeding 1000 bases to accurately characterize exon 2. Empirical data using the long-read lengths of Pacific Bioscience (PacBio) sequencing have borne out the simulated predictions of unique read mapping and accurate copy number and variation calling [19]. Although the long-read lengths and the stochastic error profile of PacBio sequencing has allowed accurate reconstruction of *CYP2D6* haplotypes, additional information can be obtained by analyzing long-range enhancers and physiological states that exert important effects upon the functional activity of CYP2D6-mediated medication metabolism [20]. To detect DNA-driven changes in gene expression, an orthogonal set of assays measuring messenger ribonucleic acid (mRNA) and/or protein abundance is required.

Due to the large degree of variation found at the *CYP2D6* locus, pharmacogenomics researchers have developed a functional activity score [21] that can be assigned to identified haplotypes and diplotypes. This functional activity score reduces the cognitive burden of associating the increasingly large number of star alleles with clinical actionability; however, this binning system reduces the quantitative precision and may ultimately be abandoned as more data are collected. *CYP2D6* is an important and complex pharmacogene, the aforementioned technologies enhance characterization and the ability to associate variation with drug-associated phenotypes.

MEASURING DRUGS

Measuring the concentration of small molecules, parent compounds, and metabolites is a critical step in the pharmacogenomics discovery workflow, but less commonly utilized in clinical contexts. Devices to quantitatively measure small molecules and their metabolites ex vivo have historically required large investments in laboratory space, financial investment, and technical training. However, innovations in scaling-down portable mass spectrometry instruments [22] and the advent of paper-based analytical devices [23] have provided an opportunity to widely capture pharmacokinetic data in more diverse contexts. The cost efficiencies provided by these products may enable the transition of pharmacokinetic study sample sizes from dozens to thousands. Although these methods generate less-precise data, the increase in sample size could offer more robust absorption, distribution, metabolism, and excretion (ADME) estimates.

An alternative, albeit less informative, method of measuring drug exposure is the use of an ingestible tracking molecule. For example, the antipsychotic medication aripiprazole has been compounded with the Proteus ingestible sensor to track medication adherence [24]. Frequent and passive collection of medication-adherence data may offer a tremendous advantage to Phase IV pharmacovigilance studies. Measuring medication adherence coupled to patient-reported adverse events at population scale may identify rare-event pharmacogenomic associations with previously unmatched speed and statistical power. The drawback of current ingestible-sensor technology is the lack of quantitative pharmacokinetic data, thereby limiting insights into ADME processes. This new type of data will require additional innovations in statistical analysis to generate unbiased associations (Fig. 10.1).

WHAT IS A THERAPEUTIC?

As we look towards the future of pharmacogenomics data technologies we would be remiss if we focused solely on traditional therapeutic categories such as small molecules, natural products (taxol), and proteins (monoclonal antibodies). Therefore, we use the term 'drug' to include more complex therapeutic interventions such as macromolecular complexes (e.g. viral-vectored nucleic acids for gene therapy), engineered cells (chimeric antigen receptor T cells), and even software interventions; all of which require novel methods for data collection and analysis.

Cells as Therapeutic

Technology to edit and design bioorganic molecules has also seen rapid advances in recent years. The FDA approval of ex vivo engineered cell therapies (Kite Pharma) and medicinal compounds developed using structure -based design (TTR) demonstrate the feasibility of personalized interventions. As drugs become more tailored to specific biological profiles pharmacogenomics will increasingly seek to confirm, not just discover, gene-drug interactions.

Gene Editing as Therapeutic

Therapies that seek to edit the genome (zinc finger nucleases, TALENs, and CRISPR) may present additional questions regarding the impact of genetic background. From alterations to single nucleotide mutations to the insertion of a synthetically derived therapeutic protein or ribozyme sequence, the ability to modify the human genome is rapidly progressing.

Software as Therapeutic

The definition of a medical device spans a range of products, from tongue depressors to pacemakers, the common principle being an intended use in the diagnosis, mitigation, or cure of disease. Software developed to diagnose and mitigate disease has demonstrated clear therapeutic benefits in randomized controlled trials (Omada Health). As most of these interventions are behavioral in nature, pharmacogenomic interactions may be enriched in neuro-psychiatric genes.

FIGURE 10.1 What is a Therapeutic?

MEASURING PHENOTYPES

The measurement and description of observable characteristics (phenotypes) is the last factor of the pharmacogenomics triad of drug, gene, and response.

Phenotypes include signs (observable states such as blood pressure, jaundice, or erythema), symptoms (such as fatigue, nausea, or hallucination), and molecular states (such as gene expression, liver enzyme concentration, or functional magnetic resonance imaging). These phenotypes have traditionally been collected at discrete time points within the context of a research study or post hoc extraction from medical records.

An emerging set of passively collected phenotypic data can be continuously reported by internet-connected devices and applications, such as search history, social media interactions, geolocation, and step counts. Passive data collection has the advantage of capturing longitudinal information at high frequencies [25]. Novel composite outcomes may be formed from various connected devices. For example, the response to a drug prescribed for depression may be measured using a combination of metrics for sleep quality (internet-connected mattress pad to collect diurnal variation, duration, and restlessness), social interactions (quantity and quality of responses within text messages, social media interactions, and voice calls), and/or weight gain/loss (internet-connected home weight scale).

As the benefits of open-data standards and data reuse become more apparent, in tandem with the recognition that publication bias may present significant clinical peril, regulations defining clinical trial reporting have moved toward enforcement of structured trial registration and results reporting. Clinicaltrials.gov trial registration is now required for any clinical study meeting a set of criteria [26,27]. This has provided machine-readable resources amenable to analyses [28] and investigations of publication bias [29]. The European Union (EU) has recently mandated release of clinical trial data for studies conducted within the member states. A future mandate will require the release of de-identified case-report forms which will dramatically increase the ability to perform individual-level meta-analyses. For studies that incorporate genetic profiling within the protocol, this could offer a vast trove of information regarding pharmacogenomic interactions with outcomes and adverse events. The release of combined genetic and clinical data may require additional security and privacy considerations and the anticipation of this future has inspired research and development of data sharing and analysis methods that preserve privacy [30–32].

In vitro technologies have also experienced a wave of innovation presenting increased sample sizes, lower costs, and greater dimensionality of data collection. For example, the Library of Integrated Network-Based Cellular Signatures (LINCS) program profiled 1000 marker genes in more than one million cells by drug by concentration combinations, generating a large corpus of drug-response data [33]. Although, an exploration of the genetic interactions was not a primary focus, valuable pharmacodynamic data may be derived from the differences in target-gene sequence/structure. Another promising in vitro technology is the organ-on-a-chip platform, which offers an increasingly complex model of human physiology to investigate multiorgan system pharmacokinetic and pharmacodynamic processes. Early ADME data from body-on-a-chip prototypes [34–36] is encouraging, but not currently ready for clinical translation.

STRUCTURING DATA FOR SHARING

Computational tools and standards for sharing information have advanced in tandem with the technology to measure and describe pharmacogenomic information.

Genomic Data Standards

Genomic data are increasingly incorporated into healthcare workflows, requiring standards for both data storage and exchange. Genomic file formats such as the binary alignment map (BAM) and variant-call format (VCF) developed by the 1000 Genomes Project consortium have become a common standard across academic, commercial, and governmental entities [37,38]. Hundreds of bioinformatic tools have been developed to manipulate BAM and VCF files, including methods to compress, subset, and transfer genomic data. Among these tools is the very useful variant (vt) normalize algorithm, which simplifies the representation of a genomic variant to facilitate dependable retrieval and comparison [39]. An alternative system for representing variants is the Human Genomic Variation Society's (HGVS) sequence variant nomenclature system (commonly referred to as hgvs g., c., and p., indicating the variant's relationship to genomic, coding, and protein coordinates, respectively). An HGVS software package to convert different variant identifiers to HGVS nomenclature is actively developed and supported [40]. Although HGVS g. nomenclature offers similarly robust variant matching as the VT normalized VCF representation, the drawback of HGVS c. and p. nomenclature is the number of potential transcripts and protein isoforms available for specification. However, the benefit to c. and p. is the increased precision for functional annotation of coding and protein variation, though not to the degree of haplotype assignment.

Internet-accessible catalogs of observed genetic variation, pharmacogenomic gene haplotypes, and disease and pharmacogenomic associations are readily available. These catalogs serve essential functions in the process of pharmacogenomic association discovery and clinical translation. Genetic variation datasets, such as the 1000 Genomes Project [41], Genome Aggregation Database (gnomAD) [42], and the Scripps Wellderly study [43], offer precise allele-frequency estimates, allowing comparison of the incidence of pharmacogenomics interactions and appropriate ancestry-based risk stratification. Haplotype frequencies can also be derived from catalogs of genetic variation that offer phased individual-level genotype calls such as the 1000 Genomes Project. The National Cancer Institute developed the linkage disequilibrium link (LDlink) website [44] to offer population-scale linkage disequilibrium estimate tools. Allele and haplotype frequencies can also facilitate the identification of spurious association due to population stratification. PharmVar, an extension of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee, is an international consortium of academics, commercial entities, and government agencies that curates and publishes a catalog of known cytochrome P450 enzyme haplotypes [17]. Catalogs of disease and pharmacogenomic associations such as ClinVar [45], Online Mendelian Inheritance in Man (OMIM) [46], Human Gene Mutation Database (HGMD) [47], and the genome-wide association study (GWAS) Catalog [48,49] provide a central repository of known interactions. This repository can reveal replication or lack of replication across studies and contexts, providing structured input or citation mapping for additional meta-analyses.

Drug Data Standards

More than 1400 therapeutic molecules have been approved by the US Food and Drug Administration (FDA). The FDA drug label includes information about: boxed warnings, indications and usage, dosage and administration, forms and strengths, contraindications, warnings and precautions, adverse reactions, drug interactions, use in specific populations, and patient counseling (Table 10.3). In 2014, the FDA began building openFDA to provide open-source application-programming interfaces and a developer community for FDA data [50]. As of 2018, openFDA offers 14 application-programming interface (API) endpoints, providing tens

TABLE 10.3 Description of Information in FDA Drug Labels

FDA Label Information	Brief Description of Section Contents
Limitations statement	Statement regarding the need for information outside of the label for the safe use of the medication.
Product names and date of initial US approval	Proprietary name and established name of drug, four digit year the drug was approved.
Boxed warning	Description of strict warning information designated within a box.
Recent major changes	Description of major changes to the labeling and approved by the FDA
Indications and usage	Description of each approved indication and major limitations.
Dosage and administration	Description of recommended dosage regimen, starting dose, dose range, critical differences among population subsets, monitoring recommendations, and other clinically significant pharmacologic information.
Dosage forms and strengths	Description of dose forms, strength or potency of the dosage form in metric system, and whether the product is scored.
Contraindications	Description of each contraindication.
Warnings and precautions	Description of the most clinically significant information that would affect decisions about whether to prescribe a drug, recommendation for patient monitoring that are critical to the safe use of the drug, and measures that can be taken to prevent or mitigate harm.
Adverse reactions	Description of the most frequently occurring adverse reactions, criteria used to determine inclusion of the adverse reactions.
Drug interactions	Description of clinically significant interactions, either observed or predicted with other prescription, over-the-counter drugs, classes of drugs, or foods. Information regarding specific practical instructions to prevent and/or manage drug interactions.
Use in specific populations	Information regarding use in pregnancy, pediatrics, and/or geriatrics.
Patient counseling information statement	Information necessary for patients to use the drug safely and effectively.

Adapted from Bodenreider, O. The unified medical language system (UMLS): integrating biomedical terminology. Nucleic Acids Research 2004;32(Database issue), D267–70; United States. Office of the Federal Register, 2005.

of millions of records regarding enforcement reports, device classifications, recalls, label information, adverse events, and regulatory filings. Although openFDA offers a breadth and depth of information about approved drugs, there are billions of additional facts available from distinct computational sources. For example, DrugBank offers hundreds of structured and curated facts for thousands of therapeutic molecules [51].

This proliferation of naming standards has given rise to several classification systems, which has prompted the National Library of Medicine to develop a number of APIs that provide a unified system of names for clinical drugs and a tool to support semantic links between drug vocabularies and pharmacy knowledge base systems. RxNorm offers more than 20 API endpoints providing unique identifiers (i.e., RxNorm Concept Unique Identifier [rxcui]) for generic names, brand names, dosage forms, precise ingredients, and National Drug Code properties. RxNorm APIs also offer search functions that map a character string to a relevant rxcui [52]. The National Drug—Reference Terminology (ND-RT) API

provides access to the National Drug File Reference Terminology dataset [53], providing information about drug class, indications, mechanism of action, and a series of identifiers from standardized vocabularies such as the Unified Medical Language System [54]. An alternative source of drug class information can be accessed through the RxClass API, which offers an assortment of mappings to standard class schemas such as the FDA's Established Pharmacologic Class (EPC), Veterans Administration (VA) class, and the NDFRT's drug classification system. As with any well-developed API, the Rx* APIs provide a number of well-documented paths to access the same information.

Phenotype Data Standards

Methods used to describe phenotypes have also advanced. A large amount of pharmacogenomics literature has been developed upon electronic medical record mining, which is commonly based upon structured billing data such as International Classification of Diseases (ICD) 9 codes [55]. The release and adoption of ICD-10 offered a more descriptive vocabulary to record patient health states; however, ICD-10 lacks the advantages of a formal ontology. The Human Phenotype Ontology (HPO) was developed to describe abnormalities associated with human disease [56,57]. HPO has quickly become a standard used by several clinical genetic consortia, and tools have been developed to enable clinicians and patients to efficiently record phenotype data, integrate with genomic information, and yield a differential diagnosis [58,59,60]. It is currently premature to conclude that the use of the HPO can enable robust federated analytics across institutions and geographic contexts; however, early results are promising [61].

Adverse drug reactions are an important class of pharmacogenomic associations and can be described using the Common Terminology Criteria for Adverse Events (CTCAE), which is a subset of the Medical Dictionary for Regulatory Affairs (MedDRA) coding system commonly utilized in clinical trials [62]. This freely available vocabulary is both detailed and comprehensive; however, it is organized by organ system and lacks a formal hierarchical structure. One benefit of the MedDRA vocabulary is the use of a well-defined severity continuum that adds an additional dimension of detail for describing phenotypes that the HPO currently lacks. Table 10.4 offers a brief comparison of commonly utilized phenotyping terminologies.

TABLE 10.4 Comparison of Phenotyping Terminologies

	Intended Use	Breadth	Depth	Ontology
ICD-9	Medical terminology to classify diagnoses and procedures	++	++	–
ICD-10	Medical terminology to classify diagnoses and procedures	++	+++	–
SNOMED-CT	Clinical terminology	+++	+++	–
MedDRA	Medical terminology for use by regulatory authorities and regulated biopharmaceutical industry	++	+++	–
CTCAE	Adverse drug event description and severity grading in oncology clinical trials	+	+++	–
HPO	Description of phenotypic abnormalities found in human disease	+++	++	+

CTCAE, common terminology criteria for adverse events; *HPO*, human phenotype ontology; *ICD*, international classification of diseases; *MedDRA*, medical dictionary for regulatory activities; *SNOMED-CT*, systematized nomenclature of medicine—clinical terms.

Data Exchange Standards

After pharmacogenomic data has been structured in a manner that facilitates clear comprehension by clinicians, scientists, and patients, a method of accessing this data is imperative to its usefulness. Enter the application-programming interface (API), which can be used to standardize the expected input(s) and output(s) for any knowledge-based resource.

An API presents an abstraction layer for data and/or functions that a computational service would like to expose. The abstraction layer typically offers a subset of data and/or services using the API parameter set. A good API clearly specifies the range of acceptable inputs and guarantees the structure and range of outputs. The value of an API arises from this standardization of input and outputs, allowing the user to create higher levels of abstraction for general and specific tasks. For example, the National Laboratory of Medicine's aforementioned drug APIs provide access to a diverse and configurable set of information about drugs, in addition to access to several computational functions (e.g., fuzzy mapping a generic drug name to a unique RxNorm rxcui identifier). APIs can also be subject to standards in their design; two popular API standards are Representational State Transfer (RESTful) and Simple Object Access Protocol (SOAP), the former can use JavaScript Object Notation (JSON) and Extensible Markup Language (XML), whereas the latter uses XML exclusively.

It is important to note the difference between an API specification (definition of the acceptable inputs and outputs) and the underlying implementation (algorithm and software that carries out the steps between input and output of the API). Standardization of pharmacogenomic API specifications will speed clinical translation, while still allowing stakeholders the flexibility to design their own implementation. A thorough review of API design, documentation, and specification is beyond the scope of this chapter, but an excellent resource for the construction of good RESTful API design patterns can be found at www.github.com/WhiteHouse/api-standards.

A plethora of data schemas and standards exist to structure medical, laboratory, and phenotype information. Of the myriad healthcare data models in use, the Health Level Seven International (HL7) organization is likely the most widely utilized. Unfortunately, the early versions of the HL7 standard model provided sufficient flexibility to allow a multitude of valid local implementations, ultimately reducing interoperability across the healthcare industry due to the lack of a common and interoperable data model.

Building upon the success of RESTful APIs, HL7 developed Fast Healthcare Interoperability Resource (FHIR) to provide a simple and flexible standard to facilitate health-data exchange without "sacrificing information integrity" [63]. FHIR's information integrity arises from the requirement to specify a permanent reference to the source vocabulary and definition for each functional term. FHIR leverages web-based APIs to exchange electronic health-record information in either JSON or XML format. Most of the major electronic healthcare vendors and federal healthcare agencies in the United States have pledged to support FHIR API data exchange, which has spurred adoption by a broad coalition of healthcare stakeholders. Although FHIR has gained momentum as a standard healthcare exchange model, FHIR does not require the use of a specific vocabulary or concept set, nor does it require backend systems to utilize an FHIR data model.

A more opinionated Common Data Model was developed by a consortium of academic investigators (Observational Medical Outcomes Partnership [OMOP]) and subsequently extended by the Observational Health Data Science and Informatics (OHDSI) program to enable consistent data sharing for research purposes [64,65]. The Common Data Model uses a relational data schema with a multitude of structured vocabularies forming a patient-centric

datastore that hosts information about clinical phenotypes, laboratory values, medications, and administrative billing. This 'batteries-included' resource has been used to transform some of the largest healthcare datasets into the CDM and allow analytic pipelines to be reused with minimal modification across institutions [66].

DISCOVERING AND QUANTIFYING PHARMACOGENOMIC INTERACTIONS

Federated Data Models

Pharmacogenomics is a broad field encompassing dozens of therapeutic areas, thousands of genetic variants, and hundreds of medications. Several of the most clinically important pharmacogenomics associations involve rare adverse reactions, in which evidence is accrued necessarily across wide geographic boundaries. Several astute scientists realized early that a federated system of evidence collection and data sharing would speed discovery and translation of pharmacogenomics knowledge among other genomic medicine-use cases. Numerous federated-data models have been developed recently and two successful open-source models of federated health data have had particular success across industry, academia, and government.

Informatics for Integrating Biology and the Bedside (i2b2) was originally funded by the National Institutes of Health (NIH) and developed in the early 2000's by scientists in Boston, Massachusetts, as an open-source java-based application suite [67]. Development of i2b2 applications has grown into an international effort with hundreds of installations and dozens of application extensions. Core i2b2 functionality includes ontology management, identity management, a workflow framework, project management, a file repository, and a clinical data warehouse. Additional i2b2 applications provide natural language processing, correlation analyses, table viewers, and a web client. Akin to i2b2's vision of federated-data sharing, the OHDSI consortia have constructed an internationally distributed research network that leverages the Common Data Model for a variety of federated tasks, including transportable cohort definitions, analysis packages, and reporting dashboards [68].

A simpler example of a federated genomic data network is the Global Alliance for Genomics and Health (GA4GH)'s Beacon project [69]. The Beacon developers envisioned a publicly accessible distributed network of open-source APIs, which provided a single bit of information in response to a query regarding a genomic variant. That single bit indicated whether the genomic variant exists within the data store, returning a true or false response. The Beacon project developers believed that the restriction to a single bit of information could provide enough information to be useful, but too little to substantially increase privacy concerns. However, several groups have shown that the Beacon architecture does not preserve privacy when queried many times, demonstrating the unique security and privacy features of sharing genetic information [70,71].

Several algorithms have been developed to maintain precision and accuracy of the resulting model while preserving privacy [30,72–74]. These algorithms leverage a federated-data model and offer the benefit of scalability. The Trusted Analytics Platform was built upon the premise that it is much cheaper to send code to data rather than data to code. It can also be shown that inspection of code, particularly the expected outputs, can minimize security and privacy concerns associated with sharing data. Federated-data analysis saves bandwidth and can avoid more cumbersome data-transfer agreements. The rise of container technologies, such as Docker [75] and Kubernetes [76], has accelerated the vision of moving code to the data. With an increasing number of nations ratifying laws prohibiting sensitive data export, federated-data analysis is likely to be

the dominant method of scientific collaboration employed by international pharmacogenomic studies. The suite of tools available through i2b2 demonstrates a successful implementation of a federated-data analysis platform yielding novel pharmacogenomics discoveries [77].

Primary Evidence

Data created by studies specifically designed to investigate a gene–drug interaction are herein defined as primary pharmacogenomic data sources. Primary pharmacogenomics data provides the most specific information about gene–drug interactions due to experimental methods that seek to preserve causal inference and offer the most important source of clinically actionable pharmacogenomics evidence. Primary pharmacogenomic studies may focus on pharmacokinetics (absorption, bioavailability, metabolism, and excretion), pharmacodynamics (mechanism of action and effects), or a combination of the two. A well-designed randomized controlled trial with a pharmacogenomics-focused prespecified primary endpoint offers the most statistically powerful study design to investigate frequent outcomes.

The Clarification of Optimal Anticoagulation through Genetics (COAG) and European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) studies are excellent examples of hypothesis-driven, randomized controlled pharmacogenomic studies yielding primary evidence. Both studies were published in the same issue of the *New England Journal of Medicine* in 2013 and both randomized patients to genotype-guided or clinically guided-warfarin dosing. The primary outcomes for COAG and EU-PACT were time within an international normalized ratio (INR) therapeutic window at 4 and 12 weeks, respectively [78,79]. The COAG trial (n = 1015) reported no improvement of anticoagulation control in the genotype-guided arm at 4 weeks, whereas the EU-PACT trial (n = 455) observed a higher percentage of time within therapeutic range at 12 weeks in the genotype-guided arm. The clinical equipoise the COAG and EU-PACT trials created was further explored in the Genetics InFormatics Trial (GIFT) trial (n = 1650) which was published 4 years later and measured a composite outcome of INR greater than 4, major bleeding, venous thromboembolism, and death over 5 years in a population at risk of deep vein thrombosis [80]. The GIFT trial demonstrated a clear benefit in clinical outcomes for the genotype-guided arm.

AltheaDx recently published a prospective, blinded, randomized controlled trial assessing the effectiveness of pharmacogenomics-guided antidepressant treatment on successive measures of depression and anxiety [81]. Six hundred and eighty-five patients were enrolled from Psychiatry, Internal Medicine, and Obstetrics and Gynecology clinics; however, the reported analysis focused on a subset of participants with moderate and severe depression. The authors report response rates, defined as a ≥50% reduction in the baseline Hamilton Rating Scale for Depression (HDRS) score ($P = .001$; OR 4.72, 95%CI 1.93–11.52, n = 261 moderate and severe depression at enrollment). The authors also found higher remission rates, defined as an HDRS score of <7 at 12 weeks, in the experimental arm ($P = .02$; OR 3.54, 95%CI 1.27–9.88, n = 93 participants with severe depression at enrollment). Importantly, the study failed to provide evidence for the prespecified primary outcome of reducing adverse events, nor did the authors utilize an intention-to-treat analysis protocol [81].

Another example of primary pharmacogenomics evidence is the Statin Response Examined by Genetic Haplotype Markers (STRENGTH) trial, which enrolled 509 subjects to investigate the efficacy and safety of statin medications (Simvastatin, Atorvastatin, and Pravastatin) in the context of reduced-function solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) variants [82]. Voora et al. reported significantly higher rates of composite adverse events (defined as discontinuation of therapy due to any side effect, myalgia,

or a creatinine kinase level greater than three times the upper limit of normal) in two groups of participants given high doses of statins for 6 weeks, females ($P < .01$), and those carrying the *SLC01B1**5 genetic variant ($P = .03$).

Rare adverse events are very difficult to investigate using prospective randomized controlled trials due to cost and thus require a different set of methods as detailed later.

Secondary Evidence

Pharmacogenomic data derived from studies originally focused on the evaluation of a medication or evaluation of genetic markers without a specific pharmacogenomics hypothesis are herein defined as *secondary* pharmacogenomics data sources. Secondary pharmacogenomics data provide a vital wellspring of hypotheses for primary pharmacogenomics studies and/or important supplementary information to primary data. Whether the initial investigation focused on a gene-based or drug-based hypothesis, the ability to analyze the samples post hoc for the complementary outcomes presents a wealth of opportunities to generate preliminary evidence of pharmacogenomics association and will likely offer the greatest number of putative associations. We offer two examples of secondary pharmacogenomics data; the first example used a drug-focused trial design, whereas the second example describes post hoc analyses of genomic biobank cohorts.

Post Hoc Drug-Focused Randomized Clinical Trial

The Effective aNticoaGulation with factor xA next GEneration in Atrial Fibrillation (ENGAGE AF)-Thrombolysis in Myocardial Infarction (TIMI) 48 trial prespecified a nested pharmacogenomics trial (n = 14,348) to investigate the effect of cytochrome P450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex subunit 1(*VKORC1*) variants on the risk of bleeding in the warfarin and edoxaban arms [83]. Patients were categorized into three phenotypes (normal, sensitive, and highly sensitive responders to warfarin) based upon variation in *CYP2C9* and *VKORC1* variant alleles. Mega et al. reported that sensitive and highly sensitive responders spent a greater proportion of time overanticoagulated in the first 90 days of treatment in comparison to normal responders. The authors also reported a reduction in bleeding risk in the edoxaban arm compared to those who were classified as sensitive or highly sensitive in the warfarin group, although this risk difference became nonsignificant by 90 days postintervention.

A common side effect of the widely prescribed angiotensin-converting enzyme inhibiting (ACEI) medications is dry cough. In 2016, Mosley et al. reported the results of a genome-wide association study (GWAS) for ACEI-associated cough, offering evidence of association with variants in an intron of voltage-gated potassium channel-interacting protein 4 (*KCNIP4*) [83,84]. The study analyzed 7080 subjects of diverse ancestries in the Electronic Medical Records and Genomics (eMERGE) network. A strong association with rs145489027 ($P = 1.0 \times 10^{-8}$; OR = 1.3, 95CI% 1.2–1.4) was observed. A replication GWAS using two separate cohorts, eMERGE (n = 926) and Genetics of Diabetes Audit and Research in Tayside, Scotland (n = 4309), identified a combined association with rs1495509 ($P = 1.9 \times 10^{-9}$; OR = 1.23, 95%CI 1.15–1.32). These studies demonstrate the usefulness of genomic biobanks connected to electronic medical records and the ability of secondary pharmacogenomics data sources to generate testable hypotheses.

Tertiary Analysis

We define tertiary evidence as the synthesis of published data from machine-readable or human-curated sources. The curation of pharmacogenomics evidence commonly begins with literature searches using structured queries of Public/Publisher MEDLINE (PubMed), the Cochrane

Database, and other catalogs of scientific literature. The National Center for Biotechnology Information (NCBI) maintains APIs that accept PubMed queries, optionally using Boolean operators, Medical Subject Headings (MeSH), and additional filters, thus allowing automation of the search process. Once pertinent manuscripts and/or data sources are identified, extraction of useful information proceeds through additional human curation and/or natural language processing (NLP). Although rapid advances in the accuracy and speed of NLP algorithms and software packages have occurred in the past several years, the task of identifying precise relationships between genes, medications, and phenotypes continues to require human review [85]. Once pertinent facts are extracted from the literature and/or datasets, summaries of effect-size estimation and association validity can be generated using qualitative or quantitative methods.

Qualitative Summary Methods

The Pharmacogenomics Knowledge Base (PharmGKB; www.pharmgkb.org), hosted at Stanford University in California, provides extensive and structured pharmacogenomics data, narrative extracts, and curated haplotype tables [86]. PharmGKB utilizes computational methods and human curation to provide information about medication dosing, drug metabolism and pharmacodynamic pathways, pharmacogenomic allele frequencies, statistical results from published manuscripts, and clinically actionable pharmacogenomics guidance. PharmGKB also provides a semiquantitative evidence level score for variant–drug pharmacogenomic associations. This score is based upon the existence of professional guidelines, number and statistical significance of published manuscripts, and in vitro evidence. PharmGKB also publishes guidelines produced by the Clinical Pharmacogenomics Implementation Consortium (CPIC).

CPIC is an international consortium dedicated to "translating genetic laboratory test results into actionable prescribing decisions for affected drugs" [87]. This is accomplished through the creation of evidence-based peer-reviewed guidelines that contain clinical recommendations and evidence grades. The CPIC guidelines are produced through literature review and expert consensus, offering a more-qualitative assessment of the evidence than a systematic meta-analysis. CPIC has recently launched an independent website that hosts published guidelines, pharmacogenomic allele tables, and searchable table of CPIC evidence levels (www.cpicpgx.org).

Quantitative Summary Methods

Rigorous individual-level meta-analyses of primary and secondary evidence yields the most robust syntheses of pharmacogenomic associations, clinical utility, and clinical effectiveness. Meta-analyses can suffer from a paucity of published evidence, heterogeneity of study designs, and variable analytic methods; however, meta-analyses can also quantify heterogeneity and uncertainty. In 2017, Lee et al. published a pharmacogenomics meta-analysis engine leveraging the Web Ontology Language 2 (OWL2) semantic web technology [88]. Although the published ontology was built to support two cardiology-focused pharmacogenomics associations, the conceptual model can be extended into a general pharmacogenomics knowledge graph that can produce semiautomated quantitative evidence summaries. This structured and semantic model of pharmacogenomics evidence offers a flexible and rigorous technology that is likely to provide the global pharmacogenomics community a continuously learning knowledge graph.

As regulatory agencies throughout the world demand public release of structured clinical trial data describing study design and individual responses, a plethora of statistical analyses by scientists unassociated with the study will likely ensue [89–91]. The availability of this data via APIs may lead to automated analyses and meta-analyses investigating pharmacogenomics

associations. One barrier to this vision of distributed pharmacogenomic analysis is the set of justified and reasonable privacy protections. Whether these protections will use a system of registered data access, statistical de-identification, or acceptance of registered analytic code remains to be seen. Regardless of the privacy protocols put in place, an increasing number of algorithms will be applied to controlled-trial data yielding discovery of new pharmacogenomic interactions and/or validation/refutation of previously reported associations.

PHARMACOGENOMICS FORECASTING

Discovery and validation of pharmacogenomics associations will continue using in vitro, ex vivo, and in vivo hypothesis-based research. Many of these focused experiments will be informed by a deluge of post hoc data mining of genomics-focused cohorts (e.g., UK Biobank, NIH All of Us Research Program [AllofUs], Geisinger Health System Community Health Initiative [MyCode], Vanderbilt University Medical Center biorepository [BioVU]) and drug-focused clinical trials. As the costs to reconstruct linear haplotypes decrease and accuracy improves, published pharmacogenomic interactions will include detailed summaries of genomic, expression, and phenotypic covariates. Models similar to the warfarin-dosing calculator will become more common as larger datasets become available and interoperable due to standardization of labeling via ontologies (e.g., HPO), structure via CDM, and exchange via FHIR. The rise of passive health sensors embedded into medications, watches, smartphones, and smart speakers will collect streams of individual-level data that will be used to minimize unobserved confounders in clinical trials, population-health interventions, and quality-of-care analyses.

The robust commercial success of rare disease and oncology therapeutics has spurred the creation of companion diagnostics as necessary components of clinical development pipelines. Due to the genetic basis of several rare diseases and oncology subtypes, many of these companion diagnostics will be classified as pharmacogenomic tests, e.g., eliglustat for Gaucher disease or cystic fibrosis transmembrane conductance regulator (CFTR) mutation analysis for ivacaftor. The use of standard phenotyping methods, genomic data structures, and federated analysis tools in primary pharmacogenomics studies will accelerate the identification of clinically meaningful pharmacogenomic efficacy and adverse reactions. Ancestry informative genetic markers will become standard covariates in average treatment effect models, similar to sex, age, and education level.

Electronic health-record systems will incorporate genetic-testing information, via the FHIR Genomics specification, as a standard laboratory result. The availability of genotype information as discrete laboratory values in an electronic health record will enable clinical decision support. Clinical decision support at the point of prescribing will be accelerated by open-source projects like CPIC's PGx Clinical Annotation Tool (PharmCAT) and integration of commercial genetic testing applications into electronic health record (EHR) application marketplaces such as Epic's App Orchard. Pharmacogenomic Clinical Decision Support Systems (CDSS) will alert clinicians to impending pharmacogenomics-prescribing interactions and assist with appropriate drug selection and dosing guidance. Once a medication and dose is selected, automated algorithms to monitor the patient for efficacy and side effects will be deployed through patient-reported outcomes surveys, ingress of sensor data, and electronic health-record mining.

CONCLUSION

Our ability to measure genes, drugs, and phenotypes will continue to improve apace with genomic and computational technology.

Massive publicly and privately funded initiatives to sequence millions of individuals are well underway. The ability to construct accurate linear haplotypes is now available, if at the moment costly and cumbersome. Digital sensors compounded with therapeutics offer the ability to passively track medication exposure. Patient phenotypes are routinely collected using structured vocabularies, transformed into a common data model, and analyzed through federated-data platforms. Technologies that standardize pharmacogenomics data collection, analysis, and interoperability will accelerate discovery of new associations and speed robust validation and clinical translation. The clinical implementation of pharmacogenomics is progressing and will continue to accelerate in tandem with the use of genomics data in reproductive health, oncology, and rare diseases.

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CHAPTER

11

The Importance of Ethnicity Definitions and Pharmacogenomics in Ethnobridging and Pharmacovigilance

Wei Chuen Tan-Koi[1], *Michael Limenta*[1], *Elsa Haniffah Mejia Mohamed*[2,3], *Edmund Jon Deoon Lee*[4]

[1]Health Products Regulation Group, Health Sciences Authority, Singapore; [2]Pharmacogenomics Laboratory, Department of Pharmacology, University Malaya, Kuala Lumpur, Malaysia; [3]Pharmacogenetics Laboratory, Department of Pharmacology, National University of Singapore, Singapore; [4]Pharmacogenetics Laboratory, Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00011-5

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OBJECTIVES

1. To emphasize the complexity of defining ethnicity and the need of a standardized definition for scientific research
2. To outline the ethnic intrinsic and extrinsic factors in determining outcome of treatment
3. To identify the factors considered in acceptability of foreign clinical data for drug approval
4. To highlight the role of pharmacogenomics in global drug development
5. To highlight the role of pharmacogenomics in pharmacovigilance

INTRODUCTION

Within the human race, variation exists between people of different ethnicities. The ethnic effect on variability of disease susceptibility and pharmacological outcome is well established and has been described extensively. This is one of the major considerations for any local drug regulatory authority when considering applications for new drug approval utilizing foreign clinical data in their region. Bridging studies were proposed to provide supplemental data on a drug's pharmacokinetics/pharmacodynamics (PK/PD), safety, efficacy, dosage, and dosage regimen in a new country/region, to determine if the foreign clinical data can be applicable to the new country/region. Discussions on the (de)merits of doing bridging studies based on ethnicity are not new. Many arguments have been put forth for various reasons, and one of the main reasons for dispute is that the terms "ethnicity" and "race" are very poorly defined. Regulatory agencies and the pharmaceutical industry have attempted to address this issue through the issuance of a harmonized guideline under the ambit of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). This coalition of regulatory agencies and pharmaceutical industries was first incepted in 1990 to discuss and harmonize scientific and technical aspects of drug registration among its founding members. These include the United States Food and Drug Administration (US FDA), the European Commission, the Ministry of Health, Labour and Welfare of Japan, the European Federation of Pharmaceutical Industries and Associations, the Japan Pharmaceutical Manufacturers Association, and the Pharmaceutical Research and Manufacturers of America.

With evolvement in global drug development, the coalition of Regulatory Members was expanded to now include other regulatory agencies such as Health Canada; the Brazilian Health Surveillance Agency (ANVISA; Brazil); Swissmedic (Switzerland); China Food and Drug Administration; Korea Ministry of Food and Drug Safety, and Singapore Health Sciences Authority. The ICH E5(R1) guideline on Ethnic Factors in the Acceptability of Foreign Clinical Data aims to provide a framework to evaluate ethnic factors on the outcome to medical

treatment(s), and hence facilitate drug-approval process, while minimizing clinical trial duplication with swift delivery of new medicines to needy patients [1]. However, not all regulatory agencies have fully adopted the guidelines. Even in the agencies in which it has been adopted, appreciable differences existed in the way the bridging concept has been applied. An approach that may be a solution to this dilemma is the multiregional parallel bridging method, or process of simultaneous drug development. Apart from having the advantage of reducing significantly the lag time in drug approval, these allow for prospective bridging of the data in various regions. In 2014, the topic on "E17: General principle on planning/designing Multi-Regional Clinical Trials" was endorsed by ICH and had been adopted by the Regulatory Members of the ICH assembly in 2017. This complements the guidance in ICH E5(R1), thus facilitating Multiregional Clinical Trials (MRCT) data acceptance by the different regulatory agencies [2]. The integration of pharmacogenomics and biomarkers in drug development is also seen as a positive attempt to better characterize ethnic factors. In the end, it is hoped that better risk–benefit consideration for drug treatment can be achieved through population data that are more relevant.

In this chapter, the relevance of ethnicity-based bridging studies is examined in three different parts. The first part reviews the difficulties in the definition of ethnicity and the ambiguous ways that in which the term has been applied. Next, ethnic factors as defined by ICH E5(R1) contributing to the variability in drug response are examined, with emphasis on the influence of ethnicity on the interpretation of clinical trial results and the learning points from pharmacogenomics studies of admixture populations. With increasing popularity of complementary medicine, the effects of dietary and use of herbal medicines in various population groups are also discussed. Thirdly, approaches by different regulatory agencies on acceptability of foreign clinical data for drug-marketing approval will also be reviewed. The chapter concludes with an update on the application of pharmacogenomics in the field of pharmacovigilance in the postmarketing phase of a drug life cycle and issues to be considered for future pharmacogenomics studies.

ETHNICITY

Ethnicity is a sociocultural construct with very vague scientific definition. The tendency to overgeneralize ethnic population groups also reduces the validity of many analyses based on ethnic stratification. The relationship between self-identified race or ethnicity and disease risk has been depicted as a series of surrogate relationships between genetic and nongenetic factors [3]. The nongenetic component includes social, cultural, educational, and economic variables, all of which can influence disease risk and response to pharmacological treatments. The distinction between the terms race and ethnicity is also highly controversial and varied, as highlighted in *Nature Genetics Editorial* (*2000*): *Census, Race, and Science* [4]. In this article, several examples of dictionary definitions of race and ethnicity were exemplified as follows:

Race:

- A vague, unscientific term for a group of genetically related people who share certain physical characteristics
- A distinct ethnic group characterized by traits that are transmitted through their offspring
- Each of the major divisions of humankind, having distinct physical characteristics
- A group of individuals who are more or less isolated geographically or culturally, who share a common gene pool, and whose allele frequencies at some loci differ from those of other populations

Ethnic group:

- A population of individuals organized around an assumption of common cultural origin
- Individuals with a common national or cultural tradition
- A social group or category of the population that, in a larger society, is set apart and bound together by commonalities of race, language, nationality, or culture

Although the definitions are ambiguous, it can be deduced that race and ethnicity give an insight to cultural, historical, and perhaps socioeconomic, as well as ancestral geographic origins. As race and ethnicity are different mixtures of biological with social constructs, they are highly complex and dynamic in nature.

Defining race and ethnicity accurately is obviously difficult, given its complex and dynamic nature and the fact that the genetic pool for a population would most likely be heterogeneous in nature and not discrete categories [5]. This makes any assumptions about purity and accuracy of the definitions fallacious.

Ethnicities Are Rarely Homogeneous in Any Nation

There is often a presumption that certain nations are ethnically homogeneous in nature, and that extrapolation of clinical data can be made to certain nations this way. In most circumstances, such references to population groups are not actually ethnicity based, but rather, nation based. Even though this is understandable because regulatory agencies are nation-based entities, such practices are fundamentally erroneous, given the ethnically heterogeneous nature of almost all nations.

This applies even for well-established safety biomarkers. Descriptions of populations at risk for well-established safety biomarkers in drug labels have been noted to vary between countries and extending ancestry information broadly across a single region may be misleading [6,7]. Even within a single country, albeit a large country such as China, correlations between geographical latitudes and genetic variation has been reported. This north–south cline in genetic differences reported concurred with the northern migratory route from Southeast Asia into East Asia [8]. With much of the diaspora drawn from the south, extrapolation of global Chinese data to northern Chinese would be difficult. Similarly, data drawn from the northern Chinese could also not be directly applied. Another large population, that of India, is also a heterogeneous group. Other than the North–South distinction between Dravidians and more Caucasoid Northerners, the Easterners have a more Mongoloid admixture.

Globally, human genetic variation projects such as the 1000 Genomes Project and the International HapMap Project have also shown genetic variation both within and between nations [9–12]. Investigations of pharmacogenomics variants involved in drug absorption, distribution, metabolism, and excretion have also reported marked variation both within and between populations grouped geographically [13]. Genetic variations between populations are further complicated by complex ancestral histories, such as those reported in African Americans [14] and findings that self-identification of ethnicity could be erroneous. Studies have reported European genetic ancestry in some self-identified African Americans and vice versa of African genetic ancestry in self-identified European Americans [15,16]. Clearly, individual genetic ancestry cannot be simplistically extrapolated from self-reported ethnicity as misclassification errors introduced by self-reporting can affect the interpretation of clinical trial results, which may be pivotal for drug-registration approval.

Some examples of genetic variation within countries are discussed in the next section.

Japan

The word “Japanese” is used collectively for a collection of ethnic groups known as the Yamato

(Hondo–Japanese, or mainland Japanese), Ainu, and Ryukyuan (Okinawan). Even in the national census [17], actual ethnicity (minzoku) is not measured, but rather Japanese nationality (kokuseki). However, the Ainu people, in particular, who are regarded as the aborigines of Japan living in Hokkaido, differ from the rest of the broader Japanese group physically, linguistically, and culturally [18]. Genetic studies evaluating ancestral origins of the Japanese people have shown that they originate from two distinct groups of people: Ainu and Ryukyuan populations are direct descendants of the Neolithic Jomon people, whereas the Hondo people are derived from the northeast of continental Asia [19]. Ancient mitochondrial analyses suggested that the gene flow was from Southeastern Siberia to the Jomon/Epi–Jomon people of Hokkaido, Sakhalin, and Kuril Archipelago, with the Okhotsk people being intermediaries [20]. Phylogenetic analyses comparing the three ethnic groups have also suggested that the Ainu and Ryukyuan samples are clustered together, whereas the Hondo–Japanese and Koreans were clustered together in the neighbour joining genetic tree [19]. More recently, with advances in genome sequencing, genome-wide study on one million single-nucleotide polymorphism (SNPs) has also supported the dual-structure model [21]. Interestingly, haplotype diversity studies have further shown that the genetic distance between the Okinawa populations and the Hondo–Japanese measured at the major histocompatibility complex are more distinct compared to the differences between the Gujarati population in North India and Tamil–Nadu in South India [22].

When a Japanese leaves Japan and migrates to some other country, the definition of Japanese assumes a slightly different context. This is important to recognize, especially if recruiting for bridging studies. The Japanese then will be categorized as [23]:

- First-generation Japanese (Issei): subject born in Japan
- Second generation (Nissei): Subject born elsewhere, but both parents born in Japan
- Third generation (Sansei): Subject and one or both parents born elsewhere, but grandparents born in Japan

To be eligible for the studies, they must have at least had all four grandparents born in Japan with no mixed descent. Thus, a Japanese of up to the third generation would be eligible to represent the Japan–Japanese populations [23].

China

Although the Han Chinese is the largest ethnic group in China, there are at least 55 officially recognized ethnic minority groups [24] totaling about 138 million people in China [25]. An equally diverse number of languages are being spoken in China, numbering up to 200, from seven linguistic families of Altaic, Austroasiatic, Austronesian, Daic, Hmong Mien, Sino–Tibetan, and Indo–European [24]. Furthermore, even within the Han Chinese ethnic group, significant genetic heterogeneity exists.

The Han Chinese is possibly the largest ethnic group in the world, making up about 20% of the human population. It is also the most prevalent ethnic group in China at more than 90% of its total population [26]. Although the Han people are now spread all over the country, the formation of the Han people began with the ancient Huaxia tribe in northern China, which spread southward in over 200 years [24]. Expansion of the Han ethnic group is the result of integration of multiple tribes and ethnic groups [27]. Mitochondrial DNA (mtDNA) studies and other genetic marker studies in China have suggested interesting variations within the Han population with obvious geographical differentiation, primarily on a north–south axis [27–29]. As a consequence of this, it may be necessary to cluster the Chinese Han population into clusters according to geographic origins. Today, there are several subgroups of the Han Chinese, speaking different dialects or arguably, different languages, and

in fact, living in different parts of the world. It is uncertain if a clinical study done in Han Chinese from the Northern part of China can adequately represent of the rest of the global Chinese ethnic group.

Similarly, it is uncertain that clinical data derived from overseas Chinese who are primarily derived from Southern Chinese group can be taken to represent a generic Han Chinese population in China. Indeed, bioinformatic measures have found clear genetic differentiation among the Han Chinese ethnic group originating from the North and those from the South [30]. Linkage disequilibrium investigations further suggests that human leucocyte antigen (HLA) imputation panel from the HapMap project, which draws solely from the Han Chinese in Beijing, located in the North, cannot be directly applied to all Chinese samples. Apart from the heterogeneity in the Han Chinese, there is evidence of west Eurasian and northern East Asian (along the Silk Road) genetic admixture with the northwest Chinese populations [31].

Malaysia

Malaysia is a multiethnic nation, with the Malays making up the majority (42.1%), Chinese (24.6%), Indians (7.4%), and native Sabah and Sarawak (24.8%). The majority of the Sabah and Sarawak native ethnic groups are the Ibans and Kadazan/Dusun ethnic groups [32].

Malays are part of a larger linguistic family of the Austronesian group, which is very widespread in Southeast Asia, and similarities have been reported with the Tagalog people of Philippines, Ryukyu islanders (located in the chain of Japanese islands that stretched southwest from Kyushu to Taiwan), and Taiwan aborigines. Similar to the Han Chinese in China, heterogeneity can be observed within the Malays in Peninsular Malaysia itself. The Malay *race* has been defined as members of peoples inhabiting the Malay Peninsula and parts of the western Malay Archipelago (*American Heritage Dictionary 5*). The migration history of the Malays suggests the Malays have several ancestral origins [33]. The *Melayu Bugis* (Bugis Malay) and *Melayu Jawa* (Javanese Malay), who are mainly in the southern peninsula, as well as the *Melayu Minang* (Minang Malay) in the western peninsula, are historically and culturally related to Indonesia (*Sumatra* and *Java*), whereas the *Melayu Kelantan* are related to Thailand (*Siam*).

Genetic admixture with the Chinese and Indians also occurred during the British colonization period. These main ethnic population groups may play important parts in the genetic heterogeneity in the Malays [34] of the Peninsula of Malaysia. Genetic differences between the four subethnic Malay groups within Peninsular Malaysia were studied by Hatin et al., utilizing 54,794 genome-wide SNPs from the four Malay subethnic groups and compared to the genetic profile of 11 other population datasets obtained from the Pan Asian database [34]. The study showed that the *Kelantanese* Malay were genetically distinct from the other three groups of Malays, who showed high resemblance to the Indonesian Malays. Not surprisingly, the results showed that the Malays could be assigned to three different clusters, with the *Melayu Minang* and *Melayu Bugis* in Cluster I, *Melayu Jawa* in Cluster II, and *Melayu Kelantan* in Cluster III. The *Melayu Kelantan* formed an independent clade, suggesting a more divergent ancestry compared to the other two clusters. Also intrinsic in this region is the native Negrito/Veddoid people, which may have similarities between aboriginal groups, the Andamanese, and indigenous people of the ethnogeographic group of Pacific Islands known as Melanesia (spanning Papua New Guinea, Solomon Islands, Vanuatu, New Caledonia, and Fiji). Genetic affinity has been identified between Andamanese and Malaysian Negritos, and it was suggested that Senoi and Proto–Malay (two of the major Malay ethnic groups) arose from genetic admixtures between Negrito and East Asian populations [35]. Genome-wide study of Peninsular Malaysian Malay (PMM) have also shown admixture with

multiple ancestries such as Austronesian, Proto–Malay, East Asian, and South Asian [36].

Other instances also exist of bias in the definition of ethnicity. Take, for example, the definition of the Malay ethnicity in Malaysia. The Malaysian Constitution (article 160) [37] defines the Malays as the following:

- Malaysian citizen born to a Malaysian citizen
- Professes to be Muslim
- Habitually speaks the Malay language
- Adheres to Malay customs
- Born, or born of parents, or resides in Malaysia or Singapore on/before the Merdeka Day

It is obvious that the Malay ethnicity definition in Malaysia is a complex sociocultural and religious construct. The Malaysian Constitution accepts "non-biological" Malays who are incorporated through lifestyle, culture, and religion, known as masuk Melayu or literally "embracing Malayness." Apart from that, a Malay person in Malaysia is no longer considered Malay by law if the person converts out of Islam.

It should be noted, though, that only the difficulties in defining the Malays have been described here. Any attempts to carry out ethnobridging studies in Malaysia have also to take into account the other ethnic populations, as described earlier. Although the situation in neighboring Singapore is not discussed here, it should be noted that the ethnic definition of Malay differs between the two countries, and complicates attempts to generalize ethnic data from one country to another.

Indonesia

Indonesia is the fourth most populous country in the world. It is made up of 17,000 islands, over 240 million people with an immensely diverse admixture of over 750 languages and 300 ethnic groups [38]. Austronesian expansion reported from west to east in East Indonesia, as well as admixture with Papuan ancestry, led to great heterogeneity in Indonesia [39,40]. Although bearing similar trading past and genetic admixture of Negrito/Veddoid and Austronesian groups with early Chinese and Indian traders, geographical isolation between island groups have resulted in genetic pockets, making it difficult to extrapolate genetic data from Malaysia to Indonesia. To promote nationalism and sense of unity in the postcolonization era, the *Pancasila* ideology was introduced in 1945, which underlines the nation identity as culturally neutral and the use of *Bahasa Indonesia* (Indonesian language) by all people in Indonesia. National censuses between 1961 and 1990 were devoid of ethnicity data. The first ethnicity data were collected only in the 2000 census. According to this census, over 100 self-identified ethnic groups exist in Indonesia. However, most of these ethnic groups are small in population size, and only 15 groups have more than one million population numbers [41]. The majority of the Indonesian people are of Javanese ethnic group, making up 41.7% of the total population. Table 11.1 lists the major ethnic groups in Indonesia, which make up more than 1% of the total population:

TABLE 11.1 List of Major Ethnic Groups in Indonesia [41]

Ethnic Group	Percentage
Javanese	41.71
Sundanese	15.41
Malay	3.47
Madurese	3.37
Batak	3.02
Minangkabau	2.72
Betawi	2.51
Buginese	2.49
Bantenese	2.05
Banjarese	1.74
Balinese	1.51
Sasak	1.30

The majority of the nonnative Indonesians are of Chinese ethnicity. In the 2000 census, it was reported that less than 1% described themselves as Indonesian Chinese. It is believed that the figure was a gross underestimate, and that the real figure was perhaps three to four times larger [42]. With the advent of assimilation of the Chinese with the native Indonesians, there is doubt that even the 1% self-reported Chinese were homogeneous with no genetic admixture with the native Indonesians.

Thailand

Thailand became the official name for the kingdom once known as Siam in 1939. It is often perceived as unique and homogeneous in culture and ethnicity [43]. The 2010 census collected spoken language and religion data but not ethnicity data. The official demographics report states that 94% of the population is Thai-speaking Buddhists, whereas 5% are Muslims [44]. In Thailand, there are over 30 distinct ethnic groups, including the Chinese, who migrated into Thailand in the 19th century when they formed significant urban communities [43]. Due to the lack of official figures, it is exceptionally difficult to get a reliable estimate of the actual numbers of ethnic groups and their composition in Thailand. The ethnic groups in Thailand can be categorized into five large groups based on the language groups spoken, as follows [45]. It is not clear to what extent language groups reflect different genetic heritages, but this population diversity needs to be acknowledged.

- Tai–Kadai [46].
  - Yuan, Lue, Khuen, Yong, Thai–Korat, Thai–Khon kaen, Thai–Chiang Mai, Phu–Thai, Lao–Song
- Austroasiatic [46].
  - Mon, Lawa, Paluang, Blang, H'tin, Khmer, Ching, Thai–Korat
- Sino–Tibetan [46].
  - Lisu, Mussur, han–Yunnan, Han–Guangdong, Han–Wuhan, Han–Qingdao, Han–Liaoning, Han–Xinjiang, Tibetan–Qinghai
- Hmong–Mien [45].
  - Hmong, Mien
- Austronesian [45].
  - Malay, Cham

Ethnicity and Race as Defined by the US FDA

A pressing need exists for standardization of terminologies for the collection of ethnicity and race information in biomedical research. Differences in response to medical products have also been observed in racially and ethnically distinct subgroups of the U.S. population. The U.S. Food and Drug Administration (FDA), mandated by the National Institutes of Health (NIH), prepared the *Guideline for Industry: Collection of race and ethnicity data in clinical trials*. This document is relevant in determining the safety and effectiveness of a drug or medical product, as well as addressing the issue of lack of inclusion of women and minority groups in NIH-sponsored clinical research. However, much criticism has arose over the racial and ethnic categories, as they are not anthropologic or scientifically based designations, but sociocultural categories as described by the Office of Management and Budget (OMB). In this guideline,

- There are five race categories: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, and White
- There are two ethnicity categories: Hispanic or Latino and Not Hispanic or Latino

The OMB standards also mention explicitly that "*the racial and ethnic categories set forth in the standard should not be interpreted as being primarily biological or genetic in reference*" [47]. Thus, when these categories are used in a defined biological or genetic context, it creates confusion in the biological and sociocultural meaning of race and ethnicity [47]. The broad categorization of race, for example, Asian, is also arbitrary, as it lumps very heterogeneous groups of people together (although the guideline does allow for

more detailed information, such as Japanese or Indian). Likewise, a "white" is defined as person having origins in any of the original peoples of Europe, North Africa, or the Middle East [23]. This would include Scottish, Greek, Welsh English, Moroccan, and Iranian, for example, which completely abandons ethnic or cultural definitions altogether but, rather, groups these populations together based on skin color.

Immigration and interracial marriages are on the increase, not only in the United States, but also globally. This creates people with a very wide geographical and sociocultural background. If ethnicity and race were to serve, to a certain extent, as a surrogate for genetic variations, grouping people along racial and ethnic lines to study functional differences in their drug-metabolizing capacity or to interpret and communicate results of research and clinical trials relating to various drugs has proven to be complex and challenging [6,48].

Conclusion

Ethnicity is a highly ambiguous and imprecise sociocultural construct. This is also complicated by erroneous self-identification due to complex ancestral histories. Careful considerations should be given not to confuse ethnicity and nationality definitions, because it could lead to fallaciously drawn conclusions, especially when considering risk–benefits of pharmacological treatment based on more biologically related processes. A useful way to describe ethnicity in scientific research would perhaps be to combine elements of geographic origins and the sociocultural context. For example, an ethnic group could be identified as Indonesian Chinese, Malaysian Chinese, American Chinese, etc. This dual element in defining ethnicity would be better able to take into consideration the *extrinsic* components of ethnicity interacting with the *intrinsic* components.

Current international regulatory guidelines have attempted to provide a framework to evaluate ethnic factors on the outcome to medical treatment(s), i.e., ICH E5(R1). The subsequent complementary pharmacogenomics guideline of ICH E17 "General principle on planning/designing Multi-Regional Clinical Trials" facilitates MRCT data acceptance by the different regulatory agencies [2], and ICH E18 further provides harmonized principles on genomic sampling and management of genomic data in clinical studies. Future areas of work to advance pharmacogenomics in regulatory science could be to integrate our insights of human genetic variation in the context of human evolutionary history and refine how ethnicity information is collected, validated, and interpreted in clinical studies. This should aim to minimize classification error and facilitate applications for new drug approval utilizing foreign clinical data.

ETHNIC FACTORS AFFECTING DRUG RESPONSE

The definition of ethnicity has elements of both biology and the environment. These have been described in the ICH E5(R1) document as intrinsic and extrinsic factors [1]. Fig. 11.1 summarizes the intrinsic and extrinsic factors, as outlined in the ICH E5(R1) guideline. It is often difficult to ascertain which of the intrinsic or extrinsic factors is causing differences in drug response. All intrinsic and extrinsic factors play important parts in influencing pharmacological outcome to treatment. In the following section, the genetics aspect of the intrinsic factors, food, and traditional medication intake, as well as medical practices aspects of extrinsic factors are discussed.

Intrinsic Factor: (Pharmaco)genetics

Interethnic, as well as interindividual, differences have been demonstrated in genetic polymorphism of various drug-metabolizing enzymes, transporters, and pharmacologic targets [13]. The clinical relevance for the known variants is not fully understood—some have

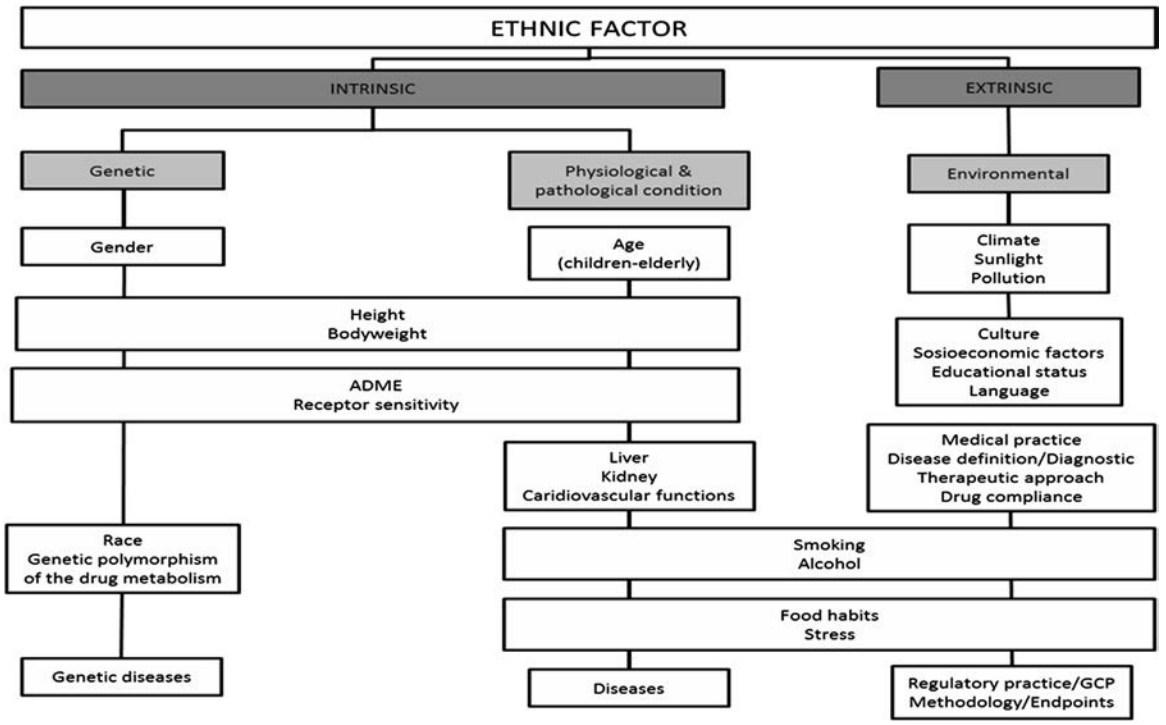

FIGURE 11.1 Classification of intrinsic and extrinsic factors [1].

very clear relevance, whereas some are still unknown. Pharmacogenetics of drug target, drug metabolism, drug transport, disease susceptibility, and drug safety have been discussed extensively elsewhere and information is available in curated repositories [49,50]. Thus, this chapter will only briefly review some examples of the pharmacogenetics of drug-metabolizing enzymes (DMEs) and pharmacologic targets.

CYP2D6

Cytochrome P450 2D6 (CYP2D6) is involved in the metabolism of approximately 25% of all drugs [51]. The first Cytochrome P450 (CYP) polymorphism was discovered for CYP2D6, which is perhaps one of the most studied and characterized CYP genes. Over 50 alleles have been described for this gene [52], with approximately 20 affecting metabolism of CYP2D6 substrates. CYP2D6 polymorphisms result in four phenotypes: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultrarapid metabolizers (UMs) [53]. Allelic variants that have associated with the phenotypes are listed in Table 11.2.

The bioavailability, systemic exposure, area under the curve (AUC), and half-life of relevant drugs for the PMs, relative to the EMs, have been reported to be between 2- and 6-fold, with metabolite clearance between 0.1- and 0.5-fold [54]. Meanwhile, the UMs experience the extreme opposite, rapidly accumulating metabolites at the highest possible doses. Clinical effects of CYP polymorphisms have been reported for various drugs, and particularly serious with the use of tricyclic antidepressants, which are primarily metabolized by this enzyme. Tricyclic antidepressants are very toxic drugs, with potentially fatal adverse effects secondary to cardiac complications [55].

TABLE 11.2 Effect of CYP2D6 Variant Allele Phenotype on Metabolism and Potential Clinical Consequences [53]

Phenotype	Effect on Metabolism and Potential Clinical Consequences	Variant Allele Examples
Poor metabolizers (PMs)	• Slowed drug metabolism • Greater potential for drug–drug interactions and adverse events • Slower conversion to active metabolites • Potentially lower efficacy	CYP2D6*3 CYP2D6*4 CYP2D6*5 CYP2D6*6 CYP2D6*10 CYP2D6*17
Extensive metabolizers (EMs)	• "normal" activity	CYP2D6*2 CYP2D6*1
Ultrarapid metabolizers (UMs)	• Accelerated drug metabolism • Greater rates of drug elimination • Potentially lower efficacy	CYP2D6*35 CYP2D6*41

In vulnerable subpopulations like the CYP2D6 PMs, as well as the elderly and adolescents, very low initial doses are recommended [56]. Table 11.3 lists some of the clinical consequences with the use of CYP2D6 substrates for persons with the PM and UM phenotypes.

Interethnic differences in CYP2D6 allelic frequencies and phenotypes have been shown in many studies. The PM phenotype occurs in about 7%–10% of European populations compared to a mere 1% of East Asians, with the *3, *4, and *5 being most commonly implicated in this phenotype [57]. The *4 variant allele is the most common variant allele in Caucasians with almost 21% frequency and, interestingly, the *4 variant is almost absent in the Chinese. The most common variant in the Chinese is the **10* (~50%), which is virtually absent in Caucasians. Other examples of differing *CYP2D6* allele variant frequencies include the CYP2D6*3 allele (no enzyme-activity phenotype), which is not found in the Eastern to Southern Asian regions [58–60], but present in Western Europeans with frequencies from 0.9% to 1.7% [61–63]. However, in some populations, for example, the Japanese, Koreans, and Chinese, studies have found small differences in the allele frequencies for most of the CYP2D6 variants (<10% difference), except CYP2D6*10 between Japanese and Chinese, with 14.7% difference. For the same variant, the difference between Japanese and Koreans as well as between Koreans and Chinese are 7.6% and 7.1%, respectively [64].

CYP2C9 and VKORC1

Warfarin is one of the most widely used oral anticoagulants globally. It acts by interrupting the regeneration of dihydroxyquinone (KH2), the reduced, active form of vitamin K by targeting Vitamin K Epoxide Reductase Complex 1 (VKORC1), leading to decreased carboxylation and activation of the vitamin K-dependent clotting factors with loss of activity. Warfarin use is hampered by more than 10-fold variability in dosing requirement [65,66] required to achieve the target the international normalized ratio (INR) in different patients. Overcoagulation causes bleeding episodes, with intracranial hemorrhage being one of the most catastrophic. The effects of genetic polymorphisms in its metabolizing enzyme, Cytochrome P450 2C9 (CYP2C9), as well as VKORC1 genes on sensitivity to warfarin, have been shown in many studies to significantly affect the dosing requirements [67–71], with the *CYP2C9*1/*1* genotype associated with a higher maintenance dose compared to the genotype containing *CYP2C9*2* or **3* alleles. There are about 28 known polymorphisms for the *VKORC1*

TABLE 11.3 Clinical Consequences for PM and UM Phenotypes of CYP2D6 [54]

POOR METABOLIZERS: INCREASED RISK FOR TOXICITY	
Drug	**Toxicity Risk**
Debrisoquine	Postural hypotension and physical collapse
Flecainide	Possibility of ventricular arrhythmias
Nortriptyline	Hypotension and confusion
Thioridazine	Excessively prolonged QT interval
Tramadol	Hyperanticoagulation from warfarin
Propafenone	CNS toxicity and broncoconstriction
POOR METABOLIZERS: FAILURE TO RESPOND	
Codeine	Poor analgesic efficacy
Tramadol	Poor analgesic efficacy
Opiates	Protection from oral opiate dependence
ULTRARAPID METABOLIZERS: INCREASED RISK FOR TOXICITY	
Drug	**Toxicity Risk**
Encainide	Possibility of proarrythmias
Codeine	Morphine toxicity
ULTRARAPID METABOLIZERS: FAILURE TO RESPOND	
Nortriptyline	Need higher dose to be effective[1]
Propafenone	Need higher dose to be effective[1]
Tropisetron	Need higher dose to be effective[1]
Ondansetron	Need higher dose to be effective[1]

[1] *Ineffective at regular doses.*

gene to date, with the 1639G>A polymorphism being most significant clinically. The –1639 AA genotype is associated with a significantly lower warfarin-dose requirement [56]. Subsequent clinical studies incorporating the use of the pharmacogenomics algorithm of CYP2C9 and VKORC1 demonstrated better overall predictions for the appropriate dosage needed to achieve target INR versus standard management approaches [72–74].

Table 11.4 shows allele distribution for *CYP2C9* and *VKORC1* variants and stabilized warfarin dose according to ethnicity [71]. It has been increasingly evident that the allele distribution may differ within a population due to interethnic admixture. Among the Brazilian population, which is formed by three ancestral groups (European, African, and Amerindian), CYP2C9*3 allele frequency varied from 3.2% in Black Brazilians to 8.1% in White Brazilians [75]. Even among Asians, warfarin-dosing variation has also been observed. Findings flagged the importance in determining genetic information from the genetic loci and the risk of broad classification based on self-reported ethnicity, especially in warfarin, which has a narrow therapeutic index and is associated with serious adverse consequences [76].

CYP2C19: Clopidogrel

Clopidogrel is an antiplatelet agent of the thienopyridine group used in the secondary prevention of myocardial infarction, ischemic stroke, and also in existing peripheral arterial disease, among other indications. It is a prodrug, which is activated mainly by the hepatic enzyme Cytochrome P450 2C19 (CYP2C19), although other CYP enzymes such as the CYP1A2, CYP2B6, CYP2C9, and CYP3A4 are also involved. Apart from polymorphisms in *CYP2C19*, effectiveness of clopidogrel has also been associated with polymorphisms in the P-glycoprotein (P-gp) efflux pump, ATP-binding cassette subfamily B member 1 (*ABCB1*), particularly the c.C3435T variant [77], which will not be discussed here. Variant *CYP2C19* alleles have been associated with a range of differing activities, from UM, to EM, IM, and PM. This has clinical implications, whereby for loss-of-function alleles, there is reduced activity of CYP2C19 rendering clopidogrel ineffective. In contrast, alleles with increased enzyme activity such as **17* may be associated with increased risk of bleeding. The *CYP2C19*1* allele is the allele related to normal functionality, whereas the *2 (c.681 G>A;

TABLE 11.4 Stabilized Warfarin Dose According to Ethnicity, Allele Distributions for *CYP2C9*, Genotype Distributions for *VKORC1* [71]

Characteristic	African American	Caucasian	Hispanic American	Asian
Mean Dose (mg/day)	5.2	4.3	4.0	2.7
CYP2C9*1 %(95% CI)	94 (89–99)	74 (66–82)	93 (85–100)	95 (89–100)
CYP2C9*2 %(95% CI)	1 (0–3)	19 (12–26)	0	0
*CYP2C9*3* %(95% CI)	1 (0–3)	6 (2–10)	7 (0–15)	5 (0–10)
VKORC1 GG %(95% CI)	82 (74–90)	37 (28–46)	32 (18–46)	7 (0–13)
VKORC1 GA %(95% CI)	12 (6–18)	45 (36–54)	41 (25–56)	30 (18–42)
VKORC1 AA %(95% CI)	6 (1–11)	18 (11–25)	27 (14–49)	63 (51–75)

rs4244285) is most commonly associated with loss of function and is observed in up to 30% in Europeans and Africans (3%–4% homozygotes) and 70% in Asians (10%–15% homozygotes) [78].

The cumulated evidence for *CYP2C19* genotyping from clinical studies appears confusing, possibly due to differences in the role of *CYP2C19* in patients with varying degrees of disease risk, with greater importance in those at higher risk for poor outcomes, such as patients undergoing percutaneous coronary intervention (PCI) [78]. Studies that enrolled patients at lower cardiovascular risk (e.g., atrial fibrillation or acute coronary syndrome managed medically) mainly found no association between *CYP2C19* genotype and treatment outcome with clopidogrel [79,80], whereas those who selected patients at higher risk found significant association [81,82]. The FDA approved a boxed warning on the product label regarding the risk of lack of clinical effect of clopidogrel in patients, who are poor metabolizers, in March 2010 [78]. The labeling does not, however, mandate genotyping for all patients who will be prescribed the medication. Several institutions are starting to embrace genotyping for loss-of-function alleles for all possible clopidogrel candidates.

Extrinsic Factors

Although intrinsic factors, especially genetic factors, are critical determinants of drug response, the impact of extrinsic factors may also be very profound. Nutritional, dietary factors, intake of over-the-counter drugs, as well as use of traditional or alternative medicines all have the potential to alter treatment outcome with drugs. Not surprisingly, drugs with narrow therapeutic index and high potency are among those that have been documented to give rise to significant pharmacokinetics and pharmacodynamics alterations.

Food

Many different types of food and drugs are substrates of CYP's enzyme, particularly CYP3A4, as well as the membrane efflux transporter protein, P-gp. Both CYP3A4 and P-gp are constituently expressed in the enterocytes and, as such, affect

bioavailability of many drugs such as digoxin, cyclosporine, midazolam, and verapamil. As chemicals contained in foods are present in high concentrations in the gut, food types that affect CYP3A4 as well as P-gp would have a significant effect on the bioavailability of these drugs [83]. Expressions of P-gp and CYP's enzyme at target tissues could also be affected by drug–food interactions in similar manner. Table 11.5 lists examples of food–drug interactions.

Cruciferous vegetables such as cabbage, broccoli, cauliflower, and Brussels sprouts are consumed by people worldwide. They are rich in glucosinolates, which can endogenously be converted to biologically active indoles, such as indole-3-carbinol (I3C) and sulforaphane (SFN) [139]. I3C, in nontoxic doses, have been shown to enhance chemoresistant K562 human leukemic cells in an experimental *in vitro* study [140]. The K562 cells were also cross-resistant to other chemotherapeutic drugs, such as doxorubicin and vincristine. The Western blot analysis in this study further showed that the P-gp expression was downregulated when the cells were treated with I3C, suggesting that I3C could alleviate chemoresistance in patients taking these drugs for treatment. This could potentially give rise to difference in chemoresistance profiles among populations consuming high amounts of I3C-containing foods to those with lower-consuming populations. The Koreans are among the largest cabbage consumers worldwide. The Korean population consumes a traditional fermented, spicy cabbage dish, *kimchi*, almost on a daily basis, totaling about 56.5 kg/person/year [141], more than 10 times the average consumption of Americans, who consume an average of 4.2 kg/person/year [142]. Similarly, there are marked differences in some parts of the world with regard to consumption of various foods with the ability of modulating P-gp function.

Grapefruit (*Citrus x paradisi*)/grapefruit juice intake and drug interactions have been widely studied. Interactions with many drugs are mediated mainly through physical interactions with CYP inhibition, specifically the intestinal CYP3A4, resulting in complete inactivation of this enzyme. This causes prolonged inhibition of the intestinal clearance of specific drug substrate of this enzyme, such as felodipine [143], as well as other drugs metabolized by this pathway. Furthermore, grapefruit has also been shown to inhibit P-gp-mediated efflux, potentiating drugs used in human immunodeficiency virus (HIV) treatment and chemotherapy [144], for example vinblastine and saquinavir [145]. However, due to significant overlap in the substrates for P-gp and CYP3A4, studies to particularly isolate P-gp mediated interactions have been challenging. The consumption of grapefruit is highest in Eastern Asia, with Japan making up a significant portion, followed by the Americas and European Union (EU) nations. However, it should be noted that CYP3A4/P-gp is affected by a large number of phytochemicals. The potential differential effects in different regions should also be considered, when potential drugs are being evaluated for market registration. The consumption of such phytochemicals, which are not all related to grapefruit, are highly ethnicity specific as they relate to dietary exposure. In some parts of Asia, although the grapefruit is not consumed regularly, the related citrus fruit, pomelo (*Citrus maxima*), is consumed in great abundance. The CYP3A4 inhibitory effect of pomelo has been reported to be as potent as that of the grapefruit [101].

Alternative, Complementary, and Traditional Medicines Use

According to the World Health Organization (WHO), some Asian and African countries utilize almost 80% of traditional medicine for primary health care [146]. In fact, even in developed countries, the use of alternative or complementary medicine is very prevalent. Herbal and other natural productswere reported to be used by one in every five US adults [147]. A follow-through study in the United States that evaluated the use of herbal and natural products revealed that use was lowest among African

TABLE 11.5 Examples of Food–Drug Interactions and Their Mechanisms

Food Type	Drug Interaction	Mechanism of Interaction	References
Piperine (black pepper constituent)	*In vitro*: Digoxin Cyclosporine A Verapamil In humans: Carbamazepine Chlorzoxazone Diclofenac	P-gp efflux inhibitor CYP3A4 inhibitor CYP2E1 inhibitor CYP2C9 inhibitor	[83–86]
Capsaicin (red chilli constituent)	*In vitro*: Digoxin *In vivo*: Fexofenadine	P-gp efflux inhibitor	[87,88]
Curcumin (turmeric constituent)	*In vitro*: Digoxin	P-gp efflux inhibitor	[89,90]
Green tea	*In vitro*: Doxorubin, Vinblastine	P-gp efflux inhibitor	[91–94]
Grapefruit juice	*In vitro*: Vinblastine, Vincristine In humans: S-ketamine, tolvaptan	CYP 1A2, 3A4 inhibition, P-gp modulation	[95–98]
Orange juice	*In vitro*: Vincristine In humans: Aliskiren	P-gp efflux inhibitor OATP2B1 inhibitor	[96,99]
Pomelo juice	*In vitro*: Tacrolimus In humans: cyclosporine	CYP3A4 inhibitor P-gp modulation	[100–102]
Russian green sweet pepper (Anastasia Green)	Verapamil	P-gp inhibitors	[103]
Seville Orange	*In vitro*: vinblastine, fexofenadine, glibenclamide, Paramark In humans: atenolol, ciprofloxacine, cyclosporine celiprolol, levofloxacin, and pravastatin	Inhibits CYP3A4, P-glycoprotein, OATP-A, OATP-B	[104–112]
Tangerine	*In vitro*: Nifedipine, digoxin	Stimulates CYP3A4 activity and inhibits P-glycoprotein	[113–115]
Grapes	In humans: Cyclosporine	Inhibits CYP3A4 and CYP2E1	[116,117]
Cranberry	In humans: warfarin *In vitro*: Diclofenac	Inhibits CYP3A and CYP2C9	[118–121]
Pomegranate	Animals: carbamazepine	Inhibits CYP3A and phenolsulfotransferase activity	[122,123]
Mango	*In vitro*: Midazolam, diclofenac, chlorzoxazone, verapamil	Inhibits CYP1A1, CYP1A2, CYP 3A1, CYP2C6, CYP2E1, P-glycoprotein (ABCB1)	[124–126]
Guava	No data available; possible P-gp-mediated drug-uptake inhibition	Inhibits P-glycoprotein (ABCB1)	[127]
Black raspberry	*In vitro*: midazolam	Inhibits CYP3A	[128]
Black mulberry	*In vitro*: midazolam; glibenclamide	Inhibits CYP3A and OATP-B	

Continued

TABLE 11.5 Examples of Food–Drug Interactions and Their Mechanisms—cont'd

Food Type	Drug Interaction	Mechanism of Interaction	References
Apple, including its juice	*In vitro*: fexofenadine In humans: atenolol, aliskiren, fexofenadine	Inhibits CYP1A1, OATP2B1	[99,129–131]
Broccoli, cauliflower	No data available; possible ABC transporters and *CYP 1A1, CYP2B1/2, CYP3A4, CYP2E1* substrates modulation	Inhibits CYP1A1, CYP2B1/2, CYP3A 4, CYP2E1, hGSTA1/2, MRP-1, MRP-2, BCRP, UDP, glucorosytransferases, sulfotransferases, quinone reductases, phenol sulfotransferases, Induces UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and quinone reductases (QRs)	[132–134]
Watercress	In humans: chlorzoxazone	Inhibits CYP2E1, P-glycoprotein, MRP1, MRP2, and BCRP	[134,135]
Spinach	*In vitro*: heterocyclic aromatic amines	Possible inhibition of CYP1A2	[136]
Tomato	*In vitro*: diethylnitrosamine, N-methyl-N-nitrosourea, and 1,2-dimethylhydrazine	Inhibits: CYP1A1, CYP1B1, UGP (Wang and Leung 2010), Increases UGT and CYP2E1	[137]
Avocado	In human: warfarin		[138]

Americans, compared to Hispanics and non-Hispanic whites, with the Hispanics using the most number of products [148].

In Asia, the use of traditional medicine systems dates back to the 12th century BCE. Almost every nation in this region has its own use of traditional medicine, and the practice of some these systems has spread worldwide. The traditional systems utilize many remedial methods, and for this chapter, only remedies utilizing herbs and natural products will be mentioned. Some of the systems used in Asia include [149]:

- Traditional Chinese Medicine (TCM)—from China
- Ayurveda—from India
- Siddha—from south Tamil India
- Unani medicine—from Persia/Middle East, popular in India
- Kampo—Japanese herbal medicine

The Southeast Asian region shares many common herbs for medicinal uses, possibly because of the natural distribution of the available plants. This regions' use of herbal medicine is also greatly influenced by the Ayurveda, Unani, Siddha, and TCM; thus, certain similarities in the use of herbs are often seen. However, it is also important to note that the use of herbal medicine is usually specific to certain ethnicities [150]. For example, the Chinese ethnic groups from various countries tend to use herbs from TCM, rather than Ayurvedic herbs, and Indians tend to use herbs from Ayurvedic/Siddha and Unani medicines. On the other hand, the Malays tend to use more herbs from the Unani system, apart from the use of Folk Medicine utilizing herbs unique to the ethnic Malays like the Tongkat Ali (*Eurycoma longifolia*) [151].

Many of the herbal medicines commonly used have been shown to have significant drug interactions. St. John's Wort has been traditionally used in the treatment of depression concomitantly with other antidepressants such as the selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors

(MAOIs), and has been implicated in the incidence of serotonin syndrome by additive effect, and also CYP3A4 induction [152,153]. *In vivo* human studies have also reported a reduction in plasma concentration of drugs such as amitriptyline, cyclosporine, digoxin, and fexofenadine, indinavir, methadone, midazolam, nevirapine, phenprocoumon, simvastatin, tacrolimus, theophylline, and warfarin, possibly due to CYP3A4 and P-gp induction [121]. *Gingko biloba* has been used to improve cognitive functions in Alzheimer's patients. A report by Galluzzi [154] highlighted a case of an Alzheimer's patient who became comatose after she was started on trazodone, an antidepressant which enhances release of Gamma-Aminobutyric acid (GABA), and *Gingko biloba*. In view of reversal of the patient's clinical condition by flumazenil, a specific benzodiazepine (BDZ) antagonist, it was postulated that her condition was possibly due to a drug interaction because of an increase in GABAergic activity mediated directly by a BDZ receptor. The usually subclinical increase in GABAergic activity of *Ginkgo biloba* became clinically enhanced through an interaction with trazodone. A list of reported herb–drug interactions are given in Table 11.6.

It is important to keep in mind that a significant number of people, regardless of ethnicity and geographic locations, use herbal and natural products as remedies or daily supplements, and ethnic groups and subpopulations tend to utilize different types of herbal and natural product-based medicines. These medicines have the potential to interact with prescribed drugs, and thus play a significant factor in determining outcome to treatment and should be given due consideration.

Differences in Medical Practice

An important extrinsic factor in determining outcome to treatment, as well as whether foreign clinical data can be extrapolated to a new region would be to evaluate if significant differences exist in medical practices between the two regions. Common examples in this respect would be the difference between medical practices in Japan and that in the US and Europe. Studies have shown that there are differences in drug dosing between the United States, Europe, and Japan [191–193]. For 32% of drugs approved between 2001 and 2007, the maximum recommended dose in the United States was at least two times higher than that in Japan [193]. However, it is not certain if the difference was due to difference in intrinsic factors, or due to difference in the pharmacogenomics biomarker information in the package inserts and the interpretation of the risk–benefit balances between the regions [194]. Differences in patient–physician relationships between Western and Asian cultures have also been highlighted, in which the Japanese/Asian culture has been viewed as being more hierarchical and paternalistic [195]. The doctor is held in great respect, such that patients sometimes do not report adverse effects to not be offensive to the doctor [196]. However, it should also be noted that recent findings in Japan shows that the Japanese patients now prefer the mutual "Western" relationship, which is considered ideal possibly also in other Asian cultures, which may potentially give rise to higher placebo effects in Asians [195].

Conclusion

Genetic variations causing differences in disease susceptibility and drug response are well established, accounting for some of the ethnic differences in drug response. Moreover, genomics on its own is yet unable to account for all population differences in drug response and, in many cases, despite its ambiguous definition, ethnicity with related differences in extrinsic factors is important to be considered for many of the differences.

TABLE 11.6 Examples of Herb–Drug Interactions and Their Mechanisms

Herb Type	Drug Interaction	Mechanism of Interaction	Note on Use and Primary Users	References
Hypericum perforatum (St. John's Wort; Seiyo-otogiri-so)	Interacts with selective serotonin reuptake inhibitors and duloxetine by additive effect. Cyclosporine, Tacrolimus, Indinavir	Induces CYP3A4, P-glycoprotein membrane transporters	Used as antidepressants; Western traditional medicine; also in Japan	[155–158]
Allium sativum (Garlic)	Saquinavir, Ritonavir, Warfarin, Chlorpropamide	Induction of CYP3A4 and P-gp, Additive effect, Platelet dysfunction	Used as antidepressants; Western traditional medicine	[159–163]
Glycyrrhiza glabra (Licorice)	Prednisolone, Hydrocortisone	Potentiation of oral and topical corticosteroids by inhibition of 11β hydrogenase of its metabolite (decreasing clearance)	Widely used in Western traditional medicine	[164]
Ginkgo biloba (Ginkgo)	Thiazide diuretic, Trazodone, Warfarin, Aspirin, Digoxin	Induce CYP2C19, Metabolic Inhibition, Increase of GABAergic activity, Inhibition of CYP3A4	Widely used in TCM, US, Europe	[154,165–168]
Panax spp. (*Panax ginseng*)	Alcohol (ethanol), Phenelzine, Warfarin	Delayed gastric emptying and enzyme induction, Additive effect	Widely used in TCM, East Asia, US	[169–172]
Silybum marianum (Milk Thistle)	Indinavir	Modulation of CYP3A and P-gp	Widely used in the Mediterranean, Northern Africa as liver tonic	[173]
Angelica sinensis (Dong Quai)	Warfarin	Contains coumarin	Widely used in TCM	[164]
Ephedra (Ma huang)	Monoamine oxidase inhibitors, caffeine, decongestants, stimulants	Enhanced sympathomimetic effects when used with other similar drugs	Used in TCM for respiratory ailments	[174]
Danshen (*Salvia Miltiorrhiza*)	Warfarin	Increases bleeding tendencies by decreasing clearance and increasing bioavailability	Used in TCM for chronic renal failure, coronary heart disease	[175]
Eurycoma longifolia (Tongkat Ali, Asian Viagra)	Reduced propranolol bioavailability; potential interaction with rosiglitazone	Possible CYP enzyme induction, Inhibits CYP2C8	Aphrodisiac, antimalarial, anti-diabetic. Used mainly by Malays in Malaysia	[176–179]
Labisia pumila (Kacip Fatimah)	No data available	Inhibits CYP2C8	Postpartum medication, treat menstrual irregularities. Used mainly by Malays in Malaysia	[180,181]

TABLE 11.6 Examples of Herb–Drug Interactions and Their Mechanisms—cont'd

Herb Type	Drug Interaction	Mechanism of Interaction	Note on Use and Primary Users	References
Andrographis paniculata (Hempedu bumi)	*In vivo*: naproxen, nabumetone, etoricoxib	Inhibits CYP2C8, CYP3A4. Weak inhibitor of CYP2C19	Treatment of infections, diabetes mellitus. Widely used in Asia—South India, Sri Lanka, Malaysia, Indonesia and South China, mainly by the Indians and Malays	[180,182–186]
Orthosiphon stamineus(Misai kucing)	Possible interactions with CYP2C19 substrates: omeprazole, citalopram, proguanil, diazepam	Strong inhibitor of CYP2C19	Kidney and urinary disorders. Used widely in Thailand, Malaysia and Indonesia	[182]
Asparagus racemosus (Satavari)	Potential interaction with drugs interacting with cholinesterase and monoamine oxidase enzymes	Nonselective competitive inhibitor for cholinesterase and monoamine oxidase enzymes	Widely used in Ayurvedic medicine as galactagogue, aphrodisiac, diuretic, antispasmodic, nervine tonic	[187,188]
Commiphora mukul (Guggul)	Enhanced the efficacy of erlotinib, cetuximab and cisplatin (in vivo and in vitro)	induced decreased expression of both phosphotyrosine and total signal transducer and activator of transcription (STAT)-3	Used in Ayurvedic medicine to promote heart and vascular health, obesity and rheumatism among others	[189,190]
Agaricus brazei Murill (Ji Song Rong; Kawariharatake)	Diltiazem and other CYP 3A4 substrates	Inhibits CYP 3A4	Used in TCM and Japan	[157]

ACCEPTABILITY OF FOREIGN CLINICAL DATA

According to the ICH E5(R1) [1], it is possible to utilize foreign clinical data in drug registration, subject to the completeness of the data package, which should include:

- Adequate characterization of pharmacokinetics, pharmacodynamics, dose-response, efficacy, and safety in the population of the foreign region(s)
- Clinical trial data of efficacy, dosing, and safety from trials conducted according to regulatory standards (Good Clinical Practice [GCP] standards), well controlled with appropriate endpoints, as well as appropriate medical and diagnostic definitions acceptable to the new region
- The foreign population in whom the clinical trials were conducted are representative of the populations in the new region

Once the clinical data package fulfills the local regulatory requirements, extrapolation of foreign clinical data to the local population would be considered. If a drug is deemed ethnically sensitive, some amount of pharmacokinetics data from local subjects would be required to "bridge" the two sets of data from different regions or ethnic populations.

Fig. 11.2 from Appendix B of the ICH E5(R1) guideline demonstrates an overview of the assessment of the clinical data package (CDP).

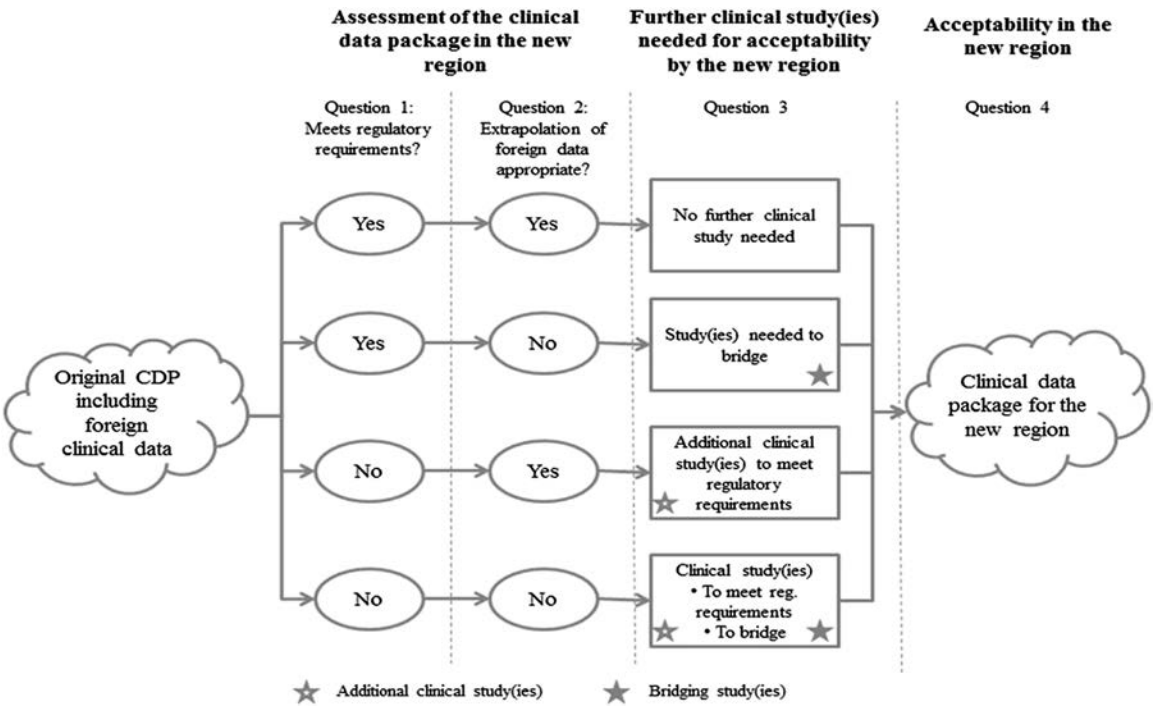

FIGURE 11.2 Assessment of the clinical data package for acceptability of foreign clinical data [1].

As can be seen from the figure, fulfillment of local regulatory requirements is mandatory, failing which, additional clinical trials may be requested for this purpose, as well as a bridging study, should the drug be deemed as ethnically sensitive. The outcome of the CDP assessment could then be:

- No bridging studies required
  - If drug is not ethnically sensitive
  - If drug is ethnically sensitive, but population is ethnically similar to ensure that the drug will behave similarly in the two populations
- Bridging studies required
  - If drug is ethnically different but with similar extrinsic factors
  - Usually requires a pharmacodynamics study (e.g., dose–response) using acceptable endpoint to ensure safety, efficacy, and dose, and dosage regimen is applicable to new region
  - Pharmacokinetic measurements for data support
- Controlled clinical trials (CCTs) required
  - If dosage is uncertain
  - Limited CCTs in the new region
  - Difference in medical practice
  - Drug is not familiar in the new region
- Safety bridging studies
  - If there are safety concerns despite adequate foreign data addressing safety and efficacy issues, for example concerns regarding possibility of higher occurrence rate of adverse events in the new region. They can be done together with efficacy studies, with adequate power
  - Separate safety study may be needed if there is no need for efficacy-bridging studies or if efficacy studies are not powered for this purpose

If pharmacodynamics data from bridging studies indicate that there is a difference in drug

TABLE 11.7 Factors Affecting a Drug's Sensitivity to Ethnic Factors [1]

Factors	Ethnically Sensitive	Ethnically Insensitive
Pharmacokinetics	Nonlinear	Linear
Pharmacodynamics	Steep curve for efficacy and safety in the range of recommended dosage	Flat effect–concentration curve for both efficacy and safety in the range of recommended dosage
Therapeutic dose range	Narrow	Wide
Metabolism	High, especially through a single pathway. Enzymes known to show genetic polymorphism Administered as a prodrug; possible ethnically variable enzymatic conversion	Minimal or distributed along multiple pathways
Bioavailability	Low; susceptible to dietary absorption effects. High intersubject variation in bioavailability	High; less susceptible to dietary absorption effects
Potential for protein binding	High	Low
Potential for interactions	High; use in multiple comedications	Low for drug–drug, drug–diet, and drug–disease
Potential for inappropriate use	Low	High; e.g.: analgesics and tranquilizers
Mode of action	Systemic	Nonsystemic

response between the two regions, a CCT will be required. If there is a difference in pharmacokinetics, a dose adjustment may be all that is needed, without the need for a CCT.

Ethnically Sensitive or Insensitive?

The ICH E5(R1) guideline indicates that a bridging study is necessary for drugs that may be ethnically sensitive. Table 11.7 lists some of the factors that may be used to evaluate whether a drug would have a high likelihood of being ethnically sensitive.

Due to the complex interaction among the drugs' pharmacological class, indication, and demography of the population [197], the ICH E5(R1) guideline does not provide a definitive criterion for evaluation of drugs' ethnic sensitivity, in terms of evaluation of the complete clinical data package or assessment of similarity of clinical results between regions [198]. Various statistical models and strategies have been proposed to assess sensitivities and similarities in ethnicities [198–200]; however, no gold standard has been established. Specific methodology for extrapolation foreign clinical data is also not provided. This has resulted in marked heterogeneity in the conduct of bridging studies in many regions, notably, heterogeneity in the criteria for bridging evaluation, trials procedure, and statistical methods adopted.

Acceptability of Foreign Clinical Data and Drug Regulatory Procedures in East and Southeast Asia

Concerns about foreign clinical data from the drug regulatory agencies' perspective vary by region. The ICH guidelines were initially intended to harmonize regulations governing drug registration in the ICH regions, but not all regions have adopted the guidelines. Some non-ICH countries/regions, such as Korea, have fully adopted and integrated the bridging concept, whereas others have not.

Southeast Asia has not fully adopted the ICH E5(R1) guidelines, although some technical aspects of evaluating the ability for foreign data to be extrapolated to their regions as outlined in the guidelines have been incorporated. The East Asian and Southeast Asian perspectives on and experiences with acceptance of foreign clinical data are reviewed in the following section.

East Asia

Japan: In Japan, the Pharmaceuticals and Medical Devices Agency (PMDA), under the Ministry of Health, Labour and Welfare, is the regulatory authority responsible for the scientific review of marketing authorization application of pharmaceutical and medical devices [201]. Japan has a huge pharmaceutical market, second only to the United States (US), and third if the European Union (EU) was put in collectively. Various reports have previously been put forth to highlight the submission gap between regulatory agencies, which refers to the date of submission at the first regulatory agency to the date of regulatory submission to the target agency. A survey of 100 top-selling drugs in 2004 reported a drug lag of 2.5 years difference between the United States and Japan. Since then, a review of new drug approvals in ICH countries from 2005 to 2014 conducted by the Center for Innovation in Regulatory Science reported a significant improvement in submission gap from 874 days (median time) for drugs approved in 2010 to 234 days (median time) for drugs approved in 2014 in Japan [202].

Japan has developed many strategies to improve the drug lag, and one is based on the use of bridging studies. Fig. 11.3 demonstrates Japan's adoption of bridging strategies to expedite the drug-approval process.

In Japan, foreign phase I results may be used to estimate the Japanese phase I studies, to enable an abbreviated study beginning with a lower maximum tolerated dose (MTD) in the foreign region, but higher than the starting dose. Foreign phase II data using the new drug as a single agent may replace the requirement for one of the two late phase II studies. At least one of the Phase II studies must be conducted in Japan, in addition, to Phase I studies, although studies conducted elsewhere may be considered [203]. In this case, the foreign phase II study must be of adequate size with dose, route, and schedule used in the study to be similar to those used in the Japanese studies. Otherwise, it will be necessary to prove that the difference will give rise to a different clinical effect, based on pharmacokinetic/pharmacodynamic studies conducted in Japanese subjects (local or abroad). Phase III studies conducted abroad may also be submitted to support a Reexamination application, with the provision that one of the studies must be conducted in Japan [203].

Hirai et al. [204] performed a detailed study to analyze factors contributing to the drug lag, and found that one of the major contributors to this was that there was a significant delay in initiating clinical development in Japan, i.e., drugs developed in the US and EU had longer lags in Japan. In about 60% of approved drugs in the US and EU, a clinical development phase had not even developed in Japan [204,205]. Apart from this, Japan's review of their bridging experience highlighted several facts that support their meticulous procedure of acceptability of foreign data, including their bridging approach. This included several examples of final drug dose approved in Japan, which was different from doses approved in the US, occurrence of higher adverse events in the Japanese population, as exemplified by induced interstitial lung disease with use of certain chemotherapeutic agents as well as differences in pharmacokinetic profile for tolterodine between Japanese and Koreans. Thus, the PMDA's issuance of the Notification of Basic Principles on Global Clinical Trials

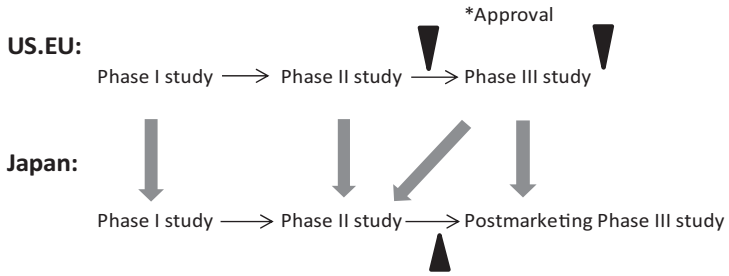

FIGURE 11.3 Overview of extrapolation of foreign clinical data to Japan. *Gray arrow* indicates possible routes of data extrapolation. *Line arrows* indicate developmental effort flow. *Solid triangles* indicate points of possible approval [203].

(GCTs) [206], which strongly recommends that clinical studies be done prior to, or in parallel with, global studies [207], was an effort to abolish the drug lag, without compromizing Japanese data. The Japanese initiatives also resulted in a revision of the ICH E5 guideline at the sixth ICH Conference, with a set of 10 questions and answers to facilitate the implementation of the E5(R1) guideline. The set of Questions and Answers (Q&A) outlines concepts for planning and implementing GCTs or MRCTs. Subsequently, a new ICH guidance "E17: General principle on planning/designing Multi-Regional Clinical Trials" was endorsed in 2014 and adopted by the Regulatory Members of the ICH assembly in 2017. This new guidance complements the ICH E5(R1) guidance, and facilitates MRCT data acceptance by the different regulatory agencies [2].

Subsequent to the publication of the guidance, there has been a marked increase seen in the number of GCTs that included Japan, with more than doubling of total numbers conducted in 2007 (17) as compared to those conducted in 2008 and 2009 (both 48) [207]. Analysis of new molecular entities approved in Japan from FY2007 to 2012 showed marked reduction of lag in drug development among drugs with clinical development strategies involving GCT [208]. Data from GCTs conducted did highlight the fact that, although there were differences in pharmacokinetic (PK), efficacy, and safety, there were also undoubtedly similarities in the data obtained across several populations. Losartan phase III trials showed superior effect when compared to placebo in overall population (Europe, Latin America, New Zealand, and North America by region) including Japan, whereas almost no effect was seen in the US population [207,209]. Global PK studies of tolterodine tartrate also showed some interesting values in the average ratio of under the serum concentration–time curve (AUC) between Japanese and Koreans, as well as the ratio between Japanese and Caucasians, which were 0.72 (95% CI: 0.62, 0.83) and 0.90 (95% CI: 0.78, 1.03), respectively. These results showed that the Japanese pharmacokinetic values were similar to those of the Caucasians, and different from those of the Koreans [207]. This exemplifies the complexity of understanding the inter-ethnic issues and in attempting to equate drug responses based on superficial ideas of ethnic differences or similarities.

Korea: The Ministry of Food and Drug Safety (MFDS) is responsible for assessing clinical data and granting approvals for pharmaceuticals in Korea. Korea has adopted the ICH bridging concept of extrapolating foreign clinical data since 2001. The MFDS defines bridging studies *as* "a trial conducted in Koreans in Korea, for the purpose of obtaining bridging data, in case it is difficult to directly apply the foreign clinical data due to differences in ethnic factors related to safety and efficacy of a drug." Bridging data, on the other hand refers to "data

of trials conducted on Koreans living in Korea or abroad, which are excerpted or selected from the clinical data package or obtained from the bridging study." Bridging data may have already been included as part of the original region of the drug, and can be used to extrapolate the foreign data. Otherwise, a bridging study must be carried out unless the drug falls into one of the seven waiver categories, which include [210]:

1. Orphan drugs (or used to be orphan drugs)
2. Drugs for life-threatening disease or Acquired Immunodeficiency Syndrome (AIDS)
3. Anticancer therapy for the following
   a. No standard therapy
   b. Therapy after failure of a standard therapy
4. New drugs for which clinical trials were conducted on Koreans
5. Diagnostic or Radioactive drugs
6. Topical drugs with no systemic effect
7. Drugs that have no ethnic differences

Basically, bridging studies must be carried out when there is absence of, or inadequate, bridging data, or if bridging data shows ethnic differences between Koreans and non-Koreans. Fig. 11.4 summarizes Korea's bridging concept.

Taiwan: In Taiwan, the Center for Drug Evaluation (CDE) under the commission of the Department of Health (now the Ministry of Health and Welfare [MOHW]) evaluates and reviews all New Drug Applications (NDAs). The bridging strategy was implemented in stages, beginning with inclusion of local (Taiwanese) clinical trials in 1993, in the "double-seven announcement" [210]. Subsequently, the "double-twelve announcement" in 12 December 2000 recommended that sponsors first apply for a Bridging Study Evaluation (BSE) to assess the necessity of carrying out a bridging study in Taiwan, which was fully implemented in 2004. The nine waiver categories requiring no verification of ethnic sensitivity are as follows [210]:

1. Drugs for the treatment of AIDS
2. Drugs for organ transplantation
3. Topical agents
4. Nutritional supplements
5. Cathartics used prior to surgery
6. Radio-labeled diagnostic pharmaceuticals
7. The only available treatment for a serious disease
8. Drugs with demonstrated breakthrough efficacy for life-threatening disease
9. Drugs for the treatment of rare diseases in which it is difficult to enroll enough subjects for a trial

Should the application not fall into the waiver category, in principle, Taiwan will accept all Asian data for consideration of NDA approval,

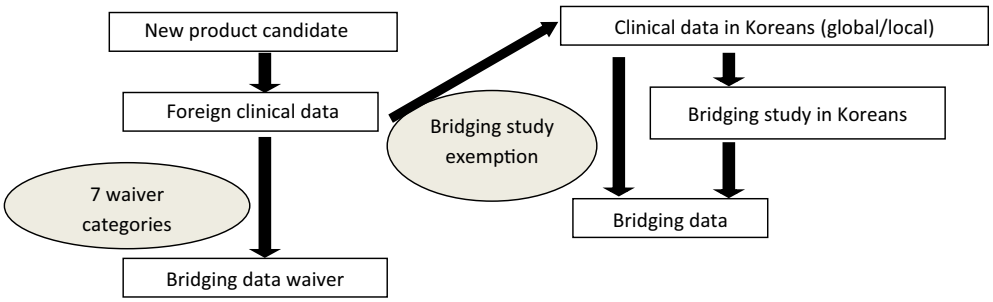

FIGURE 11.4 Overview of the new product-approval process in Korea [211].

including PK/PD study data that enable reasonable estimation of efficacy and safety of the drug. Unless the data indicated that there are ethnic sensitivities that makes extrapolation of data not possible, a bridging study would then be requested.

China: It is well known that China's current and future pharmaceutical market is very substantial. China's 1.3 billion people make up about 20% of the global population. It has grown to be the third largest pharmaceutical market and is growing rapidly. The China Food and Drug Administration (CFDA) is in charge of drug registration and evaluation, and the Center for Drug Evaluation (CDE) is responsible for the evaluation of chemistry drugs, traditional Chinese medicines, and biologic products [212]. Article 11 in the Provisions for Drug Registration SFDA No. 28 [213] defines five types of drug-registration applications; (1) new drug application, (2) generic drug application, (3) imported drug application, (4) supplemental application, and (5) renewal application. A foreign applicant shall make application according to the imported rule. For import drugs with a Certificate of Pharmaceutical Product (CPP) issued by the exporting country with a patent certificate and established Good Manufacturing Practice(s) (GMP) status [214], foreign clinical data would then be assessed for completeness in line with GMP requirements, as well as the Chinese regulatory requirements and ethnic sensitivity assessment.

Subsequently, a pharmacokinetic study and a clinical trial of 100 Chinese subjects (per arm) will be required [196,214]. Alternatively, the foreign applicant could submit a clinical trials application in accordance with Article 44 of the Provisions for Drug Registration (SFDA Order No. 28) [213], which states that the drug should already be registered in a foreign country, or in phase II/III development. However, this does not apply to an application for a new vaccine that is not registered in any country. Furthermore, CFDA may also request the applicant to first conduct a local phase I trial. Other than it has to be local, there is no explicit definition of Chinese given in biological or geographical terms.

China–Korea–Japan Tripartite: The China–Korea–Japan Tripartite cooperation was formed in 2007, following ICH E5 revision as well as Japan's Notification of Basic Principles on Global Clinical Trials. The tripartite's cooperation involved research into ethnic differences in PK/PD and genetic polymorphisms affecting them, information sharing to promote regulatory framework understanding, and creating a regional clinical guidelines protocol [215]. The pioneer activity of this group was a comparative pharmacokinetic study between China, Korea, Japan, and Caucasians for three drugs, namely, moxifloxacin, simvastatin, and meloxicam under the Kawai Project. The study demonstrated similar pharmacokinetics between all comparator populations (moxifloxacin), similar pharmacokinetics between some comparator populations (simvastatin between Japanese and Caucasians; meloxicam between Japanese and Chinese) and differences among comparator populations [216]. This cooperation is at an early stage of development as may be seen as an initial move toward genomics-based bridging, as opposed to ethnicity-based bridging.

Southeast Asia

Singapore: In Singapore, the Health Sciences Authority (HSA) is the regulatory agency responsible for drug evaluation and approval. HSA is a Regulatory Member for the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) since 2017. HSA has adopted an evidence- and risk-based approach in the evaluation processes for registration of pharmaceuticals, aligned to international regulatory requirement and standards. With regard to clinical data requirements, HSA accepts foreign clinical data with no specific

requirements for bridging studies unless there is evidence that ethnic factors could affect the efficacy or safety of the medicine in Singapore's population. HSA performs independent review of all applications, taking into account, when relevant, assessment by HSA's reference agencies (namely, Therapeutic Goods Administration of Australia, European Medicine Agency of European Union, Health Canada of Canada, Medicines and Healthcare Products Regulatory Agency of the United Kingdom, and Food and Drug Administration of the United States).

ASEAN Countries: The Association of Southeast Asian Nations (ASEAN) has 10 member countries, namely, Indonesia, Malaysia, Philippines, Singapore, Thailand, Brunei Darussalam, Vietnam, Laos, Myanmar, and Cambodia. The population size is about 634 million, with a combined gross domestic product as the sixth largest in the world at US$2.55 trillion (or US$4,021 GDP per capita and a total trade of US$ 2,218,534 million) [217]. The pharmaceutical market in Southeast Asia is relatively small, but the region remains attractive to the pharmaceutical industry due to its growth potentials. All ASEAN member countries are net pharmaceutical importers, except for Singapore.

The ASEAN's Consultative Committee for Standards and Quality (ACCSQ) – Pharmaceutical Product Working Group (ACCSQ-PPWG) was set up in 1999 to "*harmonise pharmaceuticals' regulations of the ASEAN member countries to complement and facilitate the objective of ASEAN Free Trade Area (AFTA), particularly, the elimination of technical barriers to trade posed by these regulations, without compromising on drug quality, safety and efficacy*" [218]. The topics selected for harmonization by the PPWG were safety, quality, efficacy, and administration data, which reflect the basis for drug-registration approval. PPWG was instrumental in preparing key drug regulatory harmonization documents, which include [219]:

- The ASEAN Common Technical Requirements (ACTR) for pharmaceutical product registration
- The ASEAN Common Technical Dossier (ACTD) for pharmaceutical drug registration
- ASEAN guidelines on analytical validation, bioavailability, and bioequivalence studies, process validation, and stability study

Each guidance gives cross-references to relevant ICH guidelines or pharmacopeia.

It should be noted that, although many of the ICH guidelines were adopted by PPWG, it was decided that the ICH E5(R1) was not going to be adopted due to lack of resources, to first make a scientific justification on the need for local clinical trials and, subsequently, to verify actual efficacy of drugs in local situations [220]. Instead, the ASEAN countries were strongly encouraged to participate in the "Global Drug Development Programs" [221]. In general, ASEAN capacity for evaluating and assessing drug quality, safety, and efficacy are limited. For this reason, they require a Certificate of Pharmaceutical Product (CPP) issued by the reference country as a surrogate assurance of the product reliability [221]. Furthermore, most of the drug applications reviewed by ASEAN drug regulatory authorities are generic drugs, thus much more emphasis is given to evaluations relating to quality issues such as bioavailability/bioequivalence and stability studies.

The recent trend in the shift of clinical trials to Asian emerging regions, especially in Korea, Taiwan, China, Thailand, Singapore, Philippines, and Malaysia [222], is an opportunity to provide a platform in addressing the issues of ethnic differences more objectively.

Global Drug Development and Pharmacogenomics

Currently, efforts are concentrated on developing biomarkers and pharmacogenomics information from clinical trial inception to post-market phase throughout a drug-product life

cycle. Japan has proposed the multiregional clinical trials (MRCT) model, which incorporates special consultation on pharmacogenomics/biomarker qualification to facilitate utilization of this information for regulatory decision [207]. This development was pioneered by the efforts from the FDA and EMA by first encouraging voluntary submission of genetic data (VGDS). The scope was then expanded to include nongenomic biomarkers; hence, VGDS was renamed voluntary exploratory data submissions [VXDS] for inclusion of more diverse biomarkers by pharmaceutical industries, to allow for nonthreatening discussion between the industry and regulatory authorities. For this purpose, the FDA and EMA issued the "Guidance for Industry: Pharmacogenomic Data Submissions" in 2005 [223]. This platform has encouraged novel pharmacogenomics and biomarker integration in drug development.

Subsequently, a harmonized submissions guideline was drafted and finalized by the ICH E16 working group for genomic biomarker qualifications in 2010 [224] followed by an ICH E18 Guideline on Genomic Sampling. The aim of the guideline is to provide harmonized principles of genomic sampling and management of genomic data in clinical studies. This guideline will facilitate the implementation of genomic studies by enabling a common understanding of critical parameters for the unbiased collection, storage, and optimal use of genomic samples and data among stakeholders, including drug developers, investigators, and regulators. This guideline also intends to increase awareness and provide a reminder regarding subjects' privacy, protection of the data generated, the need to obtain suitable informed consent, and the need to consider transparency of findings in line with local legislation and regulations. A pharmacogenomics-based (biomarker) success through the conduct of MRCT is perhaps best exemplified by trastuzumab. Trastuzumab is a monoclonal antibody targeting the extracellular domain of the HER2 protein, an epidermal growth factor receptor gene [225], which was found to be amplified 25–30 times in patients with an aggressive form of breast cancer, along with an increase in the expression of its protein in the malignant cells [226]. This biomarker identification coupled with reliable laboratory testing, utilizing fluorescence-in situ hybridization (FISH) assay, has enabled a more successful treatment of this subgroup of women with breast cancer, with improved disease-free survival as well as overall survival [227,228]. The design of the trial, which preselected women who were HER2-positive, has saved much time and patient numbers to provide the statistically significant benefit of trastuzumab [229,230].

Table 11.8 lists examples of drugs which have been approved in Japan based on the use of biomarkers in MRCTs.

The landscape of pharmacogenomics (PGx) in regulatory science has observed issuance of PGx-related regulatory guidance documents covering an extensive scope of topic throughout the product life cycle, setting up of pharmacogenomics working groups, as well as the publication of the summary of pharmacogenomics biomarker information in drug labeling [232]. All these are exciting developments to better risk/benefit judgment for regulatory authorities, as well as making drug therapeutic effects more predictable, effective, and safe for the end-users.

Pharmacogenomics and Ethnicity in Global Drug Development

The use of pharmacogenomics in global drug development may very well be the turning point for actual translational medicine to be realized. The adoption of the use of pharmacogenomics as a tool to evaluate differences in population groups has added tremendously to its initial value of merely looking at interindividual differences. Invariably, characterization of each and every causative factor and quantitative

TABLE 11.8 Drugs Approved Based on the Use of Pharmacogenomics (Biomarkers) in Multiregional Clinical Trials in Japan [231]

Drug Name	Indication	Biomarker
Tolterodine	Overactive bladder with symptoms of urge urinary incontinence, urgency, and frequency	CYP2D6
Trastuzumab	Adjuvant therapy for Her2-positive breast cancer	Her2/neu
Panitumumab	Metastatic colorectal carcinoma with wild-type KRAS proto-oncogene (KRAS) tumors	KRAS
Nilotinib	Newly diagnosed chronic myeloid leukemia in chronic phase	Philadelphia chromosome
Trastuzumab	Her2-positive metastatic gastric cancer	

relationship of their combinations of variability in pharmacologic treatment outcome [233] would be needed to truly personalize medical treatment. Pharmacogenomics and biomarkers are undoubtedly significant parts of this understanding. Nevertheless, it cannot be ignored that other factors constitute an integral part of drug response at the population level, which cannot be defined merely by looking at genetics and biomarkers.

In the context of using an MRCT as a bridging study, one of the key points to address is sample-size calculation to enable extrapolation of the overall trial results to the particular region. The ICH E5(R1) (Q&A) [1] emphasizes this point, and the Japanese MHLW has also provided a guideline on how to demonstrate drug efficacy in a particular region [234]. Two methods were proposed in this guideline for determination of sample size [234]:

- Method 1: D = difference between placebo and study group; D_{all} = difference in the overall study population across regions; D_{Japan} = difference within the Japanese subpopulation. The sample size is determined so that $D_{Japan}/D_{all} > 0.5$ will achieve a probability of 80% or more,
- Method 2: D_{all} = difference between placebo and entire study groups across regions, assuming inclusion of three regions; D_1, D_2, and D_3 = difference between placebo and study groups in regions 1, 2, and 3, respectively. The sample size is determined so that D for each region will show similar tendency. In the case in which $D > 0$, the number of subjects is determined so that D_1, D_2, and D_3 will exceed 0 with the probability of 80% or higher.

It has been argued that genetic clustering (which is being used in genetic ancestry) defines a population in a more robust manner as compared to ethnicity and geographical approaches [233]. However, Risch et al. [235] pointed out a very important point of how data analyses evaluating genetic clusters in isolation and ignoring race and ethnicity may lead to conclusions that are seriously confounded. As an example, they illustrated how in a study comparing the efficacy of angiotensin-converting enzyme (ACE) inhibitors between Black patients and White patients, there was a significantly better outcome to treatment in White patients. Should the study have used the genetic clustering method, the direct inference made from this study would be that this difference was due to the difference in genetic clusters between the two groups. Although it has been shown from other studies that genetic clustering is in high correlation with self-identified ethnicity/race [236], a direct inference, as is shown in this example, could lead to a grossly confounded conclusion. This is because the difference in treatment may very well be simply other extrinsic factors, which are related more to ethnicity, rather than to the actual genetics. Thus, it should be highlighted that one should not be "blinded" to ethnicity information, while carrying out studies with genetic or even nongenetic

biomarkers, to have a more complete understanding of the given scenario.

The approval of isosorbide dinitrate and hydralazine combination, for the treatment of heart failure in African Americans by FDA in 2005, illustrates that race and ethnicity are indeed very relevant. The initial application for the marketing of the isorsorbide dinitrate/hydralazine combination for all patients was rejected as the original trials, Vasodilator Heart Failure Trial I and II (V-HeFT) failed to demonstrate required statistical significance [237]. Subsequently, when the investigators reanalyzed the data, it was found that the drug may be selectively effective in the Black population. Therefore, the FDA recommended a new trial, named the A-HeFT trial (African American Heart Failure Trial), which demonstrated a 43% reduction in the rate of death from any cause, 33% relative reduction in the rate of first hospitalization for heart failure, and an improvement in the quality of life [238]. This demonstrates of how inclusion of a different specific subpopulation identification and definition can result in a different outcome. The potential of deriving less expensive, more effective, and safer drugs using pharmacogenomics stratification certainly has a special appeal for developing countries that are in desperate need of a more cost-effective healthcare strategy. Thus, close cooperation between nations for amassing and sharing genotyping data can be significantly beneficial. The Human Genome Organisation (HUGO) Pan-Asian SNP consortium is an example of such cooperative effort [239] to provide a platform for disease–population studies or pharmacogenomics research for investigators and can be leveraged by regulators alike.

An example of tapping into a population genomic database in regulatory application was the safety update of neutropenia risk for irinotecan among the main ethnic groups in Singapore [240]. On a broader approach, there is potential for incorporating quantitative population-genetics differentiation measures, such as the Wright's Fixation (Fst) index, as part of a decision tree in the evaluation of foreign data and the assessment of the transferability of clinical trial results between populations [241].

Pharmacogenomics in Pharmacovigilance

Another application of pharmacogenomics in regulatory science is in the postmarketing phase, in which rare but serious Adverse Drug Reactions (ADRs) may arise after marketing authorization, due to the inherent rarity of serious ADRs and the limitations of clinical trials. ADRs have been reported as the fourth leading cause, with more than two million hospitalized patients in the US. The use of pharmacogenomics to prospectively genotype and identify patients at risk of unpredictable but potentially life-threatening ADRs poses an attractive option as a risk management tool to avert an otherwise-unpredictable adverse and potentially life-threatening event. One success story is the recommendation of genotyping HLA-B*15:02 as standard of care for all new patients of Asian ancestry in Singapore since 2013 [242]. As a result, Singapore was able to prevent an estimate of 90 cases of serious cutaneous adverse reactions during the first 4 yrs after its implementation [243]. Other countries/regions with similar mandatory testing include Hong Kong, Thailand, and Taiwan [244,245]. Internationally in this field, the European EMA has also issued a guidance specifically addressing the use of pharmacogenomics methodologies in the pharmacovigilance evaluation of drugs.

CONCLUSION

Although ethnicity is very challenging to define, it is of utmost importance that ethnicity be defined in a standardized manner, so that an accurate scientific conclusion can be derived from any analysis that uses ethnicity/race as a variable. It remains a useful tool for regulatory authorities, practitioners, and researchers for providing a certain degree of insight to the

risk–benefit consideration to the outcome of pharmacological treatment. Bridging strategies have been useful in addressing some of the concerns in variability in drug response, as well as expediting drug approval in some countries/regions. The advent of bridging strategies using MRCTs has made it possible to address this issue in a more global manner. However, to be able to answer specific ethnicity-related questions, population selection must be clearly defined with protocols specifically catered to target population. The incorporation of pharmacogenomics and biomarkers throughout the product life cycle (including drug development and postmarket phase) may allow the stratification of patient populations in a more objective manner and further characterize and address some of the variability observed in different ethnic populations.

DISCUSSION QUESTIONS

1. Discuss the challenges of incorporating different ethnic groups in pharmacogenetic and pharmacogenomics research
2. Describe how foreign clinical trial data are utilized for drug approval in other parts of the world
3. Describe culturally related extrinsic factors that might influence the design of pharmacogenomics studies and the interpretation of study data
4. Describe the use of pharmacogenomics as a risk management tool to minimize the risks of adverse drug reactions

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CHAPTER

12

Pharmacogenomics in Latin American Populations

Mariana R. Botton[1], *Karla Claudio Campos*[2], *Jorge Duconge*[3], *Stuart A. Scott*[1,4]

[1]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [2]Department of Pharmacotherapy and Translational Research, University of Florida College of Pharmacy, Gainesville, FL, United States; [3]Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico Medical Sciences Campus, San Juan, PR, United States; [4]Sema4, Stamford, CT, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00012-7

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INTRODUCTION

Latin Americas

The term "Hispanics or Latino" is often used as a census category or label to encompass individuals from multiple Latin American countries of origin, all sharing the same language, similar culture, beliefs, habits, and history. Given that Hispanics have European (Spanish), Native American, and African ancestral contributions [1,2], they should not be considered as a single monolithic population. The percentage of contribution of each ancestry varies between countries and between the different regions within Latin American countries [3]. Intraethnic and intergroup genomic background differences, cryptic population structures, differential pattern, and extent of linkage disequilibrium (haplotype blocks), as well as stratification within these populations are expected due in part to a varying degree of ancestral contributions and ethnogeographic admixture, founder effects, genetic drifts, and other unique attributes of these populations.

Latin America comprises the Southern region of North America (Mexico), Central America, and South America. Spanish and Portuguese are the most spoken languages, whereas Portuguese is only spoken in Brazil, the biggest country of Latin America and the fifth most populous in the world (207.7 million people estimated in 2017 [4]). As a result of the great admixture in Latin America, many different skin pigmentations are observed. A huge study involving more than 7000 individuals from Latin America (Brazil, Colombia, Chile, Peru, and Mexico) showed that European and African ancestry is widespread in Brazil (except for the South of the country where the African contribution is very low), whereas in Colombia, Peru, Chile, and Mexico the most prominent contributions are the European and the Native American. However, differences within the individuals from the same countries are also observed [3]. Fig. 12.1 shows the maps from Brazil, Colombia, Chile, Peru, and Mexico, and their respective ancestry percentage contribution according to the region. The authors also showed that ancestry is strongly correlated with altitude; European and African ancestry are negatively correlated with altitude, whereas Native American ancestry is positively correlated with altitude [3]. Similarly, Via et al. observed significant differences in the average admixture proportions at the level of regions and municipalities of the island of Puerto Rico. This variation was explained by the African ancestry, which is substantially higher in the East than the rest of the island (Fig. 12.2) [5].

Latin American Admixture and Population Structure

A trihybrid admixture pattern in Latinos has been confirmed by Bryc et al. [6] after using principal component analysis (PCA) of autosomal genotype data in these populations and their corresponding putative ancestral populations. In Fig. 12.3, different ancestry depictions (mixtures) per Latino population are revealed by fitting of ellipses to the covariance matrix. The PCA showed that the Hispanic/Latino ethnic groups display distinguishable clusters according to differences in ancestral contributions, which is consistent with similar population studies [7,8]. Despite the use of ancestry as a covariate in individuals of European descents (e.g., White Americans), the admixture seems to be continuous in Latinos/Hispanics and, therefore, it cannot be grouped as a distinctive or categorical variable to appropriately represent the complex pattern of admixture among Latin Americans [7,9].

Admixture in Latin Americans has been described by Suarez-Kurtz and Parra [9] as a "*kaleidoscopic combination of individual proportions of Native American, European, and sub-Saharan African ancestries*" . The impact of admixture on genomic diversity in Latinos was revisited by these authors, who concluded that population

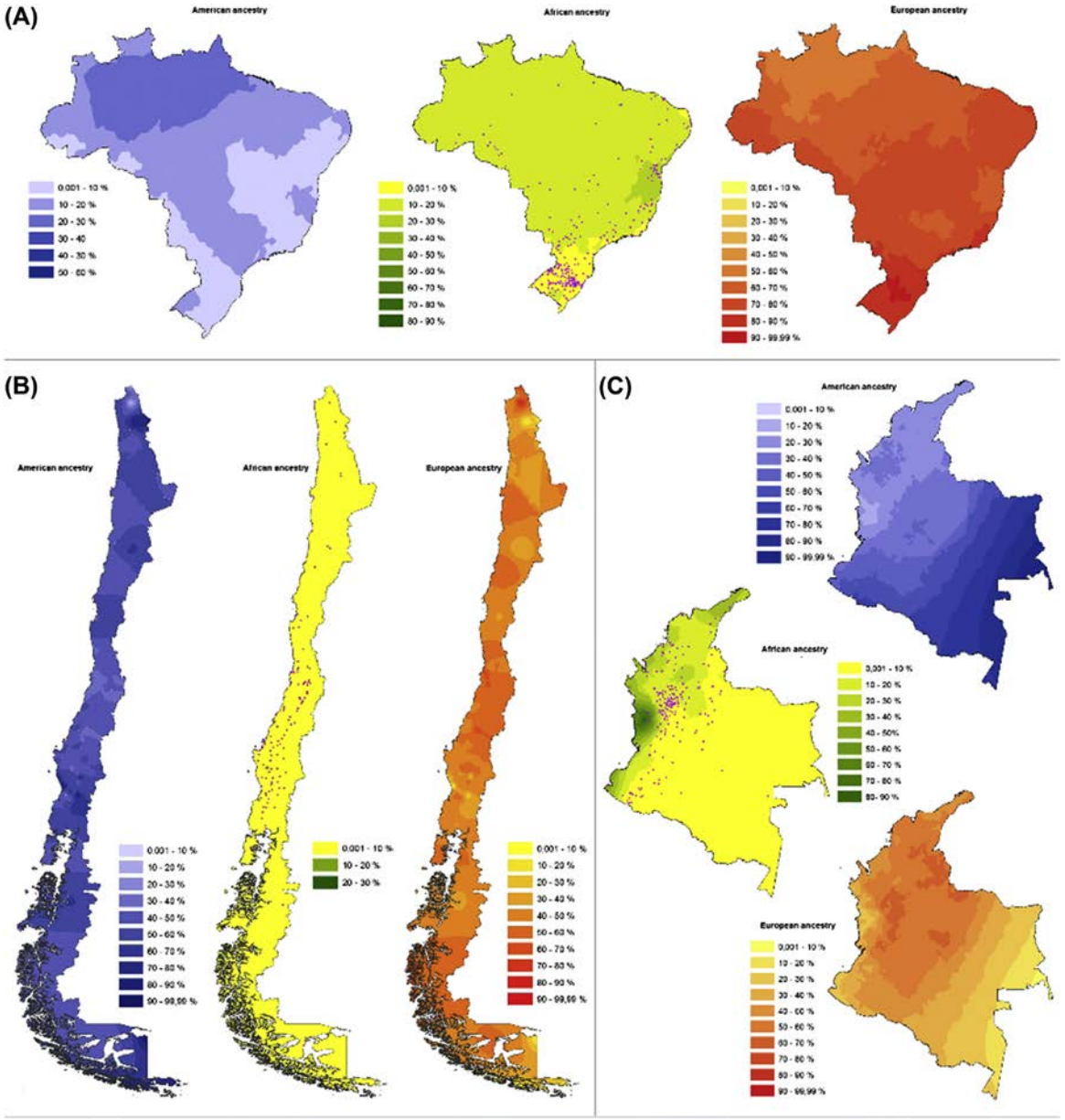

FIGURE 12.1 Geographic distribution of Native American (blue), African (green), and European (red) ancestry based on individual estimates for samples from (A) Brazil, (B) Chile, (C) Colombia,

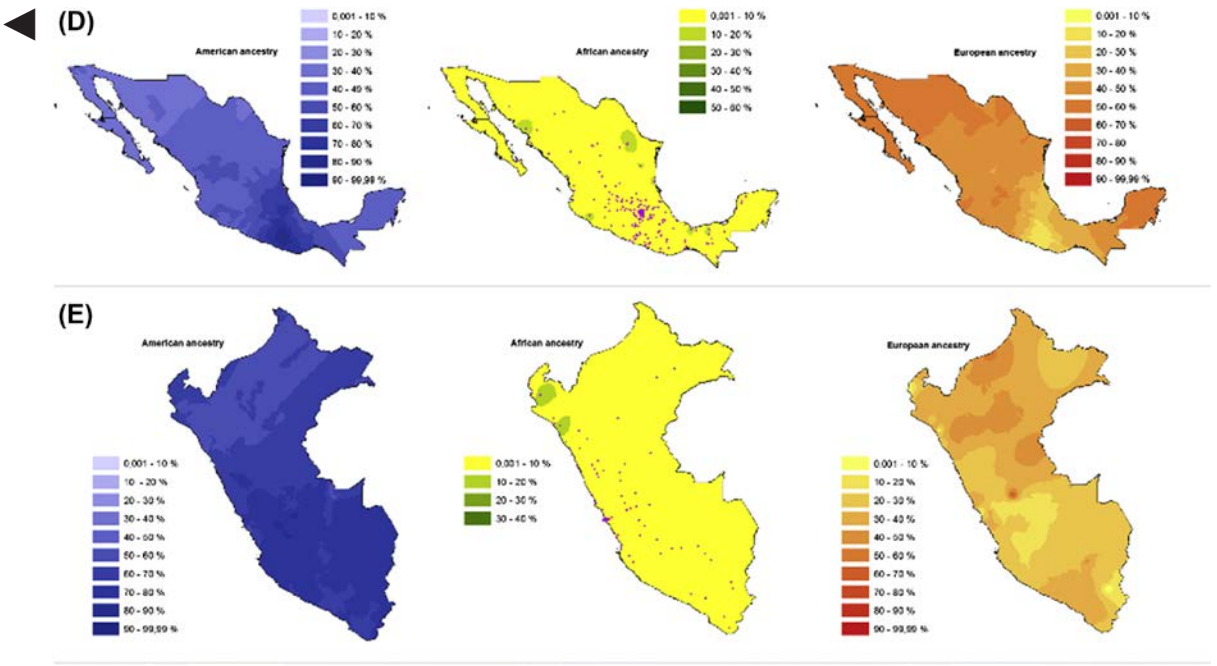

FIGURE 12.1, cont'd (D) Mexico, and (E) Peru. To facilitate comparison, color intensity transitions occur at 10% ancestry intervals for all maps. The birthplace of individuals is indicated by purple dots on the African ancestry map. Maps were obtained using Kriging interpolation as detailed in the text. https://doi.org/10.1371/journal.pgen.1004572.g001 [3].

average admixture proportions are not good predictors of the corresponding proportions at individual level [9]. Because of the extensive admixture observed in Latin America, the self-perception of genetic ancestry is often inaccurately estimated. For example, individuals who show higher skin pigmentation tend to overestimate their genetic African and Native American ancestry, and individuals with lower skin pigmentation tend to overestimate their genetic European ancestry. The same tendency is observed with other physical characteristics like eye and hair color, and hair type [3]. Similarly, a study showed that 38% of Brazilians with more than 90% European ancestry actually self-identify as brown or black [10].

The relative contribution of each ancestral group is different among Latinos: European and Native American contributions are higher in Mexicans when compared to Caribbean Hispanics, whereas African contribution is higher in Caribbean Hispanics. In admixed populations, the relative contribution of ancestral populations is expected to determine the frequency distribution of relevant variants [11]. As a result, Latinos present a large variation in ancestry proportions among different ethnogeographic groups and among individuals in a given country [5,6]. Noteworthy, both ancestry informative markers (AIMs) and physiogenomic (PG) markers have been used in pharmacogenomics studies of Puerto Ricans and other Hispanic/Latinos, as well as Brazilians, to infer the structure and ancestry pattern of these populations [12–14]. In addition, mitochondrial DNA and Y-chromosome (short tandem repeats [STRs]) markers have also been used to infer matrilineal and patrilineal ancestry, respectively, in the Latino population. One very important characteristic to consider when dealing with

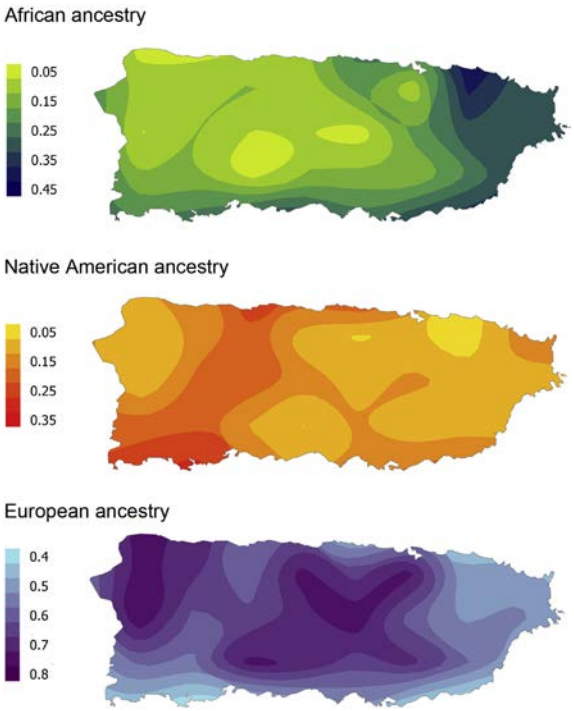

FIGURE 12.2 Population stratification in the island of Puerto Rico. Maps of Puerto Rico showing the regions with higher African (top), Native American (middle), and European (bottom) ancestry according to a color gradient. Darker colors indicate higher ancestral contributions. African ancestral contribution is significantly higher in individuals from the Northeast of the island than the rest of the island. *Reprinted from Via M, Gignoux CR, Roth LA, Fejerman L, Galanter J, Choudhry S, et al. History shaped the geographic distribution of genomic admixture on the island of Puerto Rico. PloS One January 2011;6(1):e16513. [Internet] [cited 2014 May 4].*

admixed populations like Hispanics or Latinos is the reduced number of samples required to find significant effects in genetic association studies on affected only and case-control studies [15].

Latin America and Self-Reported Ethnicity

With respect to the Brazilian population, the institution responsible for the Brazilian census (Brazilian Institute of Geography and Statistics [IBGE]) designated five categories for skin color: white, brown, black, yellow, and indigenous. According to the last official census realized in Brazil in 2010 by IBGE, brown and black individuals account for 51% of the population and white individuals account for 48%. However, the prevalence differs across the country based on differences in admixture across the distinct geographical regions of Brazil (Fig. 12.4). For example, self-declared brown individuals from the North had, on average, 68.6% European ancestry, followed by 20.9% Native American ancestry, and 10.6% African ancestry; whereas in the South region they had 44.2% European ancestry, 11.4% Native American, and 44.4% African ancestry [16]. Moreover, due to the great variability observed in skin color, the classification varies according to the observer. There is a pronounced African contribution in the Northeast; a high Native American contribution in the North; and in the South, the European contribution is predominant [3].

Although self-reported race (White or European, Black or African, Asian, and Latino) is commonly considered in clinical studies, this term may be inaccurate and misleading, especially for admixed populations [17]. Race classifies individuals according to their physical appearance (i.e., skin color) and biogeographical ancestries, which is subjective and could be ambiguous for Hispanic/Latinos. For example, skin color classification can be subjective for patients and healthcare providers as found by a study conducted in Cuba. In this study, approximately half of Cubans who were self-declared as mestizos had the same melanin levels as those Cubans declared as Whites. In addition, it was found that social workers and family doctors participating in the study classified skin color differently [18]. Association of ancestral proportions with skin pigmentation and bone mineral density in Puerto Rican women from New York City has also been reported [19]. Hispanics have genetic ancestry from Africans, Native Americans, and Europeans, but differences in these contributions are not necessarily observable. When physical

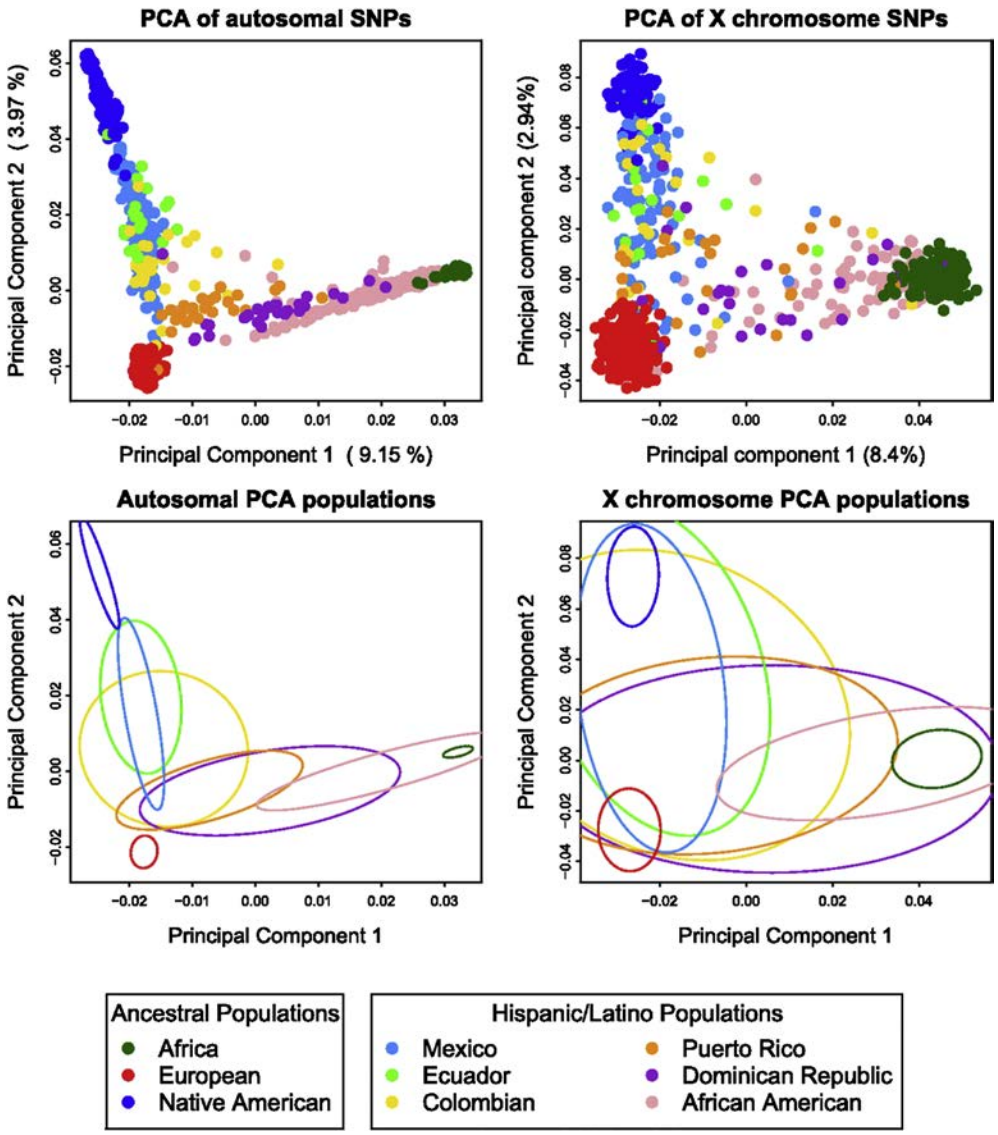

FIGURE 12.3 Principal component analysis (PCA) of the Hispanic/Latino populations performed by Bryc et al. [6]. Top: Different PCAs were performed using autosomal (left) and X-chromosome (right) single-nucleotide polymorphisms. Bottom: Clusters were fitted in *ellipses* for both PCAs. Each *dot* represents a single individual colored by their ethnic groups. Africans, Europeans, and Native Americans were used as reference populations for Hispanic/Latinos. *Reprinted from Bryc K, Velez C, Karafet T, Moreno-Estrada A, Reynolds A, Auton A, et al. Colloquium paper: genome-wide patterns of population structure and admixture among Hispanic/Latino populations. Proceedings of the National Academy of Sciences of the United States of America. May 11, 2010;107 Suppl.:8954–8961. [Internet] [cited 2014 May 1].*

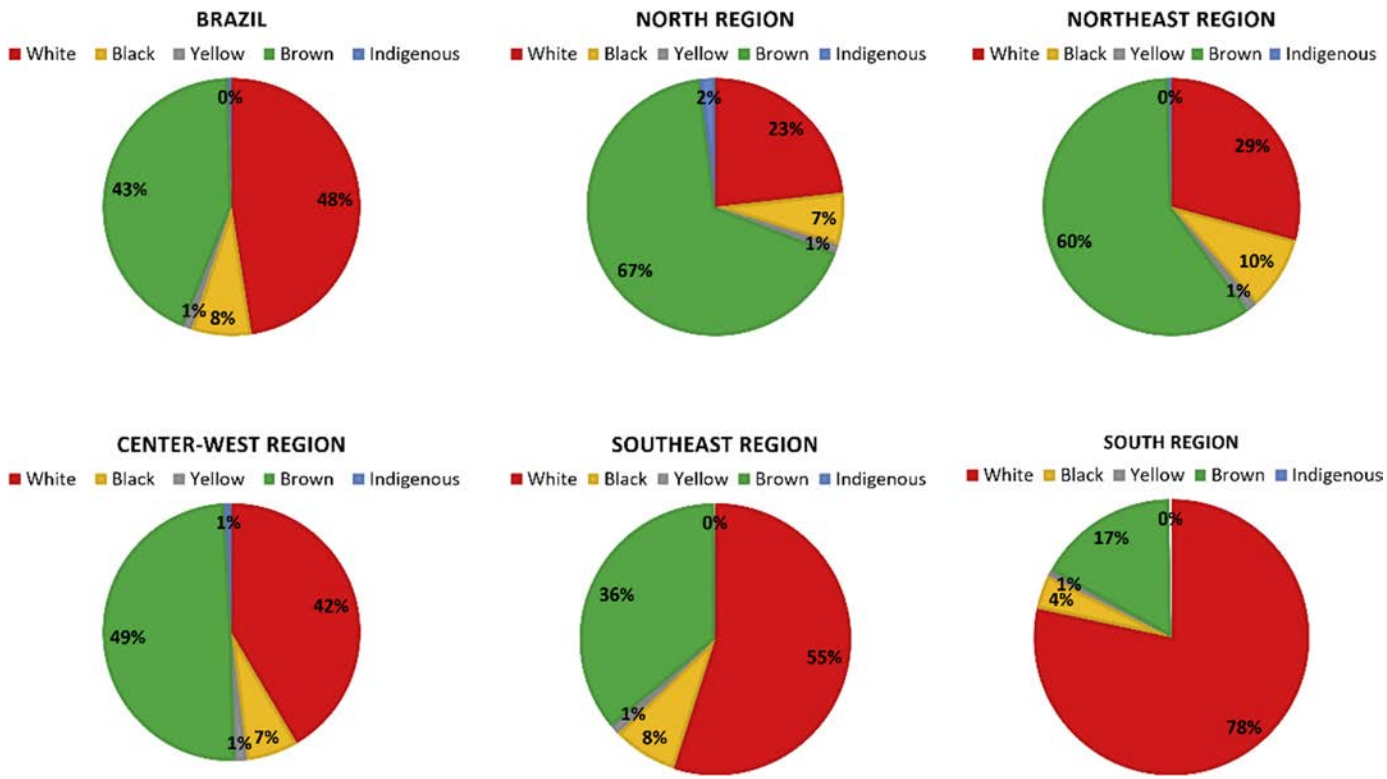

FIGURE 12.4 Distribution of self-reported skin colors in Brazil in each of the five geographical regions of the country, according to the 2010 census (https://sidra.ibge.gov.br/tabela/136) [4].

appearance is used as a method to classify individuals in races, only a limited number of genes that encode for these phenotypes are being considered, discounting the rest of the genome [20]. Furthermore, genetic ancestry is expected to reduce spurious associations because differently from race and ethnicity, genetic ancestry does not account for factors, such as education, socioeconomic status, culture, and skin color.

Ancestral contributions of Hispanic/Latinos explain differences in the prevalence of diseases across Hispanic-ethnic groups and consequently, genetic ancestry may be useful to understand these patterns and identify better healthcare services. For example, asthma has the highest prevalence in Puerto Ricans when compared to North Americans and Mexican Americans, but paradoxically, Hispanics are reported as the group with the lowest prevalence of this disease [21]. The high-prevalence of asthma in Puerto Ricans might be explained by the higher African contribution of Puerto Ricans when compared to Mexican descendants [17]. Furthermore, severity of asthma is better predicted in 4%–5% of African Americans when genetically inferred ancestry is considered, improving diagnosis of this condition in the affected population [22].

Likewise, systemic lupus erythematosus (SLE) disproportionately affects Hispanics, having a prevalence that is reportedly up to five times higher than Europeans, as well as more serious organ system involvement and active disease at the time of diagnosis. A significantly higher risk associated with Native American ancestry on overall SLE was also identified (OR=4.84, P=.0001) [15]. Consequently, it has been postulated that such a rich mixed ancestry of Puerto Ricans provides the intrinsic variability to unravel complex gene–environment interactions in disease susceptibility [23].

Latin American Pharmacogenomics

Due to the factors mentioned above, pharmacogenetics data derived from major racial populations (e.g., European and/or African) may not be appropriate to directly infer to Latin American populations. Although a study based on 50 self-reported U.S. Hispanic individuals suggested that algorithms for warfarin dose prediction derived from non-Hispanic cohorts would be appropriate for US Hispanic patients [24], other studies showed the opposite; non-Hispanic algorithms did not have a suitable performance in cohorts derived from Brazil [25–27] and Puerto Rico [28]. Moreover, a Brazilian algorithm model derived from the Southeast [26] had a similar performance in white and black patients, and a suitable execution in another admixed cohort from the same region of the country [27], but did not show strong correlation when used to predict the warfarin dose in a Southern Brazilian population of European ancestry [25]. Concurrently, the Southeast and the Southern Brazilian cohorts were combined and the algorithm derived performed well in both white and black Brazilians, being able to capture the differences observed in the admixture level between the regions [29]. The International Warfarin Pharmacogenetics Consortium (IWPC) [30] generated an algorithm with "race" included as a covariate, distinguishing White, Asian, black/African American, and mixed individuals. However, the performance was better in White (R^2=0.45) than in Asian (R^2=0.32) or black patients (R^2=0.29), supporting the idea that self-reported ancestry is not sufficient to represent the complex genetic background for Latin American individuals [29]. Accordingly, each Latino needs to be considered as an individual rather than a member of an ethnic group when applying the pharmacogenetics-guided precision medicine paradigm into this population [31,32].

PHARMACOGENOMIC VARIANTS AMONG LATIN AMERICANS

Members of the cytochrome P450s (CYP450s) play a main role in the metabolism of drugs and, therefore, research on this topic is most of the time prioritized. Studies

in cytochrome P450 2C9 (*CYP2C9*), *CYP2C19*, and *CYP2D6* are available for several Hispanic populations (see Tables 12.1 and 12.2) and some of their frequencies are comparable to other populations [33]. Although Hispanics/Latinos have higher European contribution than African or Native, the frequency, distribution, and combination of genetic variants in Hispanics may differ from Europeans and other parental populations [32]. For example, frequencies in *CYP2C9* in Hispanics compares with Europeans, however, *CYP2C19* has a higher number of variants considered rare (4% in Hispanics vs. 0.05% in Europeans) [33]. Indeed, variants like *CYP2C9*5*, **6*, **8*, and **11* (mostly found in African descendants) were found in Hispanics from the European and Ibero-American Consortium of Population Pharmacogenetics (known as CEIBA as the original acronym is in Spanish language) [57]. Furthermore, another study of Hispanics living in the United States also identified the *CYP2C9*6* [75].

Intraethnic variations in CYP450s genetic frequencies have been identified for *CYP2C9*, *CYP2C19*, and *CYP2D6*. A study on the relevance of ancestry for the variability of gene polymorphisms related to drug-metabolizing enzymes in a multiethnic Costa Rican population revealed a lower frequency of *CYP2C9*2* in self-reported Amerindian groups compared to mestizos from the Central Valley/Guanacaste, and higher frequencies of *CYP2C19*2* and *CYP2C19*17* in the self-reported Afro-Caribbean group from Limon [76]. Similarly, the presence of *CYP2D6* variant alleles (*CYP2D6*5*, *CYP2D6*17*, and *CYP2D6*29*) correlated positively with African ancestry but negatively with Native American ancestry in Costa Ricans [76].

The frequencies of CYP450s variant alleles may provide information to infer the potential risk of a population for drug-induced adverse events; however, other nongenetic factors inherent to the population and the individual may also be influencing drug response. *CYP2C19*2* encodes for a nonfunctional enzyme and individuals who are heterozygous or homozygous for the variant allele are intermediate and poor metabolizers, respectively. *CYP2C19*2* has a lower frequency in Hispanics (10%) when compared to Africans (18.1%), Europeans (18.3%), and Asians (31.0%) [33]. The reported lowest allele frequency in Hispanics when compared to other populations agrees with previous findings in Mexican Mestizos (7%–10%), in which also the frequency of poor metabolizers (as observed by s-mephenytoin metabolism) was found lower (3.2%) when compared to African Americans (5.0%), Whites (5.0%), and East Asians (16.7%) [77,78]. Another study found that 6% of Mexicans mestizos (n = 127) from Jalisco were CYP2C19 poor metabolizers according to phenotyping test with omeprazole [79]. Although *CYP2C19*2* allele frequencies data are in agreement across different studies in Mexicans [61,78,80], in Tarahumaras—Native Americans from Mexico—the *CYP2C19*2* was found with a frequency of 31%, which is at least three times higher than in Mexican mestizos (~10%) and other Native Americans from Mexico (3.6%–5.6% in Tojolabales, Purépechas, and Tzotziles) [77].

*CYP2C19*17* has been described as a regulatory variant that increases CYP2C19 expression resulting in a gain-of-function allele. *CYP2C19*17* shows high variability across Hispanics groups (4%–25%; see Table 12.2). Peruvians are the ethnic group with the lowest frequency of this variant (4.1%), whereas Colombians, Mexicans, and Puerto Ricans have frequencies approximately three times higher (~14%), and Ecuadorians have a frequency that is approximately six times higher (~25%). Individuals who have one or two alleles of *CYP2C19*17* have higher metabolism of drugs resulting in lower attainment of therapeutic concentrations of tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs) among other drugs [81–83].

TABLE 12.1 Minor allele frequencies (MAF) of clinically actionable variants on relevant pharmacogenes, as reported by studies in Hispanics

Gene	Allele	rs Number	DNA Change (ATG Start)	Effect	MAF Hispanics	N	References
CYP2C9	*CYP2C9*2*	rs1799853	c.430C>T	p.Arg144Cys	0.066	5789	[33]
	*CYP2C9*3*	rs1057910	c.1075A>C	p.Ile359Leu	0.036	5789	[33]
CYP2C19	*CYP2C19*2*	rs12769205	g.12662A>G	Splicing defect/frameshift	0.101	5789	[33]
	*CYP2C19*3*	rs4986893; rs17886522	c.636G>A; c.1251A>C	p.Trp212Ter; p.Gly417=	<0.001	5789	[33]
	*CYP2C19*17*	rs11188072; rs12248560	g. -3402C>T; g.-806C>T	Regulatory; regulatory	0.120	5789	[33]
CYP2D6	*CYP2D6*2*	rs16947; rs1135840	c.733C>T; c.1304G>C	p.Arg296Cys; p.Ser486Thr	0.327	5789	[33]
	*CYP2D6*3*	rs35742686	c.775delA	p.Arg259Glyfs (frameshift)	0.003	5789	[33]
	*CYP2D6*4*	rs3892097	g.1847G>A	Splicing defect/169 frameshift	0.157	5789	[33]
	*CYP2D6*5*	N/A	N/A	Gene deletion	0.030	5789	[33]
	*CYP2D6*6*	rs5030655	c.454delT	p.Trp152Glyfs	0.004	5789	[33]
	*CYP2D6*17*	rs16947; rs28371706	c.733C>T; c.320C>T	p.Thr107Ile	0.007	5789	[33]
	*CYP2D6*41*	rs28371725	g.2989G>A	Splicing defect	0.035	5789	[33]
CYP3A5	*CYP3A5*3*	rs776746	c.219-237A>G	Splicing defect	0.797	5789	[33]
	*CYP3A5*6*	rs10264272	c.624G>A	Splicing defect	0.023	5789	[33]
	*CYP3A5*7*	rs41303343	c.1035_1036insT	Frameshift	0.004	5789	[33]
CYP4F2	*CYP4F2*2*	rs3093105	c.34T>G	p.Trp12Gly	0.098	5789	[33]
	*CYP4F2*3*	rs2108622	c.1297G>A	p.Val433Met	0.223	5789	[33]
DPYD	*DPYD*2A*	rs3918290	c.1905+1G>A	Splicing defect	0.002	449	[34,35]
IFNL3		rs12979860	c.151-152C>T	Intron variant	0.477	2377	[34,36–38]
		rs8099917	g.1332A>C	Regulatory	0.358	1027	[34,36–38]
NAT1	*NAT1*3*	rs15561	c.1095C>A	3′-UTR variant	0.415	504	[34,39]
	*NAT1*10*	rs1057126	c.215A>T	3′-UTR variant	0.597	504	[34,39]

NAT2	*NAT2*5*	rs1801280	c.341T>C	p.Ile114Thr	0.349	504	[34,39]
	*NAT2*6*	rs1799930	c.590G>A	p.Arg197Gln	0.179	504	[34,39]
	*NAT2*7*	rs1799931	c.857G>A	Gly286Glu	0.114	504	[34,39]
	*NAT2*11*	rs1799929	c.481C>T	p.Leu161=	0.334	504	[34,39]
	*NAT2*13*	rs1041983	c.282C>T	p.Tyr94=	0.300	504	[34,39]
SLCO1B1	*SLCO1B1*5*	rs4149056	c.521T>C	p.Val174Ala	0.146	1260	[34,40,41]
	*SLCO1B1*15*	rs2306283	c.388A>G	Asn130Asp	0.495	545	[34,40,42]
	*SLCO1B1*17*	rs4149015	c.-910G>A	Regulatory region	0.037	347	[34]
TPMT	*TPMT*2*	rs1800462	c. 238C>G	p.Ala80Pro	0.003	505	[43–46]
	*TPMT*3A*	rs1800460; rs1142345	c.460C>T; c.719T>C	p.Ala154Thr; p.Tyr240Cys	0.029	505	[43–46]
	*TPMT*3B*	rs1800460	c.460C>T	p.Ala154Thr	0.002	505	[43–46]
	*TPMT*3C*	rs1142345	c.719T>C	p.Tyr240Cys	0.000	505	[43–46]
	*TPMT*4*	rs1800584	626-1G>A	Splice acceptor	0.003	147	[45]
UGT1A1	*UGT1A1*6*	rs4148323	c.211G>A	p.Gly71Arg	0.007	944	[41,47]
	*UGT1A1*27*	rs35350960	c.686C>A	p.Pro229Gln	0.011	647	[34,47]
	*UGT1A1*28*	rs8175347	c.-53-52TA[6]>TA[7]	Intron tandem repeat	0.342	1104	[41,48,49]
	*UGT1A1*36*	rs8175347	c.-53-52TA[6]>TA[5]	Intron tandem repeat	0.000	705	[50]
	*UGT1A1*37*	rs8175347	c.-53-52TA[6]>TA[8]	Intron tandem repeat	0.000	705	[50]
	*UGT1A1*80*	rs887827	c.862-10021T>G	c.862-10021T>G	0.380	705	[50]

TABLE 12.2 Reports of minor allele frequencies (MAF) of clinically actionable variants on relevant pharmacogenes across different Hispanic/Latino populations

	Hispanics											
Allele	**Colombia[a]**	**Mexican American[a]**	**Mexico**	**Peru[a]**	**Puerto Rico[a]**	**Chile**	**Argentina**	**Bolivia**	**Ecuador**	**Nicaragua**	**Costa Rica**	**Cuba**
*CYP2C9*2*	0.122	0.102	0.051 [51]	0.024	0.065 [52]	0.059 [53]	0.257 [54]	0.048 [55]	0.050 [56]	0.095 [57]	0.044 [58]	0.113 [59]
*CYP2C9*3*	0.064	0.023	0.039 [51]	0.012	0.054 [52]	0.036 [53]	0.025 [54]	0.030 [55]	0.037 [56]	0.023 [57]	0.028 [58]	0.100 [59]
*CYP2C19*2*	0.087 [60]	0.125	0.086 [61]	0.059	0.130 [62]	0.120 [53]	ND	0.078 [55]	0.078 [56]	0.065 [57]	0.087 [58]	ND
*CYP2C19*3*	0.000 [60]	0.000	0.000 [61]	0.000	0.000 [62]	0.000 [53]	ND	0.001 [55]	0.004 [56]	0.000 [57]	0.000 [58]	ND
*CYP2C19*4*	0.000 [60]	0.008	0.000 [61]	0.000	0.003 [62]	ND	ND	ND	ND	0.000 [57]	0.004 [58]	ND
*CYP2C19*17*	0.128	0.117	0.143 [61]	0.041	0.14 [62]	ND	ND	ND	0.249 [56]	0.081 [57]	0.095 [58]	ND
*CYP2D6*2*	0.370 [63]	0.258	0.178 [64]	0.324	0.389	0.407 [65]	0.174 [66]	ND	0.314 [67]	0.173 [57]	0.149 [58]	ND
*CYP2D6*3*	0.012 [63]	0.000	0.014 [64]	0.000	0.005	0.011 [65]	0.006 [66]	ND	0.004 [67]	0.015 [57]	0.006 [58]	0.000 [31]
*CYP2D6*4*	0.194 [63]	0.125	0.112 [64]	0.065	0.154	0.118 [65]	0.164 [66]	ND	0.106 [67]	0.142 [57]	0.158 [58]	0.143 [31]
*CYP2D6*5*	0.008 [63]	ND	0.027 [64]	ND	ND	ND	0.028 [66]	ND	0.021 [67]	0.046 [57]	0.042 [58]	0.016 [31]
*CYP2D6*6*	0.000 [63]–0.001	0.000	ND	0.000	0.000	ND	0.004 [66]	ND	0.000 [67]	0.000 [57]	0.003 [58]	0.012 [31]
*CYP2D6*17*	0.016 [63]	0.000	0.017 [64]	0.012	0.010	0.000 [65]	0.002 [66]	ND	0.004 [67]	0.015 [57]	0.040 [58]	0.102 [31]
*CYP2D6*41*	0.080	0.016	0.022 [68]	0.006	0.120	ND	0.077 [66]	ND	0.025 [67]	0.041 [57]	0.034 [58]	ND
*CYP2D6*1or*2xN*	ND	ND	0.050 [64]	ND	0.000 [69]	0.003 [65]	0.034 [66]	ND	0.008 [70]	0.020 [57]	0.054 [58]	0.047 [31]
*CYP3A5*3*	0.814	0.766	0.098 [71]	0.876	0.736	0.760 [53]	0.900 [72]	ND	0.88 [73]	0.762 [74]	ND	ND
*CYP3A5*6*	0.011	0.023	ND	0.012	0.043	ND	ND	ND	ND	ND	ND	ND
*CYP3A5*7*	0.000	0.000	ND	0.006	0.005	ND	ND	ND	ND	ND	ND	ND
*CYP4F2*2*	0.133	0.141	ND	0.053	0.192	ND	ND	ND	ND	ND	ND	ND
*CYP4F2*3*	0.282	0.250	ND	0.118	0.288	ND	ND	ND	ND	ND	ND	ND
*DPYD*2A*	0.000	0.000	ND	0.006	0.000	ND	0.005 [35]	ND	ND	ND	ND	ND

IFNL3 rs12979860	0.415	0.461	0.435 [37]	0.376	0.365	ND	0.400 [36]	0.643 [38]	ND	ND	ND	ND
IFNL3 rs8099917	0.271	0.352	0.408 [37]	0.329	0.192	ND	0.370 [36]	ND	ND	ND	ND	ND
*SLCO1B1*5*	0.181	0.078	ND	0.141	0.120	0.136 [40]	ND	ND	0.169 [41]	ND	ND	ND
*SLCO1B1*15*	0.479	0.375	0.510 [42]	0.471	0.529	0.547 [40]	ND	ND	ND	ND	ND	ND
*SLCO1B1*17*	0.069	0.023	ND	0.006	0.043	ND	ND	ND	ND	ND	ND	ND
*TPMT*2*	0.003 [43]	0.000	0.000 [49]	0.006	0.005	0.000 [46]	0.007 [45]	0.000 [44]	ND	ND	ND	ND
*TPMT*3A*	0.036 [43]	0.039	ND	ND	0.048	0.070 [46]	0.031 [45]	0.065 [44]	ND	ND	ND	ND
*TPMT*3B*	0.000 [43]	ND	0.040 [49]	0.090 [49]	ND	0.000 [44]	0.000 [44]	0.000 [44]	ND	ND	ND	ND
*TPMT*3C*	0.000 [43]	ND	0.040 [49]	0.080 [49]	ND	0.001 [44]	0.000 [44]	0.000 [44]	ND	ND	ND	ND
*TPMT*4*	0.021	0.047	ND	0.065	0.091	NA	0.003 [45]	0.000 [44]	ND	ND	ND	ND
*UGT1A1*6*	0.000	0.000	0.000 [47]	0.000	0.000	ND	ND	ND	0.022 [41]	ND	ND	ND
*UGT1A1*27*	0.027	0.023	0.000 [47]	0.000	0.000	ND	ND	ND	ND	ND	ND	ND
*UGT1A1*28*	ND	ND	0.36	0.480 [49]	0.000	0.310 [48]	ND	ND	0.323 [41]	ND	ND	ND
*UGT1A1*36*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
*UGT1A1*37*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
*UGT1A1*60*	0.511	0.516	ND	0.606	0.486	ND	ND	ND	ND	ND	ND	ND
*UGT1A1*80*	0.340	0.367	ND	0.453	0.361	ND	ND	ND	ND	ND	ND	ND

[a] *Allele frequencies without citations were obtained from 1000 Genomes Project (Colombians, Mexican Americans, Peruvians, and Puerto Ricans) [34]. ND stands for not determined.*

Notwithstanding, only one study has reported the frequency of CYP2C19 ultrarapid metabolizers in a Hispanic group as measured by phenotyping tests [79]. This study reported that 4% of Mexicans mestizos from Jalisco were ultrarapid metabolizers. Although it is possible to find differences in the effectiveness of drugs metabolized by CYP2C19 across ethnic groups given that this allele is more common in Europeans and Africans (minor allele frequency [MAF] = 22.4% and 23.5%, respectively), one study did not find differences in antidepressant-treatment response between Hispanics and non-Hispanics [84].

Individuals with at least one nonfunctional allele *CYP2D6*3, CYP2D6*4, CYP2D6*5*, and *CYP2D6*6* are expected to have decreased metabolic CYP2D6 capacity. *CYP2D6* in Europeans slightly diverges from Hispanics in regard to the frequency of certain variants; *CYP2D6*3* and *CYP2D6*6* are present in Europeans with frequencies higher than 3% but are rarely observed in Hispanics [33]. For example, *CYP2D6*3* is present in approximately 4% of Europeans and in 0%–1.5% of Hispanics. *CYP2D6*6* has a frequency <1% in most Hispanics groups except in Cubans (1.2%) [31,34,57,58,63–67]. Interestingly, Colombians have the highest prevalence of CYP2D6 poor metabolizers (6.6%) when compared to Mexicans (3.2%), Panamanians (2.2%–4.4%), or Nicaraguans (3.6%). *CYP2D6*4* occurs more often in Colombians (19.4%) when compared to other Hispanics [34]. Beyond the implications of the metabolic capacity of cytochromes in regard of drug's response, studies have found association between CYP2D6 and personality traits. Interestingly, CYP2D6 metabolism capacity was found to correlate inversely with psychic anxiety and directly with degree of socialization in Cubans and Spaniards [85,86].

The Clinical Pharmacogenetics Implementation Consortium (CPIC) includes recommendations for 15 genes (including those that encode for CYP450s). Among these pharmacogenes encoding for drug-metabolizing enzymes, pegylated interferon-alpha3 (*IFNL3*) rs8099917 has a higher frequency in Hispanics (~36%) versus non-Hispanics (4.2%–16.8%; see Table 12.1) as well as the highest variation (19.2%–40.8%) across Hispanics (Colombians, Mexican Americans, Peruvians, and Puerto Ricans; see Table 12.2) [87]. Genetic variants in this gene are known to be the strongest predictors of peginterferon alpha-2a or peginterferon alpha-2b combined with ribavirin treatment response in patients with hepatitis C virus. Patients having a CC genotype at rs12979860 (c.151-152C>T) and TT at rs8099917 (g.1332A>C) are more likely to respond favorably to the treatment when compared to patients heterozygous or homozygous for the variant alleles [88,89].

Because the frequency of IFNL rs12979860 differs across ethnic groups, it is possible that this explains the poor treatment response in African Americans and Hispanics in whom the frequency of the variant allele is higher than in European descendants [88,90]. However, the rs8099917 variant allele occurs less frequently in Africans (<1%), while having the highest frequency in Hispanics (36%). This apparent contradiction is explained by the strong linkage disequilibrium of these variants (rs12979860 and rs8099917) in Hispanics ($R^2 = 0.57$ and $D' = 0.99$) and other non-Hispanic populations (Africans $R^2 = 0.02$ and $D' = 1.0$, Europeans $R^2 = 0.43$ and $D' = 0.97$, East Asians $R^2 = 0.92$ and $D' = 0.98$, and all populations $R^2 = 0.26$ and $D' = 0.98$); meaning that although their frequencies present wide variation, when the less frequent variant allele rs8099917 is present, it is in combination with the rs12979860 (frequent allele). Therefore, the combination of rs12979860 and rs8099917 may predispose to the poor outcome of the treatment observed in African Americans and Hispanics or the variant rs12979860 has a higher impact in these populations [90].

Other factors, such as treatment compliance and tolerance to the side effects of the treatment, should be considered. Interestingly, a previous study in African Americans and Hispanics observed that approximately 50% of the participants dropped out from the study due to intolerance to the side effects of the treatment or the lack of compliance to the treatment [90].

Regarding pharmacogenetics of chemotherapeutic agents, *TPMT* and nudix hydrolase 15 (*NUDT15*) genetic variants have been found to affect therapeutic response to thiopurines. Thiopurine methyltransferase (TPMT) catalyzes the conversion of mercaptopurine and thioguanine nucleotides into inactive metabolites. A decreased TPMT activity results in myelosuppression due to the accumulation of mercaptopurine and thioguanine nucleotides followed by incorporation into DNA and cytotoxicity [91]. Among variants commonly studied at the *TPMT* locus, *TPMT***3A* has the highest frequency across different Hispanic groups, having a prevalence comparable to Europeans [43–45]. *TPMT**2 was also commonly interrogated in several studies but exhibits frequencies <1% across Hispanics; it has a frequency of 0.7% in Argentinians, 0.3% in Colombians, 0.6% in Peruvians, and 0.5% in Puerto Ricans, and was not found in Chileans, Bolivians, Tibetans from Bolivia, or Mexican Americans [34,43–46,92]. Nonetheless, *TPMT**2 has been reported in Mexicans from Mexico (0.3%–1.0%) [92,93]. The less-frequent variant, *TPMT***4* (nonfunction allele), has been found in 0.3% of Argentinians and 0% of Bolivians, but most of the time is not interrogated in reports from Hispanic populations [34,45]. *TPMT***4* has a higher prevalence in Africans (6.7%) than in Europeans (2.9%) and East Asians (2.2%), explaining the high prevalence in Puerto Ricans (9.1%) compared to other Hispanics [34].

NUDT15 variants have been found associated with myelosuppression induced by thiopurines in children with acute lymphoblastic leukemia (ALL) resulting in treatment intolerance [94,95]. Moriyama and colleagues studied a cohort of 159 Guatemalan children with ALL finding that *NUDT15* variants were associated with thiopurine intolerance [81]. Interestingly, the strength of the association was lower in the group of Guatemalan children, who also presented the lowest dose of tolerated drug, suggesting that other variants may have an effect in mercaptopurine intolerance within this population. Although NUDT15 deficiency (having low- or intermediate-activity diplotypes) occurs more frequently in East Asians (22.6%), its prevalence is considerably high in Peruvians (21.2%) and Mexicans (12.5%) [95].

Other genetic polymorphisms are of relevance to the Hispanics given their influence in diseases. For example, *N*-acetyltransferase-2 (*NAT2*)**5* was found to have a significantly higher frequency in cases of nonsyndromic cleft lip with or without cleft palate in Argentinians [96]. Similarly, UDP glucuronosyltransferase 1 family polypeptide A1 (*UGT1A1*)**28* was found to explain 75% of the Gilbert Syndrome as determined by total bilirubin concentration in Chileans [48]. UGT1A1 (specifically *UGT1A1*28*) has been associated with higher probability of atazanavir/ritonavir discontinuation in Hispanics, but not in White or Black participants [97]. Atazanavir/ritonavir may produce physical discomfort due to its affinity to UGT1A1 and, therefore, inhibiting the glucuronidation activity of this enzyme leading to an increase in plasma bilirubin concentrations [97].

Variant allele frequencies in Hispanic populations by ethnic group are presented in Table 12.2. Allele frequencies were collected from several studies and 1000 Genomes Project Data that includes Colombians, Mexican Americans, Peruvians, and Puerto Ricans only.

Pharmacogenomic Variants in the Brazilian Population

F_{ST} statistics—a methodology that measures the genetic variation/differentiation between populations based on the allele frequencies—was used by the Brazilian Pharmacogenetic Network (REFARGEN) [99] to evaluate the differences found between the pharmacogenes in the different regions of the country. Although having been the extensive admixture for more than 500 years, many variants have significant differences in their frequencies among the self-reported color groups [100]. F_{ST} values demonstrated that there are significant differences across regions between white and black and between white and brown, but this difference is not observed between black and brown. The difference is higher in the South and decreases from Southeast, to North, and to Northeast. The *CYP3A5*3* allele showed the biggest difference in the South, whereas a moderate divergence is described in the Southeast and Northeast. Moderate divergence is also reported for *CYP3A5*6*, *ABCB1* c.2677G>T/A (rs2032582), *SLCO1B3* c.334T>G (rs4149117), and *SLCO1B3* c.699G>A (rs7311358) SNPs in the Southeast and Southern regions. Moreover, African population diverges from the black Brazilian population largely for the *CYP3A5*3* allele and moderately for variants in *ABCB1*, *SLCO1B3*, and *VKORC1*, among others. The divergence between European population and white Brazilians is lower and, considering 39 SNPs included in this analysis, only *CYP3A5*3* allele shows a moderate difference [101]. The allelic distribution of some important pharmacogenomics or pharmacogenetics (PGx) genes will be discussed in the next paragraphs.

CYP3A5 is a gene that encodes an enzyme responsible for the metabolism of more than 35% of the drugs prescribed worldwide. CPIC recommends changing the tacrolimus dose for patients who are intermediate and normal metabolizers for *CYP3A5* [102]. However, allelic variants in the gene show a great variability in their frequency across different populations. *CYP3A5*3* allele presents a frequency range from 0.14 to >0.95 for Sub-Saharan Africans and European populations, respectively [103]. In opposition to *CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7* have a relative high frequency in Africans but is very rare in the European population [103]. In the Brazilian population, the probability of having the *CYP3A5*3* allele increases with the increase of European ancestry and with the decrease of African ancestry. In contrast, the higher is the individual African ancestry and the lower is the European ancestry, the greater are the *CYP3A5*1*, *CYP3A5*6*, and *CYP3A5*7* allele frequencies [104]. Moreover, the self-identified color, as well as the geographic region, is related to the *CYP3A5* allele distribution in Brazil [104] (Table 12.3). Considering the phenotypes, the frequency for *CYP3A5* intermediate metabolizer (IM) plus normal metabolizer (NM) (the phenotypes for which the tacrolimus dose should be adjusted) is 35% for whites and 65% for blacks, respectively [100].

CYP2D6, another important gene for PGx, encodes an enzyme involved in the metabolism of approximately 25% of the most-used drugs. This gene is highly polymorphic, and the allele frequencies vary across the populations. Although variation exists related to the ancestral contributions in the different regions in Brazil, the *CYP2D6* allele distribution is considered homogeneous and no differences exist among regions or self-reported color [105]. However, considering the genomic ancestry (instead of self-reported color), a difference is observed between the European and African genomic contributions and the metabolizing phenotypes (the difference is not observed for Native American ancestry). IMs for *CYP2D6* have higher levels of African ancestry and lower levels of European ancestry in the Brazilian population. In addition, some alleles are reported only in one region,

TABLE 12.3 *CYP3A5* Alleles and Phenotypes Frequencies Among Brazilians According to Geographic Region and Self-Reported Color

		Brazil[a]			North Brazil		
Alleles	**Overall**	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
*3	0.73	0.79	0.64	0.50	0.79	0.72	0.56
*6	0.04	0.01	0.04	0.09	0.01	0.02	0.05
*7	0.03	0.01	0.03	0.07	0.00	0.00	0.03
PHENOTYPES							
NM	0.04	0.04	0.10	0.20	0.08	0.05	0.10
IM	0.34	0.30	0.46	0.45	0.25	0.41	0.53
PM	0.62	0.66	0.44	0.36	0.67	0.54	0.38

	Northeast Brazil			Southeast Brazil			South Brazil		
Alleles	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
*3	0.72	0.69	0.53	0.84	0.64	0.45	0.85	0.55	0.49
*6	0.03	0.05	0.10	0.02	0.06	0.12	0.00	0.04	0.11
*7	0.01	0.02	0.06	0.02	0.03	0.10	0.01	0.06	0.07
PHENOTYPES									
NM	0.04	0.03	0.13	0.00	0.08	0.12	0.00	0.15	0.13
IM	0.40	0.40	0.36	0.25	0.38	0.42	0.28	0.39	0.39
PM	0.56	0.57	0.51	0.75	0.54	0.46	0.72	0.46	0.47

IM, intermediate metabolizer; *NM*, normal metabolizer; *PM*, poor metabolizer.

[a] *Data for "Brazil" section of the table (except for the *1 allele) are from a studies compilation [100].*

Adapted from Rodrigues-Soares F, Kehdy FSG, Sampaio-Coelho J, et al. Genetic structure of pharmacogenetic biomarkers in Brazil inferred from a systematic review and population-based cohorts: a RIBEF/EPIGEN-Brazil initiative. The Pharmacogenomics Journal 2018; Suarez-Kurtz G, Vargens DD, Santoro AB, et al. Global pharmacogenomics: distribution of CYP3A5 polymorphisms and phenotypes in the Brazilian population. PloS One 2014;9(1):e83472.

for example *CYP2D6*34* and *CYP2D6*35X2* alleles in the Northeast, and *CYP2D6*17X2* allele in the South (see Table 12.4) [105].

CYP2C cluster harbors four important genes: *CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*. Together, CYP2C8, CYP2C9, and CYP2C19 enzymes—the most important for drug metabolism—play a key role in the hepatic biotransformation of ~25% of most-prescribed drugs. The variant frequency in these genes varies notably across different populations. *CYP2C9* is a classic example, because the most frequent/important alleles can present great differences according to the population ethnicity. For instance, in European populations *CYP2C9*2* and *CYP2C9*3* are the most relevant alleles, whereas *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, and *CYP2C9*11* present an important role in African populations considering they have a higher frequency [106]. It was shown that the odds of a Brazilian individual to have *CYP2C8*3*, *CYP2C9*2*, and *CYPC9*3* alleles increase continuously as the proportion of European ancestry increases, whereas the opposite is observed for *CYP2C8*2* allele, which the odds decrease in this situation [10]. However, no differences are found between *CYP2C19* alleles according to genetic ancestry or self-declared color (Table 12.5) [10].

VKORC1 encodes a protein related to the pathway of vitamin K reduction and is the target of the coumarin anticoagulants (e.g., warfarin, phenprocoumon, and acenocoumarol). Variants in the gene strongly affect the response to these drugs. The c.-1639G>A (rs9923231) variant is related to reduced expression levels and lower warfarin doses; however, the percentage of dose variation explained by this variant is considerably lower in blacks (4.2%) than in whites (22.5%) due to the lower frequency of c.-1639A allele in the black group (10.1%) compared to white group (37.8%) [108]. In the Brazilian population, the same pattern is observed, the frequency decreases from white, to brown, and to black individuals [109] (Table 12.6).

ATP-binding cassette subfamily B member 1 (*ABCB1*) and solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) genes encode transporter proteins that play an important role in the transport of several drugs. The frequency for variants in these genes varies largely across the different populations and, due to the great and heterogeneous admixture in Brazil, significant association between the *ABCB1* and *SLCO1B1* genes variants/haplotypes, color, and geographical region are observed. For *ABCB1* gene, the frequencies for the c.1236T (rs1128503), c.2677non-G (rs2032582), and c.3435T (rs1045642) alleles decrease from white to black in the north, southeast, and southern regions. Regarding the *SLCO1B1* gene, c.388G (rs2306283) allele increases the frequency from black to white in the north, northeast, and southern regions, whereas the c.521C (rs4149056) allele increases its frequency from white to black in the southeast and southern regions [110]. Tables 12.7 and 12.8 show the allele frequency according to Brazilian regions and self-declared color.

TPMT, the thiopurine methyltransferase, can have a wide activity variation and many variants might be related to that. *TPMT*2* (rs1800462), *TPMT*3A* (rs1800460 and rs1142345), and *TPMT*3C* (rs1142345) were described in Euro-derived (white), African-derived (black), and interethnic admixture (brown) individuals in a Brazilian population from the Southeast region; however, no significant differences in the allele frequencies were observed. Although there is no difference among the three Brazilian ethnic groups, the authors highlighted the fact that there is a wide difference in the *TPMT* alleles frequency of the overall sample, in the Euro-derived (white) group, and in individuals having interethnic admixture (brown) when compared with frequencies in White and African Americans from other populations [111] (Table 12.9).

NAT2 is a phase II enzyme responsible for the acetylation of some drugs, such as the

TABLE 12.4 Allele Frequency for *CYP2D6* Gene in the Brazilian Population According to Geographic Regions and CYP2D6 Phenotype Frequencies According to the Self-Reported Color Groups and Geographic Regions

***CYP2D6* Alleles**	**Brazil**	**North**	**Northeast**	**Southeast**	**South**
*2	0.22	0.24	0.24	0.19	0.19
*3	0.007	0.006	0.008	0.006	0.008
*4	0.09	0.11	0.10	0.08	0.09
*5	0.05	0.03	0.05	0.05	0.05
*9	0.01	0.01	0.009	0.01	0.01
*10	0.02	0.01	0.02	0.03	0.02
*17	0.06	0.05	0.04	0.07	0.05
*29	0.03	0.02	0.02	0.04	0.05
*34	0.005	–	0.002	–	–
*35	0.03	0.02	0.03	0.03	0.02
*39	0.008	0.01	0.004	–	0.02
*41	0.06	0.04	0.08	0.05	0.05
*1X2	0.006	0.008	0.006	0.006	0.004
*1X3	0.0005	0.002	–	–	–
*2X2	0.006	0.006	0.004	0.008	0.004
*2X5	0.0005	–	0.002	–	–
*4X2	0.002	0.004	–	0.004	–
*17X2	0.0005	–	–	–	0.002
*35X2	0.0005	–	0.002	–	–
>2 copies	0.09	0.10	0.10	0.08	0.06

	North Brazil			**Northeast Brazil**		
Phenotype	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
PM	0.01	0.03	0.05	0.03	0.03	–
IM	0.04	–	0.10	0.03	0.05	0.08
NM	0.85	0.89	0.80	0.86	0.86	0.84
UM	0.07	0.03	0.05	0.03	0.02	0.06

	Southeast Brazil			**South Brazil**		
Phenotype	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
PM	0.01	0.05	0.01	0.05	0.01	0.01
IM	0.07	0.07	0.13	0.06	0.13	0.09
NM	0.05	0.05	0.01	0.87	0.83	0.79
UM	0.05	0.02	0.07	0.02	–	0.06

TABLE 12.5 Allele and Phenotype Frequency for *CYP2C8*, *CYP2C9*, and *CYP2C19* Genes in the Brazilian Population According to the Self-Reported Color Groups and Geographic Regions

	Brazil				North Brazil		
Variants	**White**	**Brown**	**Black**	**Native American**	**White**	**Brown**	**Black**
CYP2C8							
*2	0.03	0.06	0.11	0.01	0.064	0.029	0.075
*3	0.13	0.08	006	0	0.128	0.126	0.034
*4	0.04	0.04	0.02	0	0.038	0.017	0.017
CYP2C9							
*2	0.12	0.09	0.07	0.03	0.136	0.149	0.045
*3	0.05	0.05	0.03	0.01	0.052	0.017	0.023
*5	0	0	0.01	0	0	0	0
*11	0.01	0	0.01	0	0	0	0.017
CYP2C19							
*2	0.15	0.14	0.16	0.11	0.090	0.098	0.176
*3	0	0	0	0	0	0	0
*17	0.18	0.19	0.20	0.11	0.115	0.161	0.170

	Northeast Brazil			Southeast Brazil			South Brazil		
Variants	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
CYP2C8									
*2	0.040	0.057	0.099	0.017	0.069	0.118	0.028	0.097	0.098
*3	0.102	0.052	0.081	0.136	0.086	0.047	0.119	0.045	0.043
*4	0.028	0.046	0.012	0.051	0.040	0.024	0.028	0.034	0.037
CYP2C9									
*2	0.114	0.070	0.092	0.153	0.075	0.052	0.119	0.057	0.049
*3	0.023	0.058	0.046	0.045	0.080	0.012	0.074	0.034	0.018
*5	0.006	0	0.006	0.006	0.011	0.012	0	0.011	0.012
*11	0	0.006	0.011	0.017	0.006	0.006	0.006	0.011	0.006
CYP2C19									
*2	0.125	0.131	0.116	0.125	0.103	0.167	0.136	0.205	0.105
*3	0	0	0	0	0	0.006	0	0	0
*17	0.199	0.193	0.180	0.142	0.138	0.167	0.216	0.188	0.204

Adapted from Suarez-Kurtz G, Genro JP, de Moraes MO, et al. Global pharmacogenomics: impact of population diversity on the distribution of polymorphisms in the CYP2C cluster among Brazilians. The Pharmacogenomics Journal 2010; Rodrigues-Soares F, Kehdy FSG, Sampaio-Coelho J, et al. Genetic structure of pharmacogenetic biomarkers in Brazil inferred from a systematic review and population-based cohorts: a RIBEF/EPIGEN-Brazil initiative. The Pharmacogenomics Journal 2018.

TABLE 12.6 MAF for *VKORC1* c.-1639G>A Variant in the Brazilian Population Considering the Different Self-Reported Color Groups and in European, African, and Latino Populations From 1KG

Brazil [100]	White	Brown	Black	Native American
c.-1639G>A	0.46	0.38	0.24	0.39
1KG	**European**	**African**	**Latino**	
c.-1639G>A	0.39	0.05	0.41	

TABLE 12.7 MAF for *ABCB1* Variants in the Brazilian Population Considering the Different Self-Reported Colors and Geographic Regions

	Brazil				North Brazil		
Variants	**White**	**Brown**	**Black**	**Native American**	**White**	**Brown**	**Black**
c.1236C>T	0.43	0.35	0.31	–	0.38	0.40	0.38
c.2677G>T/A	0.42	0.32	0.23	–	0.44	0.39	0.34
c.3435C>T	0.46	0.40	0.32	0.51	0.49	0.47	0.38

	Northeast Brazil			Southeast Brazil			South Brazil		
Variants	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
c.1236C>T	0.38	0.41	0.32	0.40	0.30	0.31	0.45	0.30	0.21
c.2677G>T/A	0.38	0.41	0.27	0.41	0.27	0.18	0.45	0.20	0.14
c.3435C>T	0.42	0.48	0.35	0.45	0.33	0.29	0.50	0.29	0.27

Adapted from Sortica VA, Ojopi EB, Genro JP, et al. Influence of genomic ancestry on the distribution of SLCO1B1, SLCO1B3 and ABCB1 gene polymorphisms among Brazilians. Basic and Clinical Pharmacology and Toxicology 2012;110(5):460–8.

TABLE 12.8 MAF for *SLCO1B1* Variants in the Brazilian Population Considering the Different Self-Reported Color and Geographic Regions

	Brazil				North Brazil		
Variants	**White**	**Brown**	**Black**	**Native American**	**White**	**Brown**	**Black**
c.388A>G	0.50	0.59	0.65	–	0.47	0.49	0.61
c.463C>A	0.11	0.11	0.08	–	0.10	0.08	0.06
c.521T>C	0.13	0.14	0.10	0.28	0.11	0.17	0.11

	Northeast Brazil			Southeast Brazil			South Brazil		
Variants	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**	**White**	**Brown**	**Black**
c.388A>G	0.53	0.55	0.57	0.49	0.69	0.67	0.49	0.65	0.76
c.463C>A	0.10	0.14	0.12	0.12	0.12	0.09	0.12	0.10	0.06
c.521T>C	0.12	0.13	0.11	0.15	0.15	0.07	0.12	0.10	0.10

Adapted from Sortica VA, Ojopi EB, Genro JP, et al. Influence of genomic ancestry on the distribution of SLCO1B1, SLCO1B3 and ABCB1 gene polymorphisms among Brazilians. Basic and Clinical Pharmacology and Toxicology 2012;110(5):460–8.

TABLE 12.9 MAF for *TPMT* Variants in the Brazilian Population Considering the Different Self-Reported Color Compared With Frequencies From ExAC and 1KG

	Brazilian Population [111]			ExAC			1KG		
	Overall	White	Brown	Latino	European	African	Latino	European	African
TPMT*2	0.008	0.006	0.008	0.002	0.002	0.0005	0.006	0.006	0.001
TPMT*3A	0.016	0.018	0.020	–	–	–	–	–	–
TPMT*3C	0.021	0.018	0.025	0.048	0.040	0.054	0.058	0.029	0.067

antituberculosis medications (e.g., isoniazid and ethambutol). The slow, intermediate, or fast acetylator phenotypes are related with the haplotypes for *NAT2*. The frequency for the variants varies worldwide; high frequencies of slow acetylators are found in Middle Eastern and European populations, whereas low frequencies of slow acetylators are observed in East Asians [112]. In the Brazilian population, *NAT2*6* allele (decreased function) has lower frequencies in Native Americans than in black/brown and white individuals. In relation to *NAT2*14*, another decreased function allele, black Brazilians have higher frequencies compared to White, Brown, and Native Americans [100,113,114].

In addition, some Native American people in Brazil live in very closed and small groups dispersed across the country. These peoples can present very different allele frequencies due to the effect of genetic drift [115]. No poor metabolizers for *CYP2C9* enzyme were reported in Native Americans due to the very low frequency of *CYP2C9*2* and *CYP2C9*3* alleles [100]. The PGx alleles that present a significantly higher frequency compared to the other color categories are the following: *ABCB1* c.3435C>T (0.51), *SLCO1B1* c.521T>C (0.28), and *SLCO1B1* g.89595T>C. In contrast, the following alleles have significant lower frequency when compared with other color categories: *CYP2C9*2* (0.03), *CYP2C19*17* (0.11), *NAT2*13* (0.21), deletion of *GSTM1* (0.25), deletion of *GSTT1* (19%), and *CCR5 D32* (0.001) [100].

PHARMACOGENOMIC RESEARCH IN THE LATIN AMERICAS

The first study of pharmacogenetics conducted in Central America and the Caribbean was performed in 1975, with the description of *G6PD* polymorphisms in healthy volunteers from Cuba [116]. A systematic review published in 2016 on pharmacogenetics research activity in Central America and the Caribbean concluded that oncology was the most frequently studied medical therapeutic area, followed by cardiovascular and neuropsychiatry [117,118]. Notably, *CYP2D6* (and *NAT2+CYP2C19* to a lesser extent) and *HLAA/B* (and *MTHFR* to a lesser extent) are the most investigated biomarkers in healthy volunteers and patients, respectively. The review identified 132 research articles of pharmacogenomics studies conducted in 35,079 subjects (i.e., 11,129 healthy volunteers and 23,950 patients) from these populations. This number of subjects tested only represents 0.039% of people living in this ethnogeographic region. Overall, only 47 out of 104 relevant pharmacogenetic biomarkers (i.e., selected from those recommended by the US Food and Drug Administration [FDA]

and Pharmacogenomics Knowledgebase [PharmGKB] CPIC gene/drug pairs) have been studied in the population of Central America and the Caribbean. According to the authors, Costa Rica and Puerto Rico were the leaders in pharmacogenomics research among countries or territories in this region (i.e., 30 and 29 published studies with 11,596 and 8796 recruited individuals, respectively). Strikingly, only 10 out of 34 countries had conducted pharmacogenetics research in the region, evidencing that such studies are still scarce within these populations [118]. Because not all scientific research studies conducted in Latin America are reported in scientific journals due to a lack of tradition or because of linguistic barriers, a potential publication bias need to be taken into consideration. However, Latinos are often underrepresented in pharmacogenetics studies conducted in the United States, a situation that tends to exacerbate existing disparities while adopting this promising paradigm.

Accordingly, obviously a gap exists in the implementation of actionable pharmacogenetics-guided recommendations that could potentially benefit this population in clinical practice. In recent years, some strategies to promote effective collaborations among countries in this region and foster research efforts and interregional initiatives, like the Iberoamerican Network of Pharmacogenetics (RIBEF, www.ribef.com), has certainly expanded the pharmacogenetics knowledge in Latin American populations and its clinical implication by performing studies in various countries of this region.

Overall, both interethnic and intraethnic variability in frequencies of major genetic polymorphisms for metabolizing enzymes, drug transporters, receptors, and major histocompatibility loci have been found, which highlights the need to determine ancestry proportions of participants in pharmacogenomic association studies (e.g., GWAS) conducted on Latinos [7].

Pharmacogenomic Research Studies in Brazil

Many drugs have been studied in the Brazilian population. Here, you will find a brief review of some of the results found in relation to the most-studied drugs, including additional research studies summarized in Table 12.10.

Methylphenidate is the most prescribed drug used to treat Attention-deficit/hyperactivity disorder (ADHD). The disorder has different characteristics in adults and children, and the treatment effect might be different between both groups. In addition, the response to the drug can be evaluated based in more than one endophenotype/symptom. Methylphenidate PGx has been studied in both adults and children in the Southern Brazilian population. Youth boys from the South of Brazil that are homozygote for the 10-repeat allele in the *SLC6A3/DAT1* gene showed lower scores of improvement in the treatment with methylphenidate [137]; however, these results were not replicated in a validation cohort from the same region [138]. *ADRA2A* also showed an influence in methylphenidate effect in the Brazilians; children and adolescents from the Southern Brazil carriers of c.-1291G (rs1800544) allele had an improvement of inattentive symptoms during the first 3 months of treatment [139,140]; however, this effect was not observed in adults of European descent from the same region of the country [141]. Carriers of the catechol-O-methyltransferase (*COMT*) 158Met (rs4680) allele had an improvement of the oppositional defiant symptoms in children [142] but not in adults [143]. monoamine oxidase A (*MAOA*) promoter variable number of tandem repeats (VNTR) high-activity genotype was associated with improvement of the same endophenotype in children [144]. Recently, the synaptotagmin-1 (*SYT1*) gene was associated with methylphenidate response in adults, in which carriers of the rs2251214 A allele presented greater mean

TABLE 12.10 Summary of Some PGx Results Based on the Brazilian Population

Drug	Genes Associated	Variants	Effect	References
Rocuronium	*SLCO1A2*	rs3834939	PK	[119]
Bevacizumab/Ranibizumab	*CFH*	rs1061170	Efficacy	[120,121]
Glucocorticoid	*CYP3A7*	*CYP3A7*1C*	Dose	[122]
Varenicline/varenicline + bupropion/bupropion + or nicotine replacement	*CHRNA5*	rs16969968, rs2036527	Efficacy	[123]
Human recombinant growth hormone	*GHR*	Exon 3 deletion	Efficacy	[124,125]
	IGF1	Promoter (CA)n microsatellite	Efficacy	
	IGFBP3	rs2854744	Efficacy	
Clozapine	*DRD1*	rs4532	Efficacy	[126]
	SLC6A4	rs4795541 (HTTLPR), rs25531	Efficacy	[127]
	CYP1A2	*CYP1A2*1F*	Adverse event	[128]
Haloperidol/Chlorpromazine	*DRD3*	rs620, rs963468, rs763140	Efficacy	[129]
	CYP3A5	*CYP3A5*3*	Efficacy	
Risperidone	*LEP*	rs7799039	Adverse event	[130]
	HTR2C	rs3813929, rs6318	Adverse event	
	CYP2D6	rs72552269	Adverse event	
	LEPR	rs1137101	Adverse event	
	MC4R	rs17782313	Adverse event	
	DRD2	rs1799978, rsrs6277	Adverse event	
Levodopa	*SLC6A3*	rs2836371	Dose	[131]
	SV2C	rs30196	Dose	
	DRD2/ANKK1	rs1799732, rs2283265, rs1076560, rs6277, rs1800497, rs2734849	Adverse event	[132]
	SLC6A3	rs28363170	Adverse event	[133]
	SLC6A3	rs28363170	Dose	
	HOMER1	rs4704559	Adverse event	[134]
	ADORA2A	rs2298383, rs3761422	Adverse event	[135]
	DRD2	rs1799732	Adverse event	[136]
	DRD3	rs6280	Adverse event	

percentage reduction of inattention and oppositional defiant disorder symptoms [145]. In relation to the time-to-therapeutic effect, the faster response to the drug was observed in children carrying the CGC haplotype (rs6813183, rs1355368, rs734644) in the latrophilin 3 (*LPHN3*) gene [146].

Statins, the drug class most prescribed for lowering lipids, have also been studied in the Brazilian population. The results showed that, for European descent from Southern Brazil, *SLCO1B1*, SREBP Cleavage Activating Protein (*SCAP*), Cholesteryl Ester Transfer Protein (*CETP*), *ABCB1*, *N*-Methyl-D-Aspartate Receptor 1 (*NR1*)*3*, and paraoxonase-1 (*PON1*) genes are involved on simvastatin efficacy or safety. Carriers of the c.388G allele in the *SLCO1B1* gene presented a greater reduction in total and Low-Density Lipoprotein (LDL)-cholesterol [147]. Patients who are carriers of the *SCAP* c.2386G (rs12487736) allele had greater total cholesterol decrease [148]. Individual homozygotes for the B2 (rs708272) allele in *CETP* gene had greater High-Density Lipoprotein (HDL)-cholesterol increase [149]. In addition, carriers of the *ABCB1* c.1236T (rs1128503) allele had a greater reduction in total cholesterol and low-density lipoprotein cholesterol and patients with the *ABCB1* 1236T/2677non-G/3435T (rs1128503/rs2032582/rs1045642) haplotype were less frequent in the adverse drug reaction group [150]. In addition, patient homozygotes for *NR1*|*3* rs2307424 T allele had higher risk to develop adverse drug reaction to simvastatin and atorvastatin [151]. In relation to *PON1* gene, patients with the genotype p.192RR (rs662) and/or p.55LL (rs854560) attained less often the HDL-cholesterol goal during treatment with simvastatin or atorvastatin [152]. Moreover, results for individuals from the Southeast of the country showed that *SLCO1B1*, Estrogen Receptor 1 (*ESR1*), and myosin regulatory light-chain interacting protein (*MYLIP*) genes may influence atorvastatin response. *SLCO1B1* c.388GG genotype patients had higher reduction in the LDL-cholesterol levels [153], but this variant was not associated with myalgia [154]. LDL-cholesterol levels were lower, after 1 yr of treatment, for patients who have the *MYLIP* rs9370867 AA or GA genotypes [155]. Women who are homozygotes for rs2234693 C allele had greater HDL-cholesterol increase and carriers of the rs3798577 T allele had greater total cholesterol and triglyceride reduction, but the same effect was not observed in men [156].

Warfarin is an anticoagulant widely used and a huge variation in the individual dose is observed. Recently, the warfarin CPIC guideline was updated and ancestry (African vs. non-African, and European vs. non-European) was included as a factor to guide the dose [157]. For patients of African ancestry, the guideline mentions that *CYP2C9*5*, *6, *8, and *11 are important for warfarin dosing and, if these genotypes are not available, the healthcare provider should disregard the genetics-guided dosing [157]. In Brazil, warfarin PGx was studied in the South and Southeast populations. As expected, due to differences in *CYP2C9* and *VKORC1* allele frequencies, the average dose differed between white (28.9 mg/week), brown (32.9 mg/week), and black Brazilians (35.3 mg/week) [26]. Algorithms were developed for prediction of warfarin dose requirement in both Southeast (Rio de Janeiro) and South (Porto Alegre) regions. Southeast region sample comprised white, brown, and black Brazilians, whereas the South cohort comprised only European descent patients. Contrasting the poorer performance of warfarin algorithms in African Americans versus White Americans [158], the model based on the Southeast population performed equally well in self-reported both white and black patients, and it explained 51% of the dose variation [26]. The great admixture observed in the Brazilians, compared to Americans, can explain

this. Another algorithm, developed in the South region sample, explained 58% of variability observed in warfarin dosing [25]. However, the algorithms derived from Rio de Janeiro and Porto Alegre performed poorer when applied to the other cohort. R^2 values decreased from 0.58 to 0.44 and from 0.51 to 0.41 for Porto Alegre and Rio de Janeiro algorithms, respectively [25,29]. This difference may have occurred due to differences in admixture level observed between the South and Southeast Brazilian populations, whereas the South region individuals show a lower admixture level [3].

The PGx for antiretroviral human immunodeficiency virus (HIV) therapy was also studied in the Southern Brazilian population. The *ESR1* gene was associated with lipodystrophy in patients on High Active Antiretroviral Therapy (HAAR). The *ESR1* rs2813544 G allele was associated with Body Mass Index (BMI), total subcutaneous fat, and subcutaneous fat of limbs. In addition, patients homozygotes for rs3020450 A allele had an increased risk of developing lipoatrophy [159]. In addition, Apolipoprotein A5 (*APOA5*) c.-1131C allele carriers (rs662799) have higher risk of presenting higher triglycerides levels and lower HDL-cholesterol levels. Patient homozygotes for Apolipoprotein B (*APOB*) Del allele (rs17240441) had higher LDL-cholesterol levels, as well as c.7673TT (rs693) homozygotes [160]. Individual homozygotes for adiponectin receptor 2 (*ADIPOR2*) rs11061925 T allele had higher triglyceride and total cholesterol levels. Increased triglycerides levels were also observed in rs929434 AA patients [161]. Hyperbilirubinemia in patients exposed to Highly Active Antiretroviral Therapy (HAART) was higher in individuals carrying the *UGT1A1*28* allele for both African and European descent Brazilians from the South [162]. The estimated glomerular filtration rate (eGFR) is lower in carriers of *ABCC2* c.-24T allele (rs717620) [163]. In relation to efficacy, the Northeast Brazilian population was studied and *ABCB1* c.3435T (rs1045642) and *ABCC1* g.198217C (rs212091) carriers have higher risk of virologic failure [164].

Tacrolimus has also been studied in Brazilians from the Southeast region. The number of *CYP3A5*-defective alleles (*CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7*) is associated with the minimum concentration dose (C_0/dose) of tacrolimus in a gene–dose manner. The C_0/dose is higher as the number of defective alleles increase [165]. *ABCB1* TTT/TTT diplotype (c.2677G>A/T, c.1236C>T, c.3435C>T) is also associated with a higher C_0/dose when stratified by *CYP3A5*3* genotype [166,167]. Moreover, *ABCC2* c.3972T allele carriers as well as *CYP2C8*3* carriers had higher tacrolimus concentration:dose ratio (C:D) values. *CYP2C8*3* was also associated with increased estimated glomerular filtration rate in *CYP3A5*-nonexpressing patients (*CYP3A5*3C/*3C*). In relation to adverse drug reaction, *CYP2J2* c.-76T allele was associated with higher risk for treatment-induced nausea and/or vomiting [168].

Antituberculosis drugs often cause liver injury and depending on the genotype for *NAT2* gene the patients can present a higher risk of developing this drug adverse reaction. In a Brazilian sample from the Southern Brazil, rapid and intermediate acetylators had lower risk of developing drug-induced hepatotoxicity (DIH) [169]. Similar results were found in a sample from the Southeast, where 60% of the individuals were non-White [170]. Another study realized in a sample from the West-Central region where 35% of the individuals were Native Americans, showed that indigenous subjects had almost four times higher risk of hepatotoxicity than nonindigenous. Furthermore, NAT2 slow acetylation profile was associated with hepatotoxicity in indigenous, but not in nonindigenous patients [171]. In addition, the level of toxicity was associated with Glutathione S-Transferase M1 (*GSTM1*) gene. Individuals who had the *GSTM1* nonnull genotype had higher risk to develop higher grades of toxicity [172].

PHARMACOGENOMIC IMPLEMENTATION IN THE LATIN AMERICAS

At present, there are few thorough and broad PGx-guided Precision Medicine programs among the countries of Latin America. PGx-guided therapy faces many barriers to full integration into clinical practice and acceptance by stakeholders at these countries. The lack of education in pharmacogenetics and, consequently, a lack of confidence has been suggested as the principal barrier to this end. Another barrier is the lack of clinical-decision support tools for PGx guidelines-based actionable recommendations in the context of drug prescribing to optimize therapy for clinicians unfamiliar with genotyping. Indeed, clinicians often recognize that genotyping is potentially useful, but test-result interpretation is complicated, hindering wider adoption.

A survey conducted by the University of Puerto Rico (UPR) School of Pharmacy about perceptions and attitudes of healthcare providers toward pharmacogenetics showed that the lack of knowledge was one of the principal barriers to the adoption of pharmacogenetic testing by healthcare professionals in the Commonwealth of Puerto Rico [173]. As a matter of fact, pharmacogenetics knowledge has been slow and is largely reserved to specialized centers and large academic institutions in Puerto Rico, where a clinical protocol to implement for the first time ever a treatment algorithm based on platelet reactivity and genetic test results to guide DAPT in Caribbean Hispanics is under way. This includes the use of a clinical decision-support tool for prescribing advice to clinicians that will be implemented in "real-world" scenario through a web-based application at local clinical services of the Commonwealth of Puerto Rico. This tool is expected to allow the integration of relevant patient's genomic data into clinical decisions. To our knowledge, no such resource currently exists for translating genomic information of clopidogrel in admixed Caribbean Hispanics into medical decisions of clinical benefit. It will enable clinicians to receive better guidance on treating patients in real time. Although genotyping to identify high-risk patients is now available to guide drug prescriptions, clinicians in Puerto Rico do not have firsthand experience with individualized pharmacotherapy based on the patient's genotype status. Likewise, it seems to be the situation in others countries of the region.

Accordingly, an addressable barrier to adopt a PGx-guided antiplatelet care in Caribbean Hispanics is the lack of clear-cut, easy-to-understand translation of test results into specific, actionable decisions for prescribing clopidogrel versus other alternative drugs. Clinicians in Puerto Rico refrain from ordering a genetic test for clopidogrel, because of the lack of evidence-based resources and medical applications to guide them on proper implementation in their patients. This is particularly relevant in the context of percutaneous coronary interventions (PCI) at cardiac catheterization labs, in which cardiologists have to make critical decisions on what is the best course of action for a given patient within a very narrow time frame. The developed protocol will facilitate the ease of use of individual PGx information in clinical settings to guide medical decision-making and streamline widespread adoption of the PGx paradigm in the Hispanic population of Puerto Rico. Additionally, there is a multicenter collaborative effort between researchers at the UPR, University of Florida (UF), Icahn School of Medicine in Mount Sinai, NY, and University of Arizona to perform a warfarin pharmacogenomic study that seek to derive and implement the first warfarin dose prediction model (all-Latino pharmacogenetics-guided algorithm) that is exclusively for Hispanics of different backgrounds.

Another effort of implementation came from a pilot study conducted as part of an intramural practice at the UPR School of

Pharmacy to assess clinical utility of adding pharmacogenetic testing information into pharmacist-driven Comprehensive Medication Management (CMM) service. Pharmacists performing CMM identified 22 additional medication problems after the PGx-guided actionable recommendations were incorporated into the medication-related action plan of the CMM service provided to 24 participants (psychiatric patients). Although this certainly represents a small sample from which to draw final conclusions, participating pharmacists agreed on the fact that PGx helps identifying medication-related problems. They also perceived as highly desirable to add PGx reports into routine CMM service based on the expected clinical utility of this information for their decision-making. The RIBEF initiative (www.ribef.com) represents a promising step toward the inclusion of Latin American populations among those to benefit from the implementation of pharmacogenetics in clinical practice. Among current RIBEF activities, the CEIBA.FP Consortium aims to study interindividual variability of relevant genotypes and phenotypes in Latinos. To this end, populations from Mexico, Cuba, Nicaragua, Costa Rica, Ecuador, Colombia, Brazil, Peru, Chile, Uruguay, and Argentina are currently being studied [174].

Similar to previous reports in literature [175,176], hesitancy for incorporation of pharmacogenomics information into clinical management in Latin American countries has been mostly attributed to: limited evidences from prospective clinical trials that demonstrates improved medical outcomes, lack of clear-cut instructions to guide decision-making, lack of genetic results at the time of drug prescribing, inefficient use of (electronic) health records for making results available to all caregivers, and simple lack of awareness that pharmacogenomics data exist. Furthermore, some barriers to advance clinical pharmacogenomics implementation in these countries are also related to infrastructure, affordability, and cost/insurance issues as well as certain concerns about the cultural, religious, and ethical issues surrounding use of genetic information in medicine.

The absence of pharmacogenetics guidelines for Latino populations is exacerbated by the scarcity of pharmacogenetics studies in these populations. Several reasons have been previously identified for this paucity. First, poor, or lack of, financial support is available for pharmacogenetics research; second, the absence of strategies that attract funding from national and international entities; third, limited accessibility and availability of technologies at the use of trained personal; and finally, pharmacogenetics may not be an urgent or relevant research topic given that funding may be directed mostly to investigate diseases or problems that are priorities to each country (i.e., infectious and tropical diseases) [118]. Therefore, because pharmacogenetics research has been starting in the Latin American countries, this implies that implementation of pharmacogenetics guidelines with clinical-decision support relevant to these populations is still nonexistent [58].

The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have information regarding the use of genetic biomarkers to guide pharmacotherapy. However, regulatory agencies in other countries of Latin America may not include genetic information to be considered for recommendations or safety warnings of pharmacotherapy until the date. For example, the Federal Commission for Protection against Health Risks (COFEPRIS) of Mexico does not have information regarding whether is recommended to have genetic testing for drug's use or safety [177]. Puerto Rico, as a Commonwealth of the United States, applies the same regulatory statements that are used on the mainland.

The implementation of PGx in medical practice within Latin American populations requires the elucidation and comprehensive understanding of the clinical impact of relevant ethnospecific genetic variations on drug responses and medical outcomes (phenotypes). To address

the concern arisen by the paucity of data from Hispanic populations, these groups must be adequately represented in future clinical pharmacogenetic studies.

Pharmacogenomic Implementation in Brazil

In relation to Brazil, although a considerable number of PGx studies have been undertaken, implementation initiatives are still lacking. The absence of an educational program that makes the link between the scientific knowledge that already exists to the clinical practitioners is the main barrier to the advance of PGx in the Latin America. Healthcare providers still prefer to implement therapy in the "one-size-fits-all" model and much effort is needed to bring the professionals out of their comfort zone and start changing this scenario.

The great admixture observed in these countries is also an obstacle that hinders the genetics-guided therapy implementation and the establishment of public health policies related to pharmacogenomics. The population stratification is a challenge for the regulatory agencies to develop accurate guidelines to benefit the entire population. For instance, the Brazilian regulatory agency ("Agência Nacional de Vigilância Sanitária"—National Health Surveillance Agency [ANVISA]) has set pharmacogenetic recommendations for only 16 of the 121 FDA and/or EMA biomarkers, which are the following: *ABCG2* (rosuvastatin), *BCR-ABL* (imatinib), *BRAF* (vemurafenib, cobimetinib, trametinib), *CFTR* (ivacaftor), *EGFR* (afatinib, atezolizumab, cetuximab, erlotinib, gefitinib, nivolumab, orsimertinib, panitumumab), *HER-2* (trastuzumab, lapatinib, pertuzumab, palbociclib) *F5* (eltrombopag, ethinylestradiol), *G6PD* (dipyrone), *KRAS/NRAS* (erbitux, cetuximab, panitumumab), *POLG* (valproic acid), *SLCO1B1* (rosuvastatin), *CYP2D6* (imipramine), *CYP2C19* (citalopram, clopidogrel), *CYP2C9* (piroxicam, warfarin), *UGT1A1* (irinotecan), and *TPMT* (azathioprine, mercaptopurine, thioguanine). Moreover, some of the genetic tests (especially for drugs related to cancer treatment) are offered for free by the Brazilian public health system ("Sistema Único de Saúde"—Public Health System [SUS]). This is the first step achieved for the Brazilian population; however, the regulation of a larger number of genes/drugs and the development of more precise guidelines with specific recommendations are still a necessity, as well as the inclusion of the minority people in these guidelines.

CONCLUSION AND FUTURE DIRECTIONS

Implementation efforts focusing on proper adoption of pharmacogenetics-guided interventions by healthcare providers at clinical settings, or in the health systems of Latin American countries, are still at a very premature stage. Indeed, translating pharmacogenomic discoveries into better medical care in Hispanics/Latinos remains a challenge for a number of reasons, including a significant lack of relevant PGx information from well-designed studies conducted in individual representing the large genetic diversity within this ethnic group and, therefore, subsequent failures to replicate findings from other non-Hispanic populations. In addition, to such limited scientific evidence that implementing PGx-guided drug therapy in Hispanics improves patient outcomes, there are serious concerns about proper education of healthcare providers, cultural barriers, ethical and socioeconomic issues, and the lack of clinical decision support tools among others. Accordingly, such obstacles have hindered proper implementation for Hispanics, but also reveal critical knowledge gaps and possible studies needed to help addressing them.

Hispanics or Latinos show unique attributes in terms of their genetic backgrounds and population stratifications, with a rich repertoire of

combinatorial genotypes, serial founder effects, and distinctive haplotype blocks. This is due to admixture-driven linkage disequilibrium to varying extent, and a highly complex mixture pattern from at least three different parental populations that shaped a very heterogeneous mosaic of genomic structures at locus level. These complexities along with the ethnospecific nature of certain functional alleles on relevant pharmacogenes need to be factored into future developments of reliable pharmacogenetics-based implementation guidelines that can also be adopted in the underrepresented Latino population, particularly for patients at the extremes of drug response (outcomes) curves. Like Suarez-Kurtz and Parra have argued, it will probably imply "*…a shift of the current paradigm of PGx-informed prescription based on genotyping a few common variants in selected genes toward comprehensive sequencing approaches*" that also takes into consideration the abundance of rare, ethnospecific variants in this heterogeneous population [9]. At the end, future pharmacogenomics implementation must rely on human genetic diversity.

Due to the great level of admixture, PGx results from other populations should not be used for PGx implementation in Latin America. Moreover, studies for specific geographic regions are not sufficient because the interindividual genetic variability is huge among subjects from the same region. The genetic ancestry analysis is more accurate than self-reported color [16], because the self-reported color cannot capture all the PGx variation observed. However, color categories may also be informative and are four times more accurate for the variance presented in allele frequencies than the geographic regions [100]. Although the black/brown and Native American individuals represent a great proportion of Latin American population, they still are underrepresented in the PGx studies. The fact that these ethnic groups have poorer access to healthcare centers is also one of the reasons for the underrepresentativeness, because the researchers have limited access to these patients. However, an effort should be prompted to have a more inclusive and accurate implementation of PGx in these ethnically diverse continents. Collaborative partnerships between researchers and indigenous communities, for example, may help to reduce this important discrepancy.

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CHAPTER

13

Reactive, Point-of-Care, Preemptive, and Direct-to-Consumer Pharmacogenomics Testing

Aniwaa Owusu Obeng[1], *Matthias Samwald*[2], *Stuart A. Scott*[3,4]

[1]The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, Department of Pharmacy, The Mount Sinai Hospital, New York, NY, United States; [2]Center for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Vienna, Austria; [3]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [4]Sema4, Stamford, CT, United States

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00013-9

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GENETIC TESTING FOR PHARMACOGENOMICS

Pharmacogenetic discoveries are often followed by interest in clinical implementation. However, identification of genetic markers associated with drug response does not always equate to clinically useful predictors of efficacy or adverse outcomes, and independent replication of genotype–phenotype association is essential prior to pursuing clinical implementation [1]. Several academic health centers have invested in clinical pharmacogenetics and view its implementation as a first step toward incorporating genomics into routine and personalized healthcare; however, a number of challenges and barriers exist for widespread adoption.

Validity and Utility

The criteria for evaluating genetic tests are summarized by the four components of the ACCE analytic framework: Analytical validity, Clinical validity, Clinical utility, and associated Ethical, legal, and social implications [2]. Analytical validity refers to a test's ability to measure the genotype of interest accurately and reliably, which for germline pharmacogenetic variants is very robust [3,4]. More important is the appropriate selection of variants to interrogate for a particular drug-response phenotype [5], also considering the ethnic group(s) that is being tested to maximize clinical validity.

Clinical validity is a test's ability to detect or predict the clinical disorder or phenotype associated with the genotype. Because most drug-response phenotypes are multifactorial, it is not always easy to achieve the high clinical validity for pharmacogenetic testing that is typically found when DNA testing for Mendelian disorders. Consequently, the positive predictive value of many pharmacogenetic assays can be low. For example, *CYP2C19*2* is a common variant allele (~15%–25% allele frequency [6]) associated with high on-treatment platelet reactivity among clopidogrel-treated patients with acute coronary syndromes (ACS) managed with percutaneous coronary intervention (PCI) and increased risk for stent thrombosis, which is a rare clinical event (~0.5%) [7–10]. These disparate allele and adverse clinical outcome frequencies result in a low positive predictive value for *CYP2C19* testing for these patients [11]; however, many argue that genetic testing in this scenario can still be useful and help patients avoid life-threatening and unnecessary risks, particularly when taken into consideration with other high-risk clinical factors. In this scenario, pharmacogenetic testing can be viewed analogous to other nongenetic clinical variables with imperfect prediction (e.g., age, concurrent medications, comorbidities, liver function, etc.), yet still providing useful and additive information [12,13].

Clinical utility of a test is a widely used measure of its usefulness in the clinic and resulting changes in health outcomes. However, given the multidimensional nature of this kind of measurement, there is rarely consensus as to its precise definition or on how to adequately demonstrate it, particularly with regard to personalized medicine and pharmacogenetics [14]. The common benchmark for interventional evidence in medicine is a prospective randomized controlled trial, yet these are often resource prohibitive for testing pharmacogenetic hypotheses, and may be unethical to conduct for strong associations of severe adverse effects associated with high-risk genotypes. However, two prospective randomized clinical trials have recently been completed that are testing the utility of cytochrome P450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex 1 (*VKORC1*) genotyping to guide warfarin dosing [15,16]. The results of these trials will likely greatly influence the future of pharmacogenetic testing for anticoagulation control, and possibly other clinical scenarios with pharmacogenetic interactions. Nevertheless, in the absence of interventional clinical trial data, alternative evidence-gathering mechanisms are required, which include

incorporating pharmacogenomics into premarket drug development, innovative clinical trial designs, and continued postmarket observational and mechanistic studies [17–19].

Pharmacogenomic Testing Regulation

Over the past several years the US Food and Drug Administration (FDA) has revised numerous drug labels to now include relevant pharmacogenetic information; however, most do not require testing prior to initiating therapy. Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory-developed pharmacogenetic tests and FDA-regulated tests without clinical claims do not necessarily have to provide full evidence of clinical validity and utility to be offered by a clinical laboratory. Although a number of important pharmacogenetic genes can be currently tested by CLIA-certified laboratories as laboratory-developed tests, there are only a small number of DNA-based pharmacogenetic tests that are actually FDA-approved for in vitro diagnostic testing, including assays for warfarin sensitivity (*CYP2C9* and *VKORC1*), *CYP2D6*, *CYP2C19*, and UDP glucuronosyltransferase 1 family polypeptide A1(*UGT1A1*). Notably, the first sample-to-result system for genotyping *CYP2C19*2*, **3*, and **17* from a noninvasive cheek swab in under 60 min was recently granted 510(k) clearance by the US FDA.

For quality assurance, clinical laboratories also have the option of participating in the pharmacogenetics proficiency testing program by the College of American Pathologists (CAP), which provides graded and educational proficiency testing surveys [20]. To address the needs of quality-control reference materials for the alleles often included in these assays, the Centers for Disease Control and Prevention (CDC) Genetic Testing Reference Materials Coordination Program (GeT-RM), in collaboration with members of the pharmacogenetic testing community and Coriell Cell Repositories, came together to address the needs of quality-control reference materials for alleles often included in these assays. They characterized a large panel of commercially available cell lines for genes and variants commonly included in pharmacogenetic testing panels and proficiency testing surveys [21,22].

Pharmacogenomic Testing Turnaround Time

Although rapid genetic testing can be performed in some clinical scenarios, typical turnaround times for genetic testing in a clinical laboratory are usually days to weeks, depending on the testing technology. Unfortunately, for many actionable pharmacogenetic variants, these turnaround times are unacceptable for efficient implementation into routine clinical care. For example, in the cardiovascular pharmacogenetics field, both warfarin and clopidogrel require knowledge of *CYP2C9*/*VKORC1* and *CYP2C19* genotype data, respectively, at the time of drug initiation for their most effective use [23]. Moreover, given the demanding environments common to most anticoagulation clinics and cardiac catheterization laboratories, disruption of routine care by interfacing with an external clinical laboratory that has additional genetic testing logistics and unique laboratory information management systems can present further complexities for effective use of pharmacogenetic testing.

POINT-OF–CARE PHARMACOGENOMIC TESTING

To address the issue of testing turnaround time for more-efficient implementation, many commercial companies have been developing genotyping platforms that offer rapid sample-to-result assays that will be highly beneficial for integrating pharmacogenetics at the point of care [3,24,25]. A notable example is the use of *CYP2C19*2* (c.681G>A) point-of-care genetic testing for cardiac patients initiating clopidogrel therapy following PCI in the Reassessment of Antiplatelet Therapy Using

An Individualized Strategy Based on Genetic Evaluation (RAPID GENE) trial [26]. As a proof-of-concept study, patients were randomly assigned to rapid point-of-care genotyping or to standard treatment, and those in the rapid genotyping group were tested for *CYP2C19*2* using a cheek-swab genetic testing device that reported results within 60 min. Carriers were treated with prasugrel, and noncarriers and patients in the standard treatment group were treated with clopidogrel. Importantly, no carriers in the rapid-genotyping group had high on-treatment platelet reactivity (HTPR) at day seven (the primary endpoint), compared with 30% of patients given standard treatment ($P = .0092$).

Although this study showed that point-of-care genetic testing following PCI can be performed effectively by nursing staff and that personalized antiplatelet therapy can reduce HTPR in this patient population, it is still not established that this testing strategy results in better clinical outcomes. To address this important question, a number of related trials measuring clinical outcomes following rapid and/or point-of-care *CYP2C19*2* testing are currently ongoing. The ReAssessment of Anti-Platelet Therapy Using an InDividualized Strategy in Patients With ST-segment Elevation Myocardial Infarction (RAPID STEMI) trial evaluated the feasibility, efficacy, and safety of pharmacogenetics-guided antiplatelet therapy for the treatment of STEMI patients following PCI using point-of-care *CYP2C19*2*, **17*, and *ABCB1* 3435C>T genetic testing. This trial confirmed that concurrent identification of these variants in patients with STEMI receiving PCI is feasible at the point of care and that among carriers of at-risk genotypes, treatment with prasugrel was superior to an augmented dosing strategy of clopidogrel in reducing HPR [27]. Tailored Antiplatelet Therapy Following PCI (TAILOR-PCI; ClinicalTrials.gov Identifier: NCT01742117) is a large ongoing trial with an estimated enrollment of almost 6000 patients that is testing the hypothesis that following PCI and using a point-of-care *CYP2C19* genotyping strategy, ticagrelor is superior to clopidogrel in reducing a composite endpoint of major adverse cardiovascular events (MACE). The primary outcome is the occurrence of MACE within 1 year following PCI.

Despite the enthusiasm for rapid turnaround-time pharmacogenetic testing and the success of the RAPID GENE trial, issues remain when implementing this type of genetic testing from research study to routine clinical care. For example, the regulatory landscape of point-of-care testing, particularly that involving genetic testing, can be complicated. The FDA approval of the device used in the RAPID GENE trial does not support its use specifically as "point-of-care" due to the need for personnel with adequate genetics training. Point-of-care testing is, by definition, clinical laboratory testing performed at or near the site of clinical care delivery by personnel (or patients), whose primary training is not in the clinical laboratory sciences. The pathway for FDA approval of point-of-care devices includes 510(k) clearance, premarket approval applications, or CLIA waivers when a device has a negligible likelihood of erroneous results and has no risk of harm if performed incorrectly. Point-of-care pharmacogenetic testing is likely not amenable to a CLIA waiver, which highlights a potential challenge when performing clinical genetic testing at the point of care in the absence of personnel with certified genetics expertise. Depending on regional regulations, it is possible that a local CLIA-certified genetics laboratory may be required to oversee the point-of-care testing by remotely managing interpretation, performance, quality control/assurance, and participation in relevant proficiency testing programs. This could increase the complexity and overhead costs of point-of-care testing and add potential difficulties when defining the relationship between point-of-care clinical staff and CLIA-certified genetic laboratories.

A technical challenge for point-of-care pharmacogenetic testing involves the content of the genotyping assays themselves. The RAPID GENE trial was centered on a single polymorphic

allele, which for future pharmacogenetic assays will not be adequate. This is even more relevant when deploying point-of-care pharmacogenetic testing across more-diverse patient populations, as the frequencies of relevant variant alleles differ between racial groups and ethnicities. For example, the *CYP2C19*4B* loss-of-function allele has a frequency of ~2% among Ashkenazi Jewish individuals but is lower in other racial and ethnic groups [6,28]. In addition, more genes and functional variants are necessary for some currently actionable pharmacogenetic examples (e.g., warfarin) and ongoing genome-sequencing studies are likely to identify more variants with appreciable effect sizes that will justify inclusion in future point-of-care testing panels.

Some of the more robust pharmacogenetic associations at the present time involve specific human leukocyte antigen (HLA) alleles of the major histocompatibility complex (e.g., *HLA-B*5701* and flucloxacillin-induced liver injury and abacavir-induced hypersensitivity [29]; *HLA-B*1502/HLA-A*3101* and carbamazepine-induced hypersensitivity [30,31]). Unfortunately, *HLA* genotyping is one of the more challenging molecular assays, requiring combinatorial multiplexing that is beyond the technical capacity of current point-of-care platforms. Ideally, future point-of-care genetic testing platforms will overcome the technical needs for multiplexed sample-to-answer genotyping and be able to include larger variant panels with content specifically selected for clinically actionable alleles.

PREEMPTIVE PHARMACOGENOMIC TESTING

In the preemptive pharmacogenomics testing approach, data on multiple important pharmacogenes of a patient are collected prospectively and are stored for future use. This makes pharmacogenomics data relevant for a wide variety of common pharmaceuticals readily available at the point of care. In the ideal case, preemptive pharmacogenomics is combined with an electronic prescription system that alerts prescribers and pharmacists through a computerized physician order entry (CPOE) system when a drug is ordered or dispensed for a patient with an at-risk genotype.

Advantages of Preemptive Pharmacogenomics Testing

Preemptive pharmacogenomics testing has several potential advantages:

- Fewer genetic tests are required, which saves money and resources
- Less uncertainty about whether ordering a test is necessary; which also lowers barriers to use pharmacogenomics data in clinical practice
- Test results are available immediately, which means there is no delay in treatment initiation and no intermediate phase in which the patient is prescribed a standard dose while pharmacogenomics test results are pending
- Disruption of routine clinical care is minimized

Although preemptive testing could also be conducted for single genes, testing a specific set of important pharmacogenes at once is the most common way in which preemptive PGx is implemented (Table 13.1). This way, the pharmacogenomics test results can be used to optimize treatment with a wide range of common medications, whereas the extra cost of testing a panel of genes versus a single gene are small.

Challenges of Preemptive Pharmacogenomics Testing

To fully utilize the results of preemptive pharmacogenomics testing at the point of care, information technologies (ITs) that assist medical professionals in accessing and

TABLE 13.1 Examples of Preemptive Pharmacogenomics Implementation Programs That Include Clinical Decision Support Components

Implementation Program	Clinical Site, Country	Gene(s) Used for Treatment Optimization[a]
Ubiquitous Pharmacogenomics (U-PGx)	Several implementation sites in EU countries (UK, Netherlands, Spain, Italy, Greece, Austria, Slovenia)	CYP2C19, CYP2C9, CYP2D6, CYP1A2, CYP2B6, CYP3A4, CYP3A5, SLCO1B1, TPMT, DPYD, VKORC1, UGT1A1, HLAB-B
CLIPMERGE [49,61]	Icahn School of Medicine at Mount Sinai, New York, USA	CYP2C19, CYP2C9, VKORC1, SLCO1B1, CYP2D6
Personalized Medicine Program [52,54,64,65]	University of Florida and Shands Hospital, USA	CYP2C19, TPMT, CYP2D6, IFNL3
1200 Patients Project [55,56]	University of Chicago, USA	N/A
Personalized Medication Program [50,62]	Cleveland Clinic, USA	N/A
PG4KDS [45,51]	St. Jude Children's Research Hospital in Memphis, USA	TPMT, CYP2D6, SLCO1B1, CYP2C19, CYP3A5, DPYD, UGT1A1
PREDICT [57,58]	Vanderbilt University School of Medicine, Nashville, USA	CYP2C19, SLCO1B1, CYP2C9, VKORC1, TPMT, CYP3A5
RIGHT [46,48]	Mayo Clinic, USA	HLA-B, TPMT, IFNL3, CYP2C19, CYP2C9, VKORC1, CYP2D6, SLCO1B1

[a] *Gene panels might be changed or extended over the course of the project.*

understanding existing PGx results play a vital role. Unfortunately, in many settings, barriers exist that make the implementation of IT systems for preemptive PGx difficult or decrease their utility. For example, in many healthcare systems it still proves difficult to make results available at the point of care when patients transition between different healthcare institutions and systems. This difficulty would ideally be addressed by shared, functional electronic health records (EHRs), but these are still unavailable in many regions around the world. Another difficulty is to assure that healthcare professionals are aware of existing preemptive PGx results for a patient when new medications are prescribed and that treatment is optimized based on up-to-date clinical guidelines. CPOE systems that can provide active clinical decision support (CDS)—i.e., alerts—when new medications are prescribed, would be ideal for addressing these issues, but remain unavailable in many healthcare settings.

Examples of Preemptive PGx Implementation

Several studies have investigated the implementation of preemptive PGx accompanied by CDS interventions (Table 13.1).

The *Ubiquitous Pharmacogenomics (U-PGx) project* [47,63] implements and evaluates the outcomes of preemptive PGx testing and CDS at multiple implementation sits across seven European countries in a prospective, controlled clinical trial. The impact on patient outcomes and cost-effectiveness are investigated. The program is unique in its multicenter, multigene, multidrug, multiethnic, and multihealthcare system approach. Making PGx test results and decision support available throughout disparate healthcare systems is a challenge that needs to be addressed. Local EHRs, and CDS solutions are complemented by a system based on the Medication Safety Code system, in which patients can carry their PGx results on small pocket cards

that can be scanned and interpreted with mobile technologies at the point of care [59].

The *1200 Patients Project* was established at the University of Chicago in 2011 with the aim of prospectively genotyping 1200 adults receiving outpatient medical care [55,56]. Genotyping is conducted with a commercial panel (Sequenom Absorption, Distribution, Metabolism, and Excretion [ADME] panel) and an additional custom-designed panel. Genotypes that are deemed actionable are classified based on their clinical implications, are assigned a color (green, yellow, red) or symbol (sideways arrow, dose calculator), and are then transferred to the Genomic Prescribing System, a web-based portal that was specifically designed for this project.

Clinical Implementation of Personalized Medicine through Electronic Health Records and Genomics–Pharmacogenomics (*CLIPMERGE*) is a PGx implementation program at the Icahn School of Medicine at Mount Sinai [49,61]. After genotyping of 36 pharmacogenes, results are transferred to a data management system that is external to, but communicates with, the local Electronic Health Record (EHR). The project utilizes a "Clinical Risk Assessment Engine," which includes a rule engine for matching genotypes to relevant clinical PGx recommendations based on CPIC guidelines. Relevant recommendations are made available as automated alerts via the EHR.

In 2011 the University of Florida and Shands Hospital established a preemptive PGx testing program, called *Personalized Medicine Program* [52,54,64,65]. For the program, a custom and easily adaptable chip covering 256 drug-metabolizing enzyme and transporter SNPs with sound evidence of clinical utility was designed. The decision to move PGx results to the EHR is made by an expert committee upon evaluating the literature, and formulating the recommendation for incorporation into the CDS tools to enable active CDS at the time of prescribing.

Hicks et al. describe the implementation of PGx CDS within the Cleveland Clinic's *Personalized Medication Program* [50,62]. Based on CPIC guidelines, 63 custom rules and alerts for two different genes were created and incorporation into the local EHR. Alerts with PGx dosing recommendations are displayed to clinicians at the time of prescribing via the EHR. Furthermore, pretest alerts are deployed to recommend PGx testing whenever a patient is prescribed a relevant drug.

With PGx for Kids (*PG4KDs*), St. Jude Children's Research Hospital has implemented a standardized protocol for PGx testing and PGx CDS into clinical practice ([45]; Hoffman et al.). After genotyping of 1936 variants across 225 genes, diplotype results are matched with predefined consults (i.e., phenotype assignment, interpretation of the diplotype, dosing recommendations, and activity score when applicable) through two inhouse custom web-based applications. PGx results and consults are reviewed and, if deemed necessary, adapted by adding patient-specific comments. Results and recommendations are then incorporated into the local EHR to enable active CDS. Furthermore, a summary of the patient's PGx results is displayed in a special section of the patient's EHR entry.

The Pharmacogenomic Resource for Enhanced Decisions in Care & Treatment (*PREDICT*) *program*, established in September 2010 at the Vanderbilt University School of Medicine, implements preemptive genotyping for high-impact genetic variants [57,58]. Genotyping results are stored in a secure database separate from the EHR, and are incrementally released to the EHR every time a new genotype is deemed actionable by an expert committee. Actionable genotypes are then converted into a standard notation including a phenotype interpretation and stored in the EHR as a molecular diagnostic lab result. PGx results are displayed in the EHR in a "Drug-Genome Interaction" section of the patient summary page. Active CDS is integrated within inpatient and outpatient electronic prescribing application.

The Mayo Clinic implemented PGx CDS as part of the Research Institute for Genetic and Human Therapy (*RIGHT* stands for Right Drug, Right Dose, Right Time: Using Genomic Data to Individualize Treatment) project, which aims to integrate preemptive genotyping and sequencing into routine care [46,48]. The EHR was adapted to provide a variety of functionalities, such as reminding clinicians if PGx testing is recommended, notifying ordering clinicians if new test results are available, triggering a manual review process for unreadable results, and providing active CDS via automatic alerts.

When and What to Test?

Different strategies for timing preemptive PGx testing initiation are possible:

- Arbitrary initiation of preemptive PGx testing: Preemptive PGx testing can be conducted voluntarily by the patient or based on the recommendation of a treating physician without a clearly defined trigger. This scenario is most likely to occur with patients who take a proactive approach toward their healthcare and might involve direct-to-consumer PGx testing.
- "Reactive preemptive" approach: Preemptive PGx testing can be initiated at the time when a first prescription for a PGx drug is made. The preemptive results can be used to optimize treatment with the specific drug that triggered the test, whereas the results can be saved and used for optimizing other prescriptions at later time points. This is the approach chosen by many clinical studies on preemptive PGx testing. It has the drawback that for the very first prescription that is triggering PGx testing, the advantages of readily available results and minimal disruption of clinical workflows cannot be reaped.
- Demographic or group-based approach: Preemptive PGx testing is conducted for patients of certain risk groups, e.g., for senior patients. It has the drawback that certain patients might not end up receiving PGx drugs, i.e., some patients might be tested without benefitting from it.

There is currently no widely accepted consensus on which testing strategy is preferable, or which genes should be covered in a preemptive PGx panel. Table 13.2—adapted from [60]—gives an overview of the incidence of exposure of a large sample of patients from the United States to multiple drugs for which pharmacogenomic guidelines are available. It shows the incidence of new prescriptions of PGx drugs within a selected 4-yr period (2009–12). Patients enrolled in Medicare Supplemental age>=65 or Medicaid age 40–64 had the highest incidence of PGx drug use, with approximately half of the patients receiving at least one PGx drug during the 4-yr period and one-fourth to one-third of patients receiving two or more PGx drugs. These findings suggest that exposure to multiple PGx drugs within a relatively short-time window is common, which is an argument for preferring preemptive PGx testing over sequential, reactive PGx testing.

A surprisingly large number of common drug prescriptions can be addressed by testing a very limited set of genes. For example [53] report that among a large sample of patients in Austria analyzed for exposure to PGx drugs, 39.1% of all patients over 65 received at least one drug metabolized by the three important cytochrome P450 enzymes (CYP2D6, CYP2C9, and CYP2C19). This suggests that even the results of very focused (and therefore, cheap) preemptive PGx panels might be useful for a large number of patients.

DIRECT-TO-CONSUMER PHARMACOGENOMIC TESTING

Traditionally, genetic testing is initiated by a healthcare provider such as a physician, nurse practitioner, or a genetic counselor, and a sample is collected, sent to the laboratory, and results

TABLE 13.2 Incidence of Exposure to Drugs for Which Preemptive Pharmacogenomic Testing is Available Within a Four-Year Time Period

Characteristic	Private Insurance (Age 14–39)	Private Insurance (Age 40–64)	Medicaid (Age 14–39)	Medicaid (age 40–64)	Medicare (Age >= 65)
n	22,824,848	26,561,525	3,032,191	1,130,797	5,429,266
Female	57.5%	54.8%	69.3%	60.8%	55.2%
Age (median, mean)	27, 26.3	51, 51.23	21, 22.5	50, 50.57	72, 73.82
>=1 drugs	30.4%	42.2%	40.2%	55.5%	50.6%
>=2 drugs	9.1%	17.8%	15.3%	32.8%	27.5%
>=3 drugs	3.1%	7.5%	6.5%	18.5%	13.8%
>=4 drugs	1.1%	3.1%	2.9%	9.9%	6.4%
>=5 drugs	0.4%	1.3%	1.3%	5.0%	2.8%
>=6 drugs	0.2%	0.5%	0.6%	2.4%	1.1%
Rank 1	Codeine (9.4%)	Codeine (9.5%)	Oxycodone (15.0%)	Oxycodone (15.8%)	Simvastatin (13.4%)
Rank 2	Oxycodone (7.8%)	Oxycodone (8.8%)	Codeine (10.6%)	Tramadol (13.8%)	Metoprolol (10.8%)
Rank 3	Tramadol (4.0%)	Simvastatin (8.2%)	Tramadol (8.3%)	Omeprazole (10.9%)	Omeprazole (9.2%)
Rank 4	Sertraline (3.2%)	Omeprazole (6.2%)	Citalopram (4.8%)	Simvastatin (9.6%)	Tramadol (8.5%)
Rank 5	Omeprazole (3.1%)	Tramadol (6.2%)	Omeprazole (4.6%)	Citalopram (7.6%)	Codeine (8.2%)

CCAE, Truven MarketScan Commercial Claims and Encounters dataset. *PGx drugs*, drugs for which pharmacogenomics guidelines are available.

Adapted from Samwald M, Xu H, Blagec K, Empey PE, Malone DC, Ahmed SM, et al. Incidence of exposure of patients in the United States to multiple drugs for which pharmacogenomic guidelines are available. Medical and Technical Publishing Company International Review of Science Series One Physiology 2016;11(10):e0164972. https://doi.org/10.1371/journal.pone.0164972.

are returned to the clinician for interpretation and further use. Conversely, direct-to-consumer (DTC) or "at-home" genetic testing occurs when genetic tests are marketed and sold directly to the consumer via either Internet, television, radio, newspaper, and/or other media outlets without involving healthcare professionals in the process [32]. However, researchers and even the United Kingdom (UK) Human Genetics Commission have extended the definition to include genetic tests that are directly marketed to patients, yet require physician involvement. Either the physician involved in the direct care of the consumer, or one employed by the testing company, would be involved in the ordering and/or return of results processes [33,34] (https://www.cellmark.co.uk/pdfs/HGCprinciples.pdf). DTC tests normally provide insight on the patient's ancestral heritage; physical traits, such as facial features, presence of freckles, hair color, and/or cheek dimples; carrier status for conditions, such as sickle cell anemia, hereditary hemochromatosis, and hereditary hemophilia. In addition, DTC tests would assess genetic predisposition to conditions including, but not limited to, breast and ovarian cancer, familial hypercholesterolemia, celiac disease, Alzheimer's, Parkinson's disease and heart disease. When the marketed tests seek to advise consumers on their genetic predisposition to certain medication responses,

it is termed as direct-to-consumer pharmacogenomics (DTC PGx) testing. DTC tests usually require the consumer to purchase the test kit, collect, and send sample (e.g., saliva, cheek swab, or blood in some cases) to the testing company, and then receive the results via mail, online, or over the telephone. Some companies may provide additional support materials and/or access to content experts to help interpret the findings, if needed. However, because there is not a standardized collection of genes and variants offered through DTC, the reporting is, in turn, not standardized. With the majority of practicing physicians lacking the know-how to appropriately interpret and utilize pharmacogenomics results in practice [35], the additional support provided by these DTC companies may be helpful. Nevertheless, it also poses a big challenge to the healthcare community as the DTC reports tend to overestimate the relevance of the findings and/or report on genetic variants with little to no clinical validity [36].

History of Direct-To-Consumer Genetic Testing

Genetic tests are used clinically to diagnose, predict disease risk, or select appropriate therapies. This has been made possible because of successfully replicated genome-wide association studies (GWASs) linking genetic variations to many clinical phenotypes. GWASs have been made possible largely due to the collective influence of the completion of the Human Genome Project, which provided researchers access to knowledge about the entire human DNA sequence and its variations [37], and the creation of the Haplotype Map (HapMap) project [38]. Furthermore, the GWASs benefitted from the development and rapid advancement of high-throughput technologies to interrogate genetic sequences, and the emergence of large DNA biorepositories [39]. As more-robust information came to light regarding the genetic underpinnings of many clinical phenotypes, especially complex diseases and response to medications, the need to share this information with the public became apparent. The notion that patient awareness of genetic risk profiles may lead to positive life-style modifications, avoidance of "inappropriate" therapies, and/or adoption of preventative measures have been suggested as some of the main objectives for DTC genetic testing [39]. These motives, along with the fact that a majority of practicing clinicians are not familiar with genomic medicine and pharmacogenomics [35], and that some genetic variants lack strong evidence to be deemed clinically actionable, launched direct-to-consumer genetic testing. In 2002, Gollust and colleagues counted 14 DTC genetic testing companies [40]; however, this number is well over 200 as of 2015 (http://www.andelkamphillips.com/wp-content/uploads/2018/02/Genomic-Privacy-GenoPri.pdf).

Direct-To-Consumer Pharmacogenomics Testing Regulatory Landscape

Undoubtedly, one of the major concerns raised by clinicians, researchers, and even some in the public, regarding the insurgence of DTC genetic testing companies has been the regulatory oversight or lack thereof. In the United States, the Clinical Laboratory Improvement Amendments (CLIA) of 1988 requires every clinical laboratory performing testing on humans meant to aid clinical assessments, such as diagnosis, prevention, or treatment of any disease, should adhere to the predetermined standards of operation outlined in these amendments [41]. As such, these federal regulatory standards apply to clinical DTC genetic tests and, although they ensure analytical validity of these tests, they do not address the clinical validity or utility of DTC genetic tests. However, some states like the State of New York requires laboratories to produce information on the clinical validity of all laboratory tests performed for New York State residents. On a broader scale, the US Food and Drug Administration (FDA) has

expanded its scope of regulation regarding DTC genetic testing. The FDA classifies these tests as medical devices under section 201 (h) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 321 (h) and consequently requires DTC genetic testing companies to obtain the appropriate approval from the FDA for health-related genetic services. Beginning in 2013, warning letters have been sent by the FDA to many DTC companies including DTC PGx companies, such as 23andMe, Healthspek LLC, Genomic Express, Kailos Genetics, Harmonyx, and DNA4Life (https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/default.htm).

This step up in regulatory oversight by the FDA may help to moderate and ensure the clinical validity and utility of these tests, although many opponents of the FDA's actions argue that this may hinder the advancement of the field. About a year and a half later, in 2015, the FDA gave 23andMe the authorization to market their DTC carrier testing for Bloom syndrome as it had been classified as a Class II medical device, thereby not requiring premarket review from the agency (https://www.medscape.com/viewarticle/840067). Again, the administration authorized the sale of the 23andMe's Personal Genome Service Genetic Health Risk tests for 10 diseases in April 2017 and there DTC test for three breast cancer susceptibility gene (BRCA) mutations in March 2018 (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm551185.htm; https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm599560.htm). Moreover, the Genetic Information Nondiscrimination Act (GINA) was signed into law in 2008 to protect consumers from health insurers who may want to issue higher premiums or refuse coverage to individuals with "high-risk" genetic profiles. Many patient advocacy groups argue that GINA may not be enough in this era of genomic medicine as it does not apply to life, long-term care, or disability insurance [42].

Furthermore, regulations regarding DTC genetic testing including pharmacogenomics testing differ in scope and strictness from country to country around the world. For instance, laws in France, Germany, Portugal, and Switzerland restrict the ordering of genetic tests to medical doctors after adequate counseling and proper informed consenting process, which makes DTC genetic tests illegal in these countries [43]. On the other hand, DTC genetic tests are allowed in Belgium and the United Kingdom [43]. Apart from these national regulations, the European Union (EU) also has laws in place to protect the economic interests and rights of their citizens, especially concerning product safety and data protection (https://ec.europa.eu/info/law/law-topic/consumers/consumer-contracts-law/consumer-rights-directive_en). Another regulation of interest is the in vitro diagnostic medical devices (IVD) Regulation adopted in April 2017, which is currently undergoing a 5 yr transition phase and afterward will apply to all EU member states, Turkey, and the European Free Trade Association. This IVD regulation classifies all genetic tests as moderate to high-risk devices, which will require a premarket assessment of analytical validity, and clinical validity by a specified authority just as in Canada, Australia, and the United States [44]. Moreover, the IVD regulation will prohibit misleading advertisements, while enforcing the need for adequate education prior to consumer consent, the provision of genetic counseling posttesting, and will ensure that all companies around the world who provide services to EU residents adhere to these new laws once they are in effect [44].

Advantages of Direct-To-Consumer Pharmacogenomics Testing

Amid the challenges and numerous concerns with DTC genetic testing, particularly pharmacogenomics testing, there are some positive attributes of this model of genetics testing.

An undeniable advantage of DTC pharmacogenomics testing is the increased public awareness of genetics' influence on medication response among other clinical phenotypes. Generally, genetics or DNA is used in popular culture to identify criminals or for paternity testing, but this model of genetic testing offered has broadened the knowledge base of the public to view genetics as a clinical tool. The patient education resources developed by some of these companies are state of the art and may even inform ones used in clinical settings. Patients received their results directly, may have access to experts for further review and counseling, and, therefore, are empowered with their findings, which may lead to better health-related life-style choices. Additionally, the DTC model allows for consumers from all areas to participate in this cutting-edge tool, as opposed to it being available only at major academic medical centers and/or urban cities. As more and more patients receive their pharmacogenomics results through DTC companies, our aged health systems will be forced to adapt and incorporate these findings into routine care. This means that DTC genetic offerings have the potential to instigate and accelerate clinical adoption of pharmacogenomics testing as well as genomic medicine applications. Along the same lines, the high acceptance of this model by the public, regardless of the concerns raised by researchers and clinicians, have made genetic testing a regulatory priority in many nations as previously discussed. Health insurance reimbursements for pharmacogenomics tests have also become a major topic of discussion among the relevant stakeholders, mainly because of DTC testing companies.

Challenges of Direct-To-Consumer Pharmacogenomics Testing

Though DTC pharmacogenomics testing offers the convenience of providing samples at home and returning results directly to the consumer, the results may be misleading in many instances. To date, there are fewer than 20 Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for actionable drug–gene pairs and even a lower number of "actionable" pharmacogenomics genes. However, many of these companies provide medication response information on a host of medications using about two dozen genes or more, many of which may not be based on robust evidence. This is, in fact, one of the major critiques of the DTC pharmacogenomics model and a huge deterrent to clinicians who would embrace this tool otherwise. The regulatory oversight provided by the FDA in the United States and by laws in other countries may help to refine these misleading reports. Moreover, these reports tend to be very long (i.e., 20–>100 pages) depending on the combination of genes and medications, and this can be very overwhelming to patients who may have ordered the tests on their own without their doctor's supervision. When presented with these documents from patients, doctors mostly do not know how to use the information, or where to store it in the patient's medical records for effective referencing later on. Again, patients may choose to alter their medications based on these results, if not adequately counseled against it. This is especially important because nongenetic and clinical factors, such as age, weight, liver function, kidney function, diseases, smoking status, comedications, etc., influence medication response as well and this additional information is mostly left out of DTC pharmacogenomics reports because they do have not access to the consumer's medical records. To date, there are no regulations on how genetic testing companies should store or destroy consumer's data after the companies go out of business. This may lead to data safety and privacy issues if care is not taken (Table 13.3).

TABLE 13.3 Examples of Direct-To-Consumer Pharmacogenomics Testing Companies

DTC Company	Number of Genes Tested	Number of Medications or Classes of Medications	Price Range	Patient Education Materials?	Physician Order Required for Testing?	Link to Website
Dynamic DNA Laboratories	17	>200	$350–600	Yes	No	https://dynamicdnalabs.com/
Hudson Alpha Institute for Biotechnology (Partnered with Kailos for PGx)	38	21 classes of drugs	$99–299	Yes	No	https://www.kailosgenetics.com/pgxcomplete
Myriad Genetics (Genesite)	24	86	$110–1750	Yes	Yes	https://myriad.com/
Pathway Genomics	>23	>70	399	Yes	Yes	https://www.pathway.com/
GeneDX	34	21 classes of drugs	275	Yes	Yes	https://www.genedx.com/
PGxOne	50	>300	1200	Yes	Yes	https://www.admerahealth.com/pgx/?gclid=EAIaIQobChMIz8bB4cj-2gIVwkCGCh2xkQ8qEAAYAiAAEgIG4_D_BwE
OneOme RightMed	27	360	349	Yes	No, but genetic counselor needs to approve or deny order request.	https://oneome.com/
GeneAlign	19	Pain, psychiatry, cardiovascular	Not DTC	Yes	Yes	https://www.genealign.com/

CONCLUSION AND FUTURE PERSPECTIVE

The ongoing advances in point-of-care and preemptive genetic testing are greatly facilitating the ability to implement clinical pharmacogenetics. Both strategies circumvent the barrier of test turnaround time and, therefore, provide the opportunity to integrate pharmacogenetics test results at the point of care. Although these strategies are very promising, the increasing accessibility of genome sequencing suggests that in the future we may no longer have a need for targeted genotyping if patients already have their sequence data available. Preemptive pharmacogenetics testing is a progressive strategy for delivering genetics-based CDS at the point of care to help guide pharmacotherapy; however, the generalizability

of this strategy beyond large academic medical centers may be limited by the necessary institutional investments, information technology infrastructure, third-party reimbursement issues, and provider support. Regardless of how the clinical pharmacogenetics data is derived, of importance will continue to be the necessary efforts toward provider and patient education, accessible and appropriate evidence-based therapeutic recommendations, and rigorous assessment of validity and utility by the pharmacogenetics community.

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CHAPTER

14

Economic Evaluation of Pharmacogenomic Testing: Lessons From Psychiatric Pharmacogenomics

Y. W. Francis Lam[1,2]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]College of Pharmacy, University of Texas at Austin, Austin, TX, United States

OUTLINE

INTRODUCTION

Through identifying individual genetic factors contributing to variability in pharmacological response, the ultimate goal of pharmacogenomics is to maximize therapeutic benefit while minimizing toxicity. The clinical utility of pharmacogenetic tests has been evaluated and proven in different therapeutic areas, most notably, for testing genetic variations in thiopurine methyltransferase, for predicting hematologic toxicity with the thiopurines, and human leukocyte antigen (HLA) typing for predicting severe adverse skin reactions, and/or hypersensitivity drug reactions associated with use of carbamazepine and abacavir. In addition to substantial literature providing the scientific basis of test implementation in clinical practice, these highly successful examples also have a strong economic (business) case that is based on significant reduction in the incidence of costly adverse drug events, which inevitably play a major role in payer decision in reimbursing the pharmacogenomic tests.

Although pharmacogenomic testing of several genes related to treatment outcomes for

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00014-0

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psychiatric illnesses have been investigated for many years, data on evaluating the clinical utility and cost-effectiveness of such genetic tests have only been available in the literature over the last few years. Although the debate over clinical utility will continue in the literature, this chapter will provide a review and perspective on clinical outcome and economic evaluations of psychiatric pharmacogenomic interventions. In essence, how close are we for these two important aspects of pharmacogenomic testing, and what lessons can be learned from the literature so far?

VALIDITY AND UTILITY OF PHARMACOGENOMIC TESTING

Major depressive disease is a highly debilitating mental illness with antidepressant selection based mostly on trial and error and significant treatment failure following first-line treatment. Therefore, ways to optimize treatment outcome, including pharmacogenomics testing, has long been attractive to clinicians. In theory, the use of pharmacogenomics tests could potentially reduce healthcare costs associated with avoidance of severe adverse drug reactions and/or use of inappropriate expensive pharmacological treatment.

As discussed in the chapter "Translating Pharmacogenomics research to Therapeutic Potentials," the established components of the analytical validity, clinical validity, clinical utility, and associated ethical, legal, and social implications; see Table 14.1 (ACCE) Model Project has been applied to the evaluation of pharmacogenomic tests. In general, the analytical validity of most pharmacogenomic tests is high (>95%), especially for the pharmacokinetic genes [1].

As the sponsor for the ACCE Model Project, the Center for Disease Control launched the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative. Three years later, based on lack of data to support clinical utility, the EGAPP Study Group did not recommend the *CYP2D6* genetic test for selective serotonin reuptake inhibitors (SSRIs) [2]. However, given what we know about the contribution of multiple-gene variants affecting pharmacokinetics and pharmacodynamics of the SSRIs, it is not at all surprising that *any* single-gene testing (for *CYP2D6* in the case of the EGAPP recommendation) would have any real impact on the therapeutic response to SSRIs, let alone demonstrating clinical utility in a real-world setting. Even with testing for gene variants for CYP2D6 and CYP2C19, Steimer et al. highlighted the limitation of single-gene testing. The investigators showed that the correlation between drug concentrations and side effects in amitriptyline-treated patients is more robust for nortriptyline than for amitriptyline [3], which relies on multiple cytochrome P450 isoenzymes, including CYP2C19, for its own metabolic conversion to nortriptyline. This essentially means that the risk of increased side effects is related to different possible combinations of metabolic phenotypes, with the highest risk being those patients who are CYP2C19 ultrarapid metabolizers (UMs) and CYP2D6 poor metabolizers (PMs).

Combinatorial Pharmacogenomic Testing

Clinical Utility

Within the field of psychopharmacogenomics, the concept of combinatorial gene testing to simultaneously evaluate the effect of multiple genes, has been proposed and advocated to improve clinical utility of pharmacogenomics testing, primarily with several commercially available gene panels. Although not having the optimal study design of double-blind, randomized-control clinical trials with large sample size, several studies have attempted to address the clinical utility of combinatorial pharmacogenomic testing as compared to the standard of care (Table 14.1).

TABLE 14.1 Summary of Selected Studies of Antidepressant Pharmacogenomics Testing

Subject Cohort	Genes Tested in PG Test Panel	Study Setting	Main Findings	Ref
OBSERVATIONAL STUDIES				
44 total, all Caucasians. 22 in intervention group, 22 in control group	*CYP (2D6, 2C19, 1A2), SLC6A4, HTR2A*	Outpatient behavioral health clinic	30.8% and 31.2% reduction in HAM-D17 and QIDS-C16, respectively, in intervention group versus 18.2% and 7.2%, respectively, in control group	[4]
227 total, all Caucasians. 165 completors 72 (intervention), 93 (control)		Hospital	46.9% and 44.8% reduction in HAM-D17 and QIDS-C16, respectively, in intervention group versus 29.9% and 26.4%, respectively, in control group	[5]
116 total, all Caucasians. 58 (intervention), 58 (control)	*ABCB1*	Hospital. Participants of MARS study	83.6% with HAM-D <10 from intervention group versus 62.1% in control group	[10]
RANDOMIZED CONTROLLED TRIALS				
51 total (95% Caucasians). 26 (intervention), 25 (control)	*CYP (2D6, 2C19, 1A2), SLC6A4, HTR2A*	Outpatient clinic	20.0% with remission (HAM-D ≤7) from intervention group versus 8.3% in control group	[6]
152, ethnicity not specified. 148 completors 74 (intervention), 74 (control)	*CYP2D6, CYP2C19, ABCB1, ABCC1*	Not specified	72.0% with remission (HAM-D≤7) from intervention group versus 28.0% in control group	[9]
COST-EFFECTIVENESS STUDIES				
Initial cohort (111 cases, 222 control). Replication cohort (116 cases, 232 control).	*CYP (2D6, 2C19), DRD2, COMT, SLC6A4, MTHFR, CACNA1C*	Claims data 2010 to 2012	Medical cost saving of $562 in case versus control. 6.3% increase in adherence in case compared to control.	[18]
13,048 total, ethnicity not specified. 2,168 (guided), 10,880 (usual care)	*CYP (2D6, 2C19, 1A2), SLC6A4, HTR2A*	Pharmacy benefits claims data 2011 to 2013	$1,035.60 cost saving and medication adherence improvement of 0.123 in guided group compared to usual care.	[19]

ABC, ATP-binding cassette; *CACNA1C*, Calcium Voltage-Gated Channel Subunit Alpha1 C; *COMT*, catecho-o-methyltransferase; *CYP*, cytochrome P450; *DRD2*, dopamine D2 receptor; *HAM-D*, Hamilton Rating Scale for Depression; *HAM-D17*, HAM-D 17-item; *HLA*, human leukocyte antigen; *HTR*, 5-hydroxytryptamine receptor; *MARS*, Munich Antidepressant Response Signature; *MTHFR*, methylene tetrahydrofolate reductase; *PG*, pharmacogenomics; *QIDS-C16*, Clinician Rated Quick Inventory of Depressive Symptomatology 16-item; *SLC*, solute carrier.

In an openlabel, non-randomized, pilot prospective trial, Hall–Flavin et al. compared two cohorts of patients, one with (n = 22) and one without (n = 22) pharmacogenomic information (*CYP2D6*, *CYP2C19*, *CYP1A2*, *SLC6A4*, and *HTR2A*) provided to the psychiatrists at study initiation. The investigators reported a significant reduction in scores from two clinical rating scales in the pharmacogenomics-guided group and the non-guided group, respectively: 30.8% versus 18.2% ($P = .04$) for the seventeen-item Hamilton Rating Scale for Depression [HAM-D17], and 31.2% versus 7.2% ($P = .002$) in the sixteen-item Clinician Rated Quick Inventory of Depressive Symptomatology [QIDS-C16] [4]. The result of this smaller study was replicated

in another open-label study with identical study design but included a larger sample size of 227 patients. Comparing the 72 patients completing the study in the pharmacogenomics-guided group versus 93 patients in the unguided group, there was a significant difference in improvement of HAM-D17 depression scores from baseline: 46.9% and 29.9%, respectively ($P < .0001$). The study also demonstrated a statistically significant difference in reduction of the QIDS-C16 score ($P < .0001$) and in the patient self-reported nine-item Patient Health Questionnaire ($P < .0001$). There was also a smaller difference in remission rate, with 26.4% of patients in the pharmacogenomics-guided group versus 12.9% in the unguided group ($P = .03$) [5].

The same group of investigators also reported the findings of a smaller placebo-controlled, double-blind, prospective study, and reported a trend toward clinical significance showing improvement in HAM-D17 scores from baseline between the pharmacogenomics-guided group (n = 26 with 30.8% improvement) versus the unguided group (n = 25 with 20.7%), although not at a statistical significance level ($P = .28$). In addition, a trend also favors the guided group with respect to remission rate, but the difference again is not statistically significant [6]. Although the result is promising, the study conclusion is limited by small sample size with insufficient power to detect statistical significance. In a pooled analysis of the clinical studies, Altar et al. showed that the combinatorial pharmacogenomic approach, as opposed to single-gene analysis, has good predictive ability of an increased likelihood of adverse outcomes (hence, clinical validity). This occurred in patient-prescribed medications affected significantly by gene variants assessed in the pharmacogenomic panel [7], conceptually much akin to the study of Steimer et al. [3].

The largest evaluation of clinical utility of combinatorial pharmacogenomics is a naturalistic, unblinded, prospective study involving 685 patients with no control group. The pharmacogenomic information on 10 genes was available for use by the clinicians for dosing of the entire study cohort. The investigators indicated that in 93% of the clinicians, the antidepressant-prescribing pattern for the patient was influenced by the pharmacogenomic information. Based on QIDS-C16 rating change, 77% of the study cohort had improvement after 3 months, with 38% achieving full remission [8]. The limitation of the naturalistic study, of course, is the absence of a control group, which complicates interpretation of how much of the reported improvements is attributed to the pharmacogenomic information.

In the absence of large, randomized controlled clinical trials, the combinatorial approach categorically has been shown to possess much greater predictive value for response to antidepressant treatment than the single-gene approach. This is even true for the study by Singh, which utilizes a pharmacogenomic approach that only assesses the impact of a combination of pharmacokinetic genes (*CYP2D6*, *CYP2C19*, *ABCC1*, and *ABCB1*) involved in antidepressant dosing. This 12-week prospective, randomized, double-blind study compared pharmacogenomics-guided versus non-guided groups. At the end of the 12-week trial period, 74 patients receiving genomic-guided dosing achieved a 72% remission rate versus 28% for the unguided group ($P < .0001$), which translated into one additional remitting patient per every three genotyped [9]. Given what is generally accepted that both pharmacokinetic and pharmacodynamic gene variants *partially* contribute to the variance in antidepressant response, it is interesting that the significant difference in remission rate was achieved with a panel that only assess pharmacokinetic genes. Although the remission rate from this study is notably high [9], the clinical utility of the commercially available pharmacogenomic panel used has not been evaluated in any additional study. Therefore, at the very least, replication data confirming similar remission rates need to be generated before consideration of this specific pharmacogenomic panel to be utilized on

a wider scale. In this respect, the observational study by Breitenstein et al. also demonstrated a statistical difference in remission (based on HAM-D17) between ABCB1-guided therapies versus usual care (Table 14.1) [10].

Most of the studies reviewed here have the usual study limitations and/or criticisms, including small sample size; flaws in design and analysis; and issue of generalizability of results. In addition, study subjects could arguably been given more attention with closer follow-up, which might contribute to some of the improvement in symptomatology. Not all studies provided information on which specific gene-variant alleles are tested, which is important given their differential impact on enzyme, target, or transporter expression, as well as ethnic variation in frequencies of these variants. Finally, most of the evidence supporting the regular use of commercial pharmacogenomic panels were primarily or solely performed by organizations and/or sponsors whose viability depends on the commercial success of the panels [4,5]. Similar potential bias and/or conflict of interest had been highlighted before [11,12]. In addition, Clinical Laboratory Improvement Amendments (CLIAs) regulations allow marketing of tests without proof of clinical validity and/or utility, and there is lack of oversight of studies by regulatory agencies or other independent qualified entity. These concerns have led to additional skepticism and negative perception of the evidence so far for clinical utility, and the recommendation that pharmacogenomic testing should not be part of the standard of psychiatric care [13].

The Center for Medical Service approved the Diagnosing Adverse Drug Reactions Registry (DART) in 2013 (ClinicalTrials.gov NCT01970709). DART is the first multicenter observational cohort study involving 250,000 patients. Rather than focusing on just psychiatric patients, the registry spans patients with all medical specialties, and the primary study objective is to evaluate whether the use of pharmacogenomic data will result in a meaningful change in the drug choice and/or dose. In addition, the study also aims to determine the relationship between adverse-drug reactions and patients' genotypes, as well as healthcare utilization (e.g., emergency room visits, hospitalizations). Pending results from this study should provide additional data and insight on the clinical utility of pharmacogenomic testing.

COST-EFFECTIVENESS OF PSYCHOPHARMACOGENOMIC TESTING

Health Technology Assessment (HTA) is commonly employed in European healthcare systems to evaluate innovative pharmacotherapeutic modalities and the associated reimbursement decision. Based on the principles of evidence-based medicine, HTA essentially compared two interventions from the perspective of benefits and costs [14,15]. In the current era of limited healthcare resource, cost-effectiveness is an important aspect to be evaluated beyond proof of clinical utility, even though it is either expected or perceived that precision medicine should be cost-effective because of either decreasing the time to clinical improvement and/or improving the extent of positive therapeutic outcome.

In the current healthcare environment, the randomized controlled clinical trial, comparing per-patient cost for specific clinical outcome between pharmacogenomics-guided therapies versus standard of care, is the gold standard considered by many investigators the only acceptable proof of cost-effectiveness. Although this approach had been successfully demonstrated for the proton pump inhibitors [16], the study design associated with showing this direct cost-saving impact remains, most of the time, cost-prohibitive. One can also assert that results from well-controlled and structured settings are not necessarily predictive

of outcomes in real-world setting. Therefore, alternative approaches need to be considered for assessing the value of pharmacogenomic implementation. One such approach is utilization of real-world claim data from different clinical databases to compare direct health costs between usual care versus the care when clinicians and/or patients have access to pharmacogenomics test results.

Within psychopharmacogenomics, several economic studies utilize claims data to assess the cost-effectiveness of combinatorial pharmacogenomics. A blinded retrospective study by Winner et al. first demonstrated that in 96 depressed patients, individuals who are at risk for adverse drug-related outcomes, because of receiving a drug identified by the combinatorial pharmacogenomic interpretive report as problematic, had 67% more-frequent medical visits. These patients had 69% higher total healthcare costs, and three- and four-fold greater numbers of medically related absence days and disability claims, respectively, compared to patients receiving "non-problematic" drugs, with an economic translation of approximately $5,200 difference in healthcare expenditure between the two groups [17]. Nevertheless, the absence of cost-effectiveness measures other than increased cost associated with the "problematic" drugs could be perceived as a study limitation.

In a much larger, yet retrospective, analysis of patients' health claims data, Fagerness et al. evaluated direct health costs associated with availability of pharmacogenomic information to clinicians and patients. They reported an outpatient cost saving of $562 per patient over a four-month time period in 227 case patients who received genetic testing, compared to 454 propensity score-matched control patients who received standard of care. In addition, patients who were provided genetic testing were found to be more adherent (6.3% increase in adherence rate) to their medications, as compared to a 0.3% increase with patients receiving standard of care [18]. This is relevant because, in general, healthcare cost reduction is associated with increased medication adherence. Surprisingly, pharmacy cost was found to increase in both study groups. However, the increase for the case patients could be related to the group having more prescription refills because of adhering to their medications [18]. The use of propensity score matching in the study enables adjusting for confounding variables (for example, payer type, practitioners' specialty) and minimizes potential bias. This retrospective analysis demonstrates that even observational studies utilizing real-world claim data could be used to form a foundation for further assessment of evidence of clinical and economic benefits of pharmacogenomic testing and ultimately more-extensive clinical adoption.

In a 1-year prospective evaluation of cost saving, Winner et al. also reported lower pharmacy cost (medication expenditure) of $1,035.60 per patient in the pharmacogenomics-guided group (n = 2168), compared to the propensity-matched standard-of-care control group (n = 10,880) ($P = .007$). In addition, the guided group also showed improved medication adherence compared to the standard-of-care group ($P < .0001$) [19]. Interestingly, the total medication saving comprised $321.36 for psychiatric medications with the remaining $714.24 accounted for by nonpsychiatric medications, and the cost per quality-adjusted life-year (QALY) associated with the combinatorial pharmacogenomic approach was not estimated. In a subsequent subanalysis of the 2,168 pharmacogenomics-guided patients, Brown et al. also focused on evaluating cost-saving and reported positive results with the combinatorial approach for psychiatric patients managed by their primary care physicians [20]. However, it is unclear the extent of utilization of the pharmacogenomic information by the primary care physicians.

In addition to evaluating cost, another important parameter for assessing cost-effectiveness is to determine QALYs. In analyzing the three prospective clinical studies that provided evidence

of clinical validity and utility of combinatorial pharmacogenomic testing [4–6,21], Hornberger et al. evaluated cost-effectiveness (quality of life, and direct and indirect cost savings) related to the three studies. They concluded that the combinatorial approach not only improved treatment response rate by 70% compared to standard of care, but also increased QALY by 0.316 years. The expected savings per patient over lifetime amounts to $3,711 in direct medical cost and indirect cost of $2,553 related to work productivity [22]. Although it could be argued that the projected cost savings were based on study results from primarily open-label, nonrandomized studies, the study by Hornberger et al. [22] nevertheless provided more robust cost-effectiveness data for interpretation of the three studies.

Compared to cost-effectiveness studies reviewed here for antidepressants, the literature data for antipsychotics are relatively few. Herbild et al. [23] recently conducted a prospective trial and reported that genotyping for *CYP2D6* and *CYP2C19* resulted in 28% cost reduction in healthcare costs among schizophrenic patients with extreme metabolic phenotypes (PM and UM) and randomized to receive the pharmacogenomic intervention (95% CI: 0.53, 1.13). The cost-reduction result is similar to that shown with the pilot data from Chou et al., which show higher healthcare cost in PMs and UMs compared to patients with normal metabolic capacity [24]. However, neither of these studies evaluated treatment response.

Many of the studies just described [4–6,18,19] were included in a recent evidence-based critical review of clinical outcome and cost-effectiveness of pharmacogenomics-guided antidepressant treatment [25]. Among the two randomized controlled trials [6,9] and five controlled cohort studies reviewed [4–6,18,19,26], the study by Singh [9] demonstrated improved outcome (one additional remitting patient in 12 weeks per three genotyped) and reduced tolerability. On the other hand, the study by Winner et al. [6] was determined not to significantly improve remission with inconclusive evidence on tolerability, perhaps a reflection of its small sample size. Although the cohort-controlled study by Breitenstein et al. [26] also showed improved outcome (one additional remitting patient in 5 weeks per three genotyped), no tolerability data were reported. As demonstrated by the evidence review, a major limitation of clinical utility evaluations of combinatorial pharmacogenomic panel is based on a change in depression scores from baseline and not improving remission, with no study evaluating the impact on the time course of the clinical outcome. This is also evident in the most-recent randomized controlled trial [27]. In addition, only two studies evaluated outcome of test implementation by healthcare providers [6,27]. With respect to cost-effectiveness, Peterson et al. [25] reported no clear evidence for cost-effectiveness from the studies reviewed, as there was a lack of prospective or retrospective comparison of directly observed cost-effectiveness data in depressed patients, *rather than* cost savings and claim data, between the genomic testing and standard-of-care cohorts.

LESSONS LEARNED AND MOVING FORWARD

Although the literature provides more than sufficient evidence of the value of pharmacogenomics in optimizing drug therapy, widespread adoption of pharmacogenomic testing in practice needs more than just clinical benefits. Economic benefits in both direct and indirect costs associated with therapeutic failure, improved quality of life, and enhanced medication adherence should ideally be demonstrated. Yet no uniform agreement exists in what should be the scope of economic evaluations of pharmacogenomic testing. For example, is demonstration of value of testing genetic variants before drug administration compared to no testing (in

essence the intrinsic value of the test itself) sufficient? On the other hand, would it be reasonable to expect a demonstration of the value of testing genetic variants with the drug compared to other treatment modalities? In addition, how should the impact of an improper diagnosis, especially psychiatric in nature, be incorporated into any economic model to analyze the cost-effectiveness?

In addition, the review of evidence presented in the aforementioned sections also identified some gaps in economic evaluations and/or issues for consideration. First, undoubtedly the adherence of the clinicians to the pharmacogenomic test results and recommendations would have an impact on the effectiveness of the tests and/or decision-making regarding implementation, but are seldom addressed in cost-effectiveness analysis. Furthermore, the adherence to recommendation would likely be affected by the time frame of test results availability for decision-making but the information for test results turnaround time is seldom provided or assumed immediately available, which is unrealistic. Second, most of the evaluations focus on cost-effectiveness analysis at the time of the study, without considering potential impacts on future health costs. For example, the simple case of specific CYP-variant alleles identified for any patient would be valuable information that can be used to optimize future therapy for that same individual. Yet that potential cost saving is seldom incorporated into economic analysis of pharmacogenomic data, and the impact of this potential future use of genetic information will be compounded several fold with the combinatorial pharmacogenomic testing approach discussed in earlier sections.

Third, is it realistic to require clinical utility and cost-effectiveness evidence for every genetic variant? Currently HLA testing of variants for risk of Steven-Johnson Syndrome in allopurinol-treated patients faces an up-hill battle, whereas similar testing is acceptable and reimbursed by most payers without hard evidence of clinical utility and cost-effectiveness until 2008 (for abacavir) and 2011 (for carbamazepine). Another related example would be that are specific or highly prevalent for one specific ethnic group, e.g., *CYP2C9*8* for warfarin in patients with African ancestry. Fourth, when applicable, the issue of variation among study populations and/or disease type needs to be considered for study design, as cost-effectiveness could be also dependent on genetic variation for specific variant, even among study populations residing in the same geographical location. For example, testing for *HLA-B*15:02* was cost-effective for Chinese and Malays but not for Indians, despite that they all reside in Singapore [28], which is likely related to the difference in allele frequency of among the three populations.

Finally, the disease type might also affect the cost-effectiveness of genotyping. The study by Rattanvipapong et al. suggested that testing for *HLA-B*15:02* was cost-effective for management of seizure but not for neuropathic pain [29]. This disease impact on health-related costs associated with *HLA-B*15:02* screening was consistent with other studies when considering the use of carbamazepine versus other antiepileptic agents [30,31]. These results highlight the relevance of the prevalence of alleles of interest in specific populations [28] and/or disease types [29–31] when evaluating potential cost benefits associated with genetic screening. With a correlation between cost saving and allele prevalence, the importance of considering allele prevalence when assessing cost-effectiveness has also been demonstrated for *HLA-B*57:01* screening [32], which would be relevant for populations and/or geographic areas with low *HLA-B*57:01* prevalence [32,33].

Even with the previous gaps and/or issues, current literature suggests a role for pharmacogenomics-guided information to allow clinicians to tailor antidepressant treatment to attempt optimizing response as well as minimizing toxicity and increasing medication

adherence. Even though most of the available utility and cost-effectiveness is associated with some of the usual study limitations, such as lack of randomization and blinding, baseline differences between study groups, and inclusion of multiple diagnoses [4,5,34], these arguably represent intrinsic components of routine clinical practice. As such, these study results should not be simply dismissed as low-quality data that cannot be used for supporting a role of pharmacogenomic panel for optimization of drug therapy. Nevertheless, the level of evidence currently demanded by many investigators is, on one hand, rigorous but yet not very well defined in terms of specificity. What is clear is that, given no unified agreement as to what should be assessed in clinical utility and cost-effectiveness data [22,25,35,36], perhaps a silver lining of the critical review of current literature on pharmacogenomics-guided antidepressant treatment is the highlighting of what still needs to be done for confirming clinical utility with improved health outcomes and demonstrating evidence-based cost-effectiveness [25].

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CHAPTER

15

Pharmacogenomics Education and Clinical Practice Guidelines

Roseann S. Gammal[1,3], *Henry M. Dunnenberger*[2], *Kelly E. Caudle*[3], *Jesse J. Swen*[4]

[1]Department of Pharmacy Practice, MCPHS University, Boston, MA, United States; [2]Pharmacogenomics, Center for Molecular Medicine, NorthShore University Health System, Evanston, IL, United States; [3]Pharmaceutical Sciences Department, St. Jude Children's Research Hospital, Memphis, TN, United States; [4]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands

OUTLINE

Pharmacogenomics, Second Edition
https://doi.org/10.1016/B978-0-12-812626-4.00015-2

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Implementation of pharmacogenomics in the clinic has not kept pace with the rate of scientific discovery of clinically relevant gene–drug interactions. Many challenges have slowed progress in this arena and are broad in scope, pertaining to evidence, cost, infrastructure, test interpretation, and education, among others [1–3]. In particular, the process of translating a genotype result into a clinical action can be complicated, even with appropriate training. Therefore, evidence-based clinical practice guidelines are necessary to facilitate routine use of pharmacogenetics test results in patient care. Some argue that one of the greatest challenges to integrating personalized medicine into healthcare is the lack of education and awareness among providers and patients [2,4]. Providers require education to understand how to effectively apply pharmacogenomics guidelines to clinical practice, which includes using that information in the context of other clinical variables. Patients need to be informed about the availability, utility, and limitations of pharmacogenetics tests, as well as about the implications of their specific test results. Current implementation efforts clearly demonstrate that clinical practice guidelines and clinician/patient education are critical to the advancement of precision medicine in everyday clinical practice [5–7]. In this chapter, we will explore currently available international consensus guidelines for pharmacogenomics as well as educational strategies for both clinicians and patients.

CLINICAL PRACTICE GUIDELINES

The application of pharmacogenomics in clinical practice requires the interpretation of a pharmacogenetics test result followed by a translation into clinical action. Although this process may appear trivial at first glance, 95% of clinicians indicate that this process is one of the most challenging aspects of implementing pharmacogenomics in routine clinical care [4]. In recent years, the number of pharmacogenomics publications and amount of pharmacogenomics information in drug labels [8] has increased at an unprecedented pace, making it impossible for any individual to keep fully up-to-date. To assist clinicians in making the best pharmacogenomics-informed treatment decisions for their patients, the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have developed clinical practice guidelines. These guidelines are based on systematic literature review and expert opinion and aim to provide evidence-based recommendations regarding selection of drug and dose based on genetics. Differences in genetic test interpretation and clinical recommendations exist between these guidelines and are discussed in a recent publication [9].

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines

Background

The Clinical Pharmacogenetics Implementation Consortium (CPIC) was established in 2009 and is a resource of the Pharmacogenomics Research Network (PGRN) (www.pgrn.org) [4,10]. CPIC is an international consortium of over 250 clinicians and scientists from over 150 institutions and 23 countries, who provide actionable, genotype-based prescribing recommendations. As of May 2018, CPIC has published 20 clinical practice guidelines including recommendations for 20 genes covering over 40 drugs (Table 15.1) and are freely available on the CPIC website (www.cpicpgx.org).

An important distinction separates CPIC guidelines from other clinical society guidelines that address pharmacogenetics testing. CPIC guidelines are developed to help clinicians understand how available genetic test results should be used to guide drug therapy (i.e., selection of alternative dosing or medications) and not whether to order a genetic test. For

TABLE 15.1 Genes/Drugs With Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines and/or Dutch Pharmacogenetics Working Group (DPWG) Guidelines as of May 2018

Gene	Drug/Drug Class	CPIC Guideline?[a]	DPWG Guideline?
CACNA1S	volatile anesthetic agents and succinylcholine	In progress	No
CFTR	ivacaftor	Yes	No
CYP1A2	clozapine	No	Yes
CYP2B6	efavirenz	In progress	Yes
CYP2C9	phenytoin	Yes	Yes
CYP2C9	warfarin	Yes	Yes
CYP2C9	acenocoumarol	No	Yes
CYP2C9	celecoxib	In progress	No
CYP2C9	phenprocoumon	No	Yes
CYP2C19	clopidogrel	Yes	Yes
CYP2C19	PPIs	In progress	Yes
CYP2C19	SSRIs	Yes	Yes
CYP2C19	TCAs	Yes	Yes
CYP2C19	voriconazole	Yes	Yes
CYP2D6	5-HT3 receptor antagonists	Yes	No
CYP2D6	antipsychotics	No	Yes
CYP2D6	atomoxetine	In progress	Yes
CYP2D6	codeine	Yes	Yes
CYP2D6	eliglustat	No	Yes
CYP2D6	flecainide	No	Yes
CYP2D6	metoprolol	No	Yes
CYP2D6	oxycodone	Yes	Yes
CYP2D6	pimozide	No	Yes
CYP2D6	propafenone	No	Yes
CYP2D6	SSRIs	Yes	Yes
CYP2D6	tamoxifen	Yes	Yes
CYP2D6	TCAs	Yes	Yes
CYP2D6	tramadol	Yes	Yes
CYP2D6	venlafaxine	No	Yes
CYP3A5	tacrolimus	Yes	Yes

Continued

TABLE 15.1 Genes/Drugs With Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines and/or Dutch Pharmacogenetics Working Group (DPWG) Guidelines as of May 2018—cont'd

Gene	Drug/Drug Class	CPIC Guideline?[a]	DPWG Guideline?
CYP4F2	warfarin	Yes	No
DPYD	fluoropyrimidines	Yes	Yes
FVL	hormonal contraceptives	No	Yes
G6PD	rasburicase	Yes	No
HLA-A	carbamazepine	Yes	In progress
HLA-B	abacavir	Yes	Yes
HLA-B	allopurinol	Yes	Yes
HLA-B	carbamazepine	Yes	Yes
HLA-B	flucloxacillin	No	Yes
HLA-B	phenytoin	Yes	Yes
IFNL3	peginterferon alfa-2a	Yes	No
NUDT15	thiopurines	Yes	No
RYR1	volatile anesthetic agents and succinylcholine	In progress	No
SLCO1B1	atorvastatin	No	Yes
SLCO1B1	simvastatin	Yes	Yes
TPMT	thiopurines	Yes	Yes
UGT1A1	atazanavir	Yes	No
UGT1A1	irinotecan	No	Yes
VKORC1	acenocoumarol	No	Yes
VKORC1	phenprocoumon	No	Yes
VKORC1	warfarin	Yes	Yes

5-HT3, 5-hydroxytryptamine type 3; *FVL*, factor V Leiden; *PPI*, proton pump inhibitor; *SSRI*, selective serotonin reuptake inhibitor; *TCA*, tricyclic antidepressant.

[a] *For a full list of CPIC guidelines, see https://cpicpgx.org/guidelines/.*

example, CPIC's guideline for *CYP2C19*/voriconazole offers genotype-based voriconazole-prescribing recommendations for patients with a known *CYP2C19* genotype [11]. Clinicians are faced with having patients' genotypes available even if they did not order the test with the drug in mind (i.e., preemptive genotyping, direct-to-consumer testing, etc.), and CPIC guidelines provide clinical recommendations on how to use this information to inform prescribing.

Guideline Writing Process

As described by Caudle et al. [12], the CPIC guideline-development process uses established methods that closely follow the Institute of Medicine's Standards for Developing Trustworthy Clinical Practice Guidelines [13]. The guideline development process includes a rigorous evidence review and grading of the relevant scientific literature, input of a writing committee composed of experts in the guideline subject including clinicians

and basic science researchers, and an extensive pre- and postsubmission peer-review approval process. The CPIC Steering Committee and the CPIC Director provide oversight for the guideline development process and manage and approve any potential conflicts of interest. Experts with significant financial conflicts are not included on the writing committee and any potential conflicts of interest are reported in the guideline. Published guidelines are updated whenever critical new evidence emerges that changes test interpretation or prescribing recommendations. On an ongoing basis, but at least every two years, CPIC documents the date last reviewed on the CPIC site and any immediate changes to guidelines are posted online. For example, the FDA-approved drug label for ivacaftor was updated in 2014 and 2017 to include additional variants that were not included the 2014 CPIC guideline [14]. These changes are documented on the CPIC guideline webpage (https://cpicpgx.org/guidelines/guideline-for-ivacaftor-and-cftr/). CPIC guideline users should regularly check the CPIC website for updates to the guidelines.

Guideline Components and Recommendations

Each CPIC guideline adheres to a standard format and includes detailed information for:

- interpretation of the genetic test
- incidental findings (i.e., diseases or conditions that have or have not been linked to variation of the gene, unrelated to medication use)
- other considerations for critical issues about the gene or drug
- genotype-based prescribing
- evidence linking genetic variability to variability in drug-related phenotypes
- potential benefits and harm for the patient (i.e., the toxicities or adverse reactions that may be avoided by pharmacogenetics-based dosing) as well as any potential risks from incidental findings or use of alternative drugs or dosing (e.g., differences in efficacy)

Tables are also provided with information a clinician needs to translate patient-specific diplotypes into clinical phenotypes (e.g., CYP2D6 ultrarapid metabolizer) or drug-prescribing groups (e.g., HLA-B*57:01 positive) (see Table 15.2) and a phenotype-specific therapeutic recommendation (see Table 15.3). To assign strength to a recommendation, CPIC uses a transparent three-category system for rating recommendations. Therapeutic recommendations are graded as "strong" in which "the evidence is high quality and the desirable effects clearly outweigh the undesirable effects"; "moderate" in which "there is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects"; "optional" in which "the desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations and there is room for differences in opinion as to the need for the recommended course of action"; and "no recommendation" in which "there is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice at this time" [4]. Each recommendation also includes an assessment of its usefulness in pediatric patients [4].

Additional resources are available online and include allele definitions, frequencies of alleles in major racial/ethnic groups, allele functionality information, and additional tables that support the adoption of the CPIC guideline into the electronic health record (EHR) (see "Application in Clinical Practice" section below for more information).

Applications in Clinical Practice

CPIC guidelines are widely used by institutions implementing pharmacogenetics into clinical care (see https://cpicpgx.org/implementation/for list of institutions currently using CPIC guidelines as part of a program to facilitate use of genetic tests to guide prescribing). Not only do CPIC guidelines provide critical information needed to translate a patient's genotype into an actionable

TABLE 15.2 Example Diplotype to Phenotype Translation Table for a Clinical Pharmacogenetics Implementation Consortium Guideline

Likely Phenotype	Genotypes	Examples of *CYP2C19* Diplotypes
CYP2C19 ultrarapid metabolizer	An individual carrying two increased-function alleles	*17/*17
CYP2C19 normal metabolizer	An individual carrying two normal-function alleles	*1/*1
CYP2C19 intermediate metabolizer	An individual carrying one normal-function allele and one no-function allele, or one no-function allele and one increased-function allele	*1/*2,*1/*3,*2/*17
CYP2C19 poor metabolizer	An individual carrying two no-function alleles	*2/*2,*2/*3,*3/*3

Adapted from Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, Clinical Pharmacogenetics Implementation C. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clinical Pharmacology and Therapeutics 2013;94(3):317–323. https://doi.org/10.1038/clpt.2013.105.

TABLE 15.3 Example Phenotype to Clinical Recommendation Translation Table for a Clinical Pharmacogenetics Implementation Consortium Guideline

CYP2C19 Phenotype	Implications for Clopidogrel	Therapeutic Recommendations	Classification of Recommendations
CYP2C19 ultrarapid metabolizer (UM) and normal metabolizer (NM)	Normal (NM) or increased (UM) platelet inhibition; normal (NM) or decreased (UM) residual platelet aggregation	Use label-recommended dosage of clopidogrel	Strong
CYP2C19 intermediate metabolizer	Reduced platelet inhibition; increased residual platelet aggregation; increased risk for adverse cardiovascular events	Use alternative antiplatelet therapy (if no contraindication) (e.g., prasugrel or ticagrelor)	Moderate
CYP2C19 poor metabolizer	Significantly reduced platelet inhibition; increased residual platelet aggregation; increased risk for adverse cardiovascular events	Use alternative antiplatelet therapy (if no contraindication) (e.g., prasugrel or ticagrelor)	Strong

Adapted from Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, Clinical Pharmacogenetics Implementation C. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clinical Pharmacology and Therapeutics 2013;94(3):317–323. https://doi.org/10.1038/clpt.2013.105.

prescribing recommendation, but they also provide additional resources to support the adoption of CPIC guidelines into the EHR with clinical decision support (CDS) [15]. In 2013, CPIC formed an Informatics Working Group to create resources that support the translation of CPIC's recommendations into the clinical electronic environment. Resources include gene-specific information figures and tables that include full diplotype-to-phenotype

tables, diagram(s) that illustrate how pharmacogenetics test results could be entered into an EHR, example EHR consultation/genetic test interpretation language, and widely used nomenclature systems for genes relevant to the CPIC guideline. Furthermore, point-of-care resources are also provided such as diagrams that illustrate how point-of-care CDS should be entered into the EHR, example pre- and post-test alert language, and widely used nomenclature systems for drugs relevant to the CPIC guideline.

Dissemination and Impact

All CPIC guidelines are published in a specific journal (in partnership with *Clinical Pharmacology and Therapeutics*) with simultaneous posting to www.cpicpgx.org, in which they are regularly updated. Once published online, CPIC guidelines are also incorporated online at PharmGKB (www.pharmgkb.org) and publicized to the pharmacogenetics community via a blog posting. Each guideline has its own webpage with all tables and figures posted (https://cpicpgx.org/guidelines/and www.pharmgkb.org). CPIC guidelines are also freely available on guidelines.gov and PubMed Central and indexed in PubMed as clinical guidelines. Many of the CPIC guidelines are endorsed by the American Society of Health-System Pharmacists (https://www.ashp.org/) and the American Society for Clinical Pharmacology and Therapeutics (http://www.ascpt.org/), and referenced in Clinical Genome Resource (ClinGen; https://www.clinicalgenome.org/).

The Dutch Pharmacogenetics Working Group (DPWG) Guidelines

Background

Anticipating an imminent future in which both physicians and pharmacists would be confronted with patients with a known genotype, the Royal Dutch Pharmacists Association (KNMP) established the Dutch Pharmacogenetics Working Group (DPWG) in 2005 [16]. The main objectives of the DPWG are (1) to develop pharmacogenetics-informed therapeutic (dose) recommendations based on systematic literature review, and (2) to assist physicians and pharmacists by integrating the recommendations into computerized systems for drug prescription, dispensing, and automated medication surveillance. The DPWG is multidisciplinary and represented by (clinical) pharmacists, physicians, clinical pharmacologists, clinical chemists, epidemiologists, and toxicologists. Since 2005, the DPWG has systematically reviewed 86 potential gene–drug pairs of which 47 guidelines provide therapeutic recommendations for one or more aberrant phenotypes [17] (Table 15.1).

Guideline Writing Process

The guideline writing process consists of a number of steps. Members continuously propose gene–drug pairs and the compiled list is prioritized during group meetings that are organized four to six times per year. For selected gene–drug pairs, curators from the DPWG perform systematic literature searches in PubMed. Identified papers are assessed for two core parameters based on a previously described method [18]:

- Level of evidence of the gene–drug interaction. This indicates the quality of the evidence found in literature for the gene–drug interaction and is scored on a scale ranging from 0 (lowest evidence) to 4 (highest evidence).
- Clinical relevance of the potential adverse drug event (ADE). Clinical relevance is scored on a scale ranging from AA (nonsignificant clinical or pharmacokinetic effect) to F (death). This scale is originally derived from the National Cancer Institute Common Toxicity Criteria v2.0 and new events are added after assessment by the DPWG.

A draft score is prepared by the scientific curator, reviewed by two independent DPWG members, and discussed during DPWG meetings. Results are then used to synthesize a so-called risk evaluation report, presenting an overview of key findings of selected literature articles and scores of level of evidence and clinical relevance. After final assessment of the information presented in the report, it is then decided whether a gene–drug pair is indeed present and whether a therapeutic (dose) recommendation is necessitated. Composed therapeutic recommendations include (1) dose adjustments, (2) advice on therapeutic strategy (e.g., the advice for therapeutic drug monitoring or a warning for increased risk of ADE or diminished therapeutic efficacy), or (3) the recommendation to select an alternative drug. Gene–drug pairs are updated if required with a maximum interval of 5 yrs.

Calculation of Dose Adjustments

For a number of gene–drug pairs the DPWG provides specific dose recommendations. Calculation of these recommendations is based on the following rules:

- Only pharmacokinetic data from articles with a level of evidence of 3 or 4 are used.
- Statistically significant, as well as not statistically significant, data are used. However, dose recommendations are calculated only if statistically significant data are available.
- Dose calculations are based on the sum of parent drug and active metabolites (e.g., clomipramine + desmethylclomipramine, imipramine + desipramine, etc.)
- For prodrugs, pharmacokinetics of the active metabolite are used (e.g., morphine when codeine is used for analgesia).

The DPWG assumes that currently used standard doses are representative for normal metabolizers. For the calculation of dose adjustments for CYP2D6 poor-metabolizer (PM) phenotype (D_{pm}), we proceeded as follows. A dose adjustment was calculated from each individual article using the following formula:

$$D_{PM}(\%) = (AUC_{EM}/AUC_{PM}) * 100\,\%$$

After calculating dose adjustments for each individual article, a final dose recommendation was calculated as the population-size weighed mean of the individual dose adjustments.

$$D_{PM}(\%) = \frac{\left(N_{(a)} * D_{PM(a)}\right) + \left(N_{(b)} * D_{PM(b)}\right) + \left(N_{(c)} * D_{PM(c)}\right) \ldots + \left(N_{(x)} * D_{PM(n)}\right)}{N_{(a)} + N_{(b)} + N_{(c)} \ldots + N(x)}$$

N = number of subjects with corresponding phenotype in article a,b,c,…x.

Dose recommendations for other genotypes and phenotypes were calculated by using analogous equations with the exception of prodrugs (e.g., codeine for analgesia) and drugs with metabolites with unknown contribution to the clinical effect (e.g., tamoxifen).

Applications in Clinical Practice

The second objective of the DPWG is to assist physicians and pharmacists by integrating pharmacogenetics recommendations into computerized systems for drug prescription. In The Netherlands, the G-standard, a unique national drug database, is used by all electronic prescribing and medication surveillance systems. The information in the G-standard supports the prescribing, dispensing, ordering, and reimbursement of drugs, and is used by physicians, pharmacists, health insurers, government, and drug wholesalers in The Netherlands (https://www.knmp.nl/producten-en-diensten/gebruiksrecht-g-standaard/informatie-over-de-g-standaard/the-g-standaard-the-medicines-standard-in-healthcare). By integrating the DPWG guidelines with the G-standard, the guidelines are available at point of care throughout the entire country. As soon as a physician or pharmacist records a patient's genetic

information in the electronic medical record and prescribes a drug with a relevant DPWG recommendation, the system will fire an alert.

Dissemination and Impact

The DPWG guidelines are initially written in Dutch and distributed in The Netherlands. However, English versions of the DPWG guidelines have been published in 2008 [16] and 2011 [17], and a subset is currently available through the Pharmacogenomics Knowledgebase (PharmGKB) (www.pharmgkb.org). As part of the Ubiquitous Pharmacogenomics Project (U-PGx; www.upgx.eu), the DPWG guidelines have been translated by certified professionals into six other languages including English, German, Greek, Slovenian, Spanish, and Italian [19]. Within U-PGx, the DPWG guidelines are used to test the impact of a preemptive panel approach consisting of a panel of 50 variants in 13 pharmacogenes.

CLINICIAN EDUCATION

Clinician Education in the Healthcare Setting

Despite growing interest and awareness of pharmacogenomics, healthcare providers continue to report lack of confidence and preparedness in applying their pharmacogenomics knowledge to patient care [20–22]. Pharmacogenomics education programs need to be scalable and sustainable. Utilization of pharmacogenomics testing and referral services increased in the wake of education efforts, but the response was not sustained, highlighting the need for continual education [23]. Institutions commonly report using multiple methods for education because of the various types of material to be covered (e.g., test interpretation and deployment of new initiatives) and familiarity level with the content by the learner [24]. Education related to pharmacogenomics lies in two major domains: operational knowledge and clinical knowledge. Operational knowledge can be specific for an institution and targeted to specific workflows or initiatives. Clinical knowledge can be targeted to specific drug–gene pairs or general genomic information. These two domains facilitate implementation of pharmacogenomics by informing clinicians on both how and when to use pharmacogenomics. Regardless on which domain is focused, some features to consider when developing education are the needs of the target audience, delivery methods, and potential barriers and challenges.

Potential Needs

The potential educational needs of the target audience are dependent on multiple factors including: their care role, prior knowledge, and educational delivery method. There is extensive literature on education needs [3,25–27]. Less well described is the content needed to meet these needs. General information on pharmacogenomics is a reasonable starting point for any educational program and should include content regarding the clinical value and importance of pharmacogenomics and information regarding available resources (e.g., clinical practice guidelines). Clinicians report needing help communicating with patients about pharmacogenomics [3,24]. Providing predetermined answers for commonly asked patient questions can help reduce clinician resistance and increase clinical uptake. From the operational side, general information about what additional resources are available, such as consultant services and how to access these resources, is important. Once clinicians have been presented the potential value of pharmacogenomics, education on specific gene–drug pairs that is more detailed is more likely to be useful. For all providers involved in the therapeutic decision process, education needs to cover the clinical knowledge of the importance of a given pharmacogenomics interaction and how might the interaction

impact patient care. Operationally, these providers will need to know the next step in patient care in their system. Some clinicians may need to be competent in areas such as interpreting test results, drug metabolism, and ethical concerns related to genetic testing [28,29]. The level of detail provided for this clinical and operational education will depend on previous education and the delivery method.

Delivery Methods

There are various delivery methods for pharmacogenomics education in both domains. Independent of the delivery methods, practical examples such as case series can be used to teach both clinical and operational knowledge [30]. Providing a layered approach with increasing amount of details can ensure the needs of your entire audience are met regardless of the delivery method. In-person education can be in the form of lectures such as Grand Rounds, interactive practice site-based educational meetings, and focused one-on-one sessions. These in-person trainings can cover both operational and clinical knowledge. Some of these activities may be recorded and stored for future On-Demand use. This provides some scalability for these resource-intensive methods. On-Demand resources, including FAQs and online training modules, can provide clinicians a way to learn at their desired time and pace. These resources can be updated over time as new content is generated and reused, particularly as staff evolves in size and composition. For operational knowledge, timely emails or handouts can be used to alert clinicians to upcoming changes to workflow or functionality.

Clinical decision support (CDS) tools offer a way to integrate education into the EHR and routine clinical practice. These tools allow clinicians to receive education at a time when it might be most impactful: alongside patient-care activities. CDS tools can be passive or active. Passive CDS require clinicians to seek them out and are used to provide more information and context around a specific pharmacogenomics result [31]. Context-specific passive CDS such as "infobuttons" can connect the user to targeted external education, which may help to provide a layered educational approach [32,33]. Active CDS tools such as interruptive alerts are actively displayed to the user based on a predefined set of conditions [34]. They are a way to provide education in both domains in a just-in-time manner (Fig. 15.1). The content needed for effective active CDS includes patient-specific data, specific therapeutic recommendations, and evidence from a trustworthy source [35,36]. Groups such as CPIC Informatics Working Group (see earlier section), Implementing Genomics in Practice (IGNITE) Network, and the Electronic Medical Records and Genomics (eMERGE) Network help clinical implementation efforts by providing tools for creating passive and active CDS (Table 15.4).

Barriers and Challenges

Creating all of the necessary educational material is an enormous barrier to implementing pharmacogenomics. There are a number of online resources to help organizations develop this material (Table 15.5). Although clinical knowledge may easily be applied from online resources without modification, learning how and why another institution applied pharmacogenomics (operational knowledge) can also be valuable. Utilizing shared online resources are a key piece to a sustainable education program.

Once the materials are created, having the resources to deliver them is a challenge. Although methods that use humans as the primary mode for delivering education may be preferred by learners, they are resource intensive and difficult to scale [37]. Additionally, motivating clinicians to participate in education activities is commonly reported as a challenge for successful programs [24]. When these two challenges combine, an ineffective education program can be the result. For example, multiple institutions have reported low attendance at

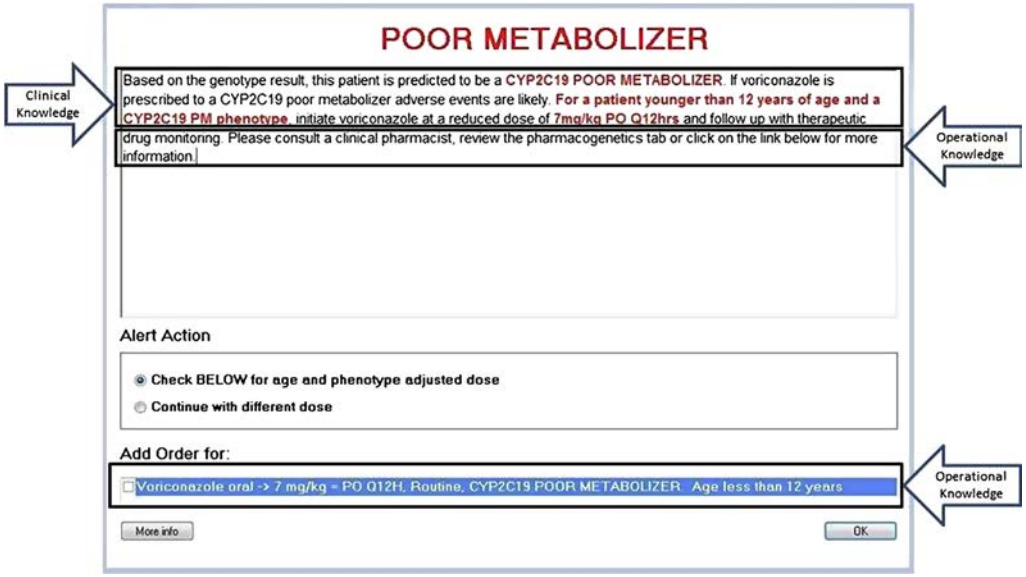


FIGURE 15.1 Example clinical decision support interruptive alert that contains both clinical and operational education.

TABLE 15.4 Clinical Decision Support Development Resources

Name	Description	Web Address
IGNITE Network SPARK Toolbox	The SPARK toolbox contains a variety of resources for implementing pharmacogenomics, including educational materials for clinicians.	https://ignite-genomics.org/spark-toolbox/
Clinical Decision Support Knowledgebase (CDS-KB)	This website contains resources for pharmacogenomics clinical decision support implementation created and shared by various contributors.	https://cdskb.org/
Clinical Pharmacogenetics Implementation Consortium (CPIC) Informatics	This website provides resources for building clinical decision support tools to aid in the application of CPIC guidelines.	https://cpicpgx.org/informatics/

IGNITE, implementing genomics in practice; *SPARK*, supporting practice through application, resources, and knowledge.

meetings and Grand Rounds, which can be time intensive to prepare and deliver [24].

Another challenge is determining the right time and place to deliver the education. For operational knowledge, if education is delivered too late, a clinician may be faced with a situation in which they cannot provide effective clinical care resulting from a clinician not knowing how to resolve an interruptive alert. If the education is delivered too early, the clinician may forget how to resolve the interruptive alert before it is encountered. Clinical education delivered too early may not be as impactful to the provider because it is not yet relevant. Clinical education delivered too late may result in an adverse outcome because a provider did not know the importance of pharmacogenomics. Equally important as timing is delivering education at the right pace. If a clinician is faced with a specific therapeutic interaction, providing general pharmacogenomics education may prove worthless and providing too-detailed education

TABLE 15.5 Provider-Focused Educational Content Resources

Name	Description	Web Address
Pharmacogenomics Knowledgebase (PharmGKB)	This resource is focused on providing clinicians and researchers comprehensive, curated pharmacogenomics information.	https://www.pharmgkb.org/
Genetics/Genomics Competency Center (G2C2)	This online repository houses genomic education materials for various healthcare professionals and these resources are mapped to discipline-specific competencies.	http://genomicseducation.net/
St. Jude Children's Research Hospital Pharmacogenetic Competencies	This website contains links to gene-specific pharmacogenetics competencies tailored to pharmacists.	https://www.stjude.org/research/clinical-trials/pg4kds-pharmaceutical-science/implementation-resources-for-professionals.html
American Society of Health-System Pharmacists (ASHP) Pharmacogenomics Resource	This website contains links to publications, presentations, and competencies related to pharmacogenomics.	https://www.ashp.org/Pharmacy-Practice/Resource-Centers/Emerging-Sciences/Pharmacogenomics
Ubiquitous Pharmacogenomics Consortium (U-PGx)	This website provides educational materials developed through the U-PGx project in Europe.	http://upgx.eu/category/documents/
Pharmacogenomics Education Program (PharmGenEd)	This is an evidence-based pharmacogenomics curriculum targeted to healthcare providers.	http://pharmacogenomics.ucsd.edu/

may cause a clinician to ignore or omit key points. Finding the optimum combination of effective education methods is a major challenge for developing a successful education program.

Clinician Education in Professional Schools

As clinical adoption of pharmacogenomics grows, healthcare providers with skills to integrate this knowledge into their practice will be important. Although on-the-job training (discussed previously) is important, training during professional school will most likely be compulsory in the future. Currently, pharmacogenomics is not routinely incorporated in the formal education of healthcare providers. There are a number of reasons for this including uncertainty in application in practice, lack of foundational knowledge, and lack of subject matter experts [24]. Given the rapid rate of new discoveries in the field, teaching each clinically relevant gene–drug pair may not be as important as teaching the skills needed to interpret and apply the published literature to patient care. For each healthcare profession, how to apply the knowledge and degree of mastery required may differ.

Pharmacy is taking a lead in integrating pharmacogenomics into professional school training. The number of pharmacy schools with pharmacogenomics in the curriculum has grown from 39% in 2005 to 89% in 2010 [38,39]. The Accreditation Council for Pharmacy Education added specific requirements related to pharmacogenomics in the 2016 standards [40]. To help clarify the educational content needed in the pharmacy school curriculum, multiple groups have described the role of the pharmacists in pharmacogenomics and/or created competencies [41–43]. Still, pharmacy education faces challenges, such as inconsistent implementation across the country, limited breadth of instruction,

and lack of faculty with teaching expertise in pharmacogenomics [44]. Pharmacogenomics content has been deployed in multiple types of courses, including as a standalone course, integrated in the therapeutics-focused courses, and integrated into laboratory-focused courses [39,45,46]. Novel and interactive teaching models have been used to teach pharmacogenomics include student genotyping, flipped classroom, case-based problems, and shared curriculums [46–48]. As seen with practicing pharmacist, a variety of approaches in a variety settings across the entire pharmacy curriculum are most likely required for effective pharmacogenomics education.

Although other professions such as medicine, nursing, and genetic counseling have not added specifics on pharmacogenomics to their accreditation standards, these professional schools are still faced with similar challenges as pharmacy education is today [49–51]. Clinical education of pharmacogenomics in all professional schools will continue to evolve as clinic implementation becomes more established into routine care, and better education at the professional school level will increase the capacity for pharmacogenomics to expand across care domains.

PATIENT EDUCATION

The successful implementation of pharmacogenomics into clinical practice requires not only adequate provider education, but patient education as well [2,27,52]. Consensus has yet to emerge regarding the content and delivery of pharmacogenomics-related patient education, but lessons can be learned from best practices shared by institutions that are actively implementing.

Patient education may occur prior to pharmacogenetics testing (to explain what it is, benefits vs. risks, limitations, cost considerations) and after (return of test results with interpretation and implications for pharmacotherapy). Currently, the general public's knowledge about pharmacogenomics is limited; however, once patients learn the basic principles, they value using test results to maximize medication efficacy and minimize adverse effects [53,54]. In the current US healthcare system, patient education about pharmacogenetics testing is particularly important because, unlike many other laboratory test results, these results may be useful throughout the patient's life. If a patient changes healthcare providers or systems, they may need to communicate their pharmacogenetics test results to other healthcare providers to ensure that future medications are selected and dosed using a gene-based approach.

There are several challenges associated with educating patients about pharmacogenomics that should be considered when developing effective educational strategies. The first is health literacy, which may vary considerably among patients and will have a significant impact on comprehension [55,56]. Complicating matters is the variable terminologies currently used in the field, though there has been a push in recent years to standardize pharmacogenetics terms [57]. Resources are available to guide clinicians in translating technical health information into helpful patient-education materials with appropriate readability for general audiences [58]. Pharmacogenomics is an ever-evolving field, so it is important that all patient-education materials not only be written/presented in layman's terms, but also revisited periodically for updates to reflect current evidence and practice.

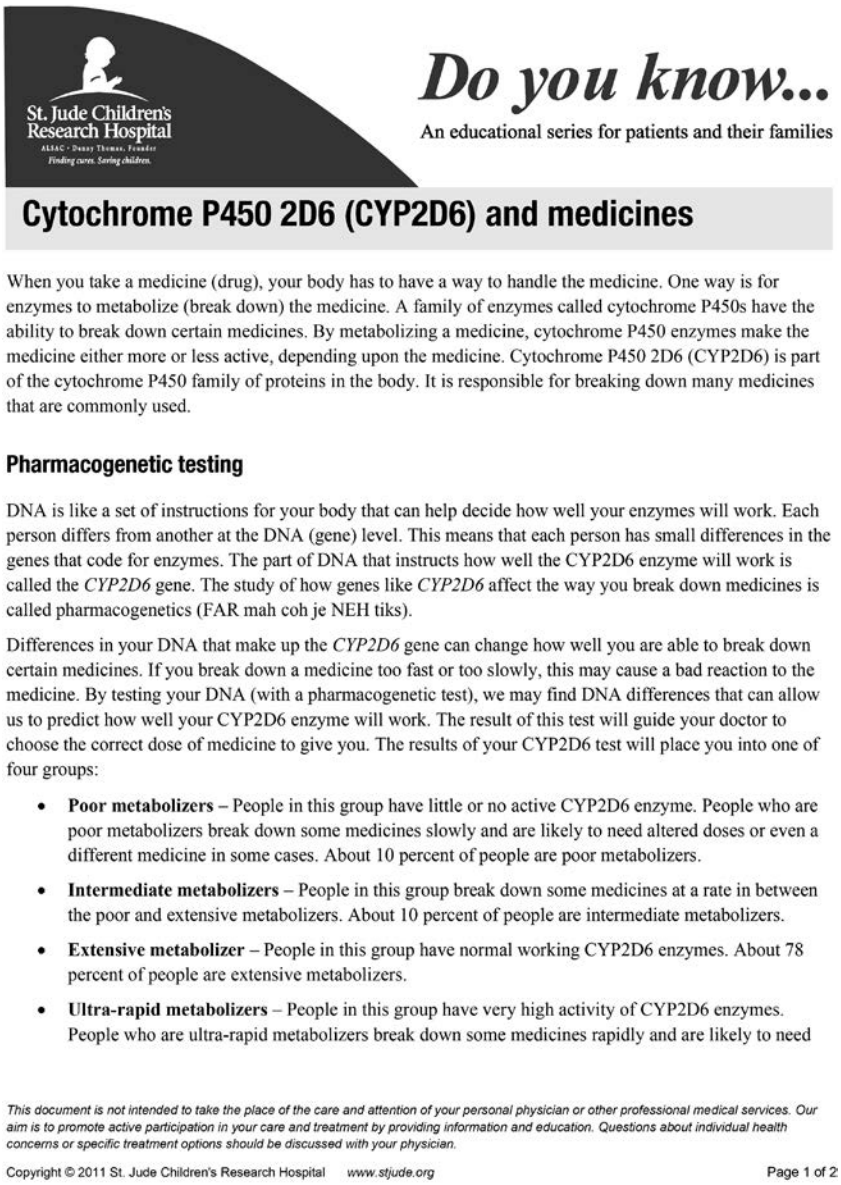
Genetic testing of any kind raises ethical and legal issues, which may prevent patients from participating. Patients may have preconceived notions about genetic testing, which can contribute to privacy, confidentiality, and discrimination concerns [53,54]. These concerns may be alleviated with adequate education by using a framework of clinical utility and data privacy [27]. It is good practice to

counsel patients on the differences between genetic testing for disease risk versus medication response, as well as the likelihood and types of incidental findings (less common with pharmacogenetic tests). In some cases, it may also be helpful to discuss antidiscrimination laws pertaining to genetic testing (e.g., the federal Genetic Information Nondiscrimination Act [GINA] of 2008).

With the diverse models for pharmacogenomics implementation come diverse patient-education strategies. Depending on the model of pharmacogenomics implementation (e.g., reactive vs. preemptive; research protocol vs. standard of care) and the setting (inpatient vs. outpatient; academic medical center vs. community hospital or clinic), the approach to patient education can vary. Some institutions may choose a primary educational modality; others may use several. A common approach is to have one or more formal pharmacogenomics counseling sessions to explain the clinical utility of testing, address any patient concerns, and/or to return test results [6,23,27,59]. If pharmacogenetic testing is implemented in the context of a research protocol, patient education may be integrated into the informed-consent process [7,60]. Patients prefer that pharmacogenomics information be delivered in a personalized way, by explaining results in the context of their current medications [55]. When discussing predicted drug response with patients, it is important to also include the impact of other relevant, nongenetic factors (e.g., drug–drug interactions, organ function, diet, etc.) [61]. Patient-education sessions are typically led by a pharmacist, physician, nurse, or genetic counselor. Pharmacists in particular are well-suited to counsel patients on the implications of genetic variation on drug therapy given their extensive training in pharmacology. Genetic counselors' expertise in additional aspects of genetic testing, including incidental findings and implications for family members, can also play a role in offering comprehensive patient counseling. A multidisciplinary, team-based approach to patient education may be preferred [61,62].

In addition to verbal communication, patient education of pharmacogenomics may be delivered through writing. In some models, pharmacogenetic test results are communicated to patients through mailed letters (which patients are encouraged to share with future providers) and/or through an online patient portal [7,63,64]. Through patient portals, pharmacogenetic testing laboratory reports may also be accessible to patients and, therefore, be a source of patient education. The way results are communicated in text and through visual representations in these reports can have a significant impact on patient understanding [65,66]. In addition, many institutions have also created custom pharmacogenomics information sheets (general or gene-specific; Fig. 15.2), which may be first screened by an advisory board for content and readability before dissemination to patients [7,27,59,63,67].

Technology allows for innovative patient-education strategies beyond the standard counseling sessions and written materials. Some institutions have explored the use of videos to explain pharmacogenetics testing [7,68]. As part of the Ubiquitous Pharmacogenomics Project in Europe, preemptive pharmacogenetic test results are provided to patients on a personal "Safety-Code Card" (Fig. 15.3) that displays an individual's results and a quick response (QR) code that is linked to web-based, patient-specific pharmacogenetics-dosing recommendations [19]. Furthermore, several online resources are available to educate the public about pharmacogenomics. The National Human Genome Research Institute (NHGRI)-funded Electronic Medical Records and Genomics (eMERGE) network created www.myresults.org, a website dedicated to "helping people understand genetic test results and to provide resources for making informed health decisions." The site contains drug-specific information pages for commonly used drugs affected by pharmacogenetics variants, including azathioprine,



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An educational series for patients and their families

Cytochrome P450 2D6 (CYP2D6) and medicines

When you take a medicine (drug), your body has to have a way to handle the medicine. One way is for enzymes to metabolize (break down) the medicine. A family of enzymes called cytochrome P450s have the ability to break down certain medicines. By metabolizing a medicine, cytochrome P450 enzymes make the medicine either more or less active, depending upon the medicine. Cytochrome P450 2D6 (CYP2D6) is part of the cytochrome P450 family of proteins in the body. It is responsible for breaking down many medicines that are commonly used.

Pharmacogenetic testing

DNA is like a set of instructions for your body that can help decide how well your enzymes will work. Each person differs from another at the DNA (gene) level. This means that each person has small differences in the genes that code for enzymes. The part of DNA that instructs how well the CYP2D6 enzyme will work is called the *CYP2D6* gene. The study of how genes like *CYP2D6* affect the way you break down medicines is called pharmacogenetics (FAR mah coh je NEH tiks).

Differences in your DNA that make up the *CYP2D6* gene can change how well you are able to break down certain medicines. If you break down a medicine too fast or too slowly, this may cause a bad reaction to the medicine. By testing your DNA (with a pharmacogenetic test), we may find DNA differences that can allow us to predict how well your CYP2D6 enzyme will work. The result of this test will guide your doctor to choose the correct dose of medicine to give you. The results of your CYP2D6 test will place you into one of four groups:

- **Poor metabolizers –** People in this group have little or no active CYP2D6 enzyme. People who are poor metabolizers break down some medicines slowly and are likely to need altered doses or even a different medicine in some cases. About 10 percent of people are poor metabolizers.
- **Intermediate metabolizers** – People in this group break down some medicines at a rate in between the poor and extensive metabolizers. About 10 percent of people are intermediate metabolizers.
- **Extensive metabolizer** – People in this group have normal working CYP2D6 enzymes. About 78 percent of people are extensive metabolizers.
- **Ultra-rapid metabolizers** – People in this group have very high activity of CYP2D6 enzymes. People who are ultra-rapid metabolizers break down some medicines rapidly and are likely to need

This document is not intended to take the place of the care and attention of your personal physician or other professional medical services. Our aim is to promote active participation in your care and treatment by providing information and education. Questions about individual health concerns or specific treatment options should be discussed with your physician.

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Revised 11/11

FIGURE 15.2 Example gene-specific patient information sheet from St. Jude Children's Research Hospital. *Copyright © St. Jude Children's Research Hospital 2012.*

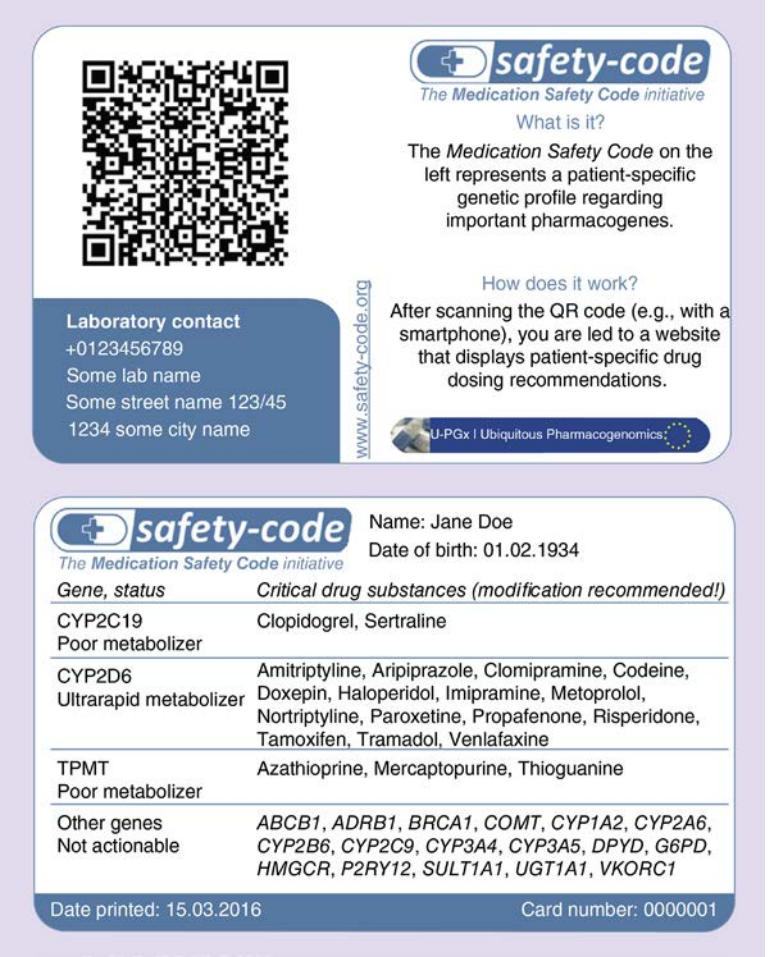

FIGURE 15.3 An example "safety-code card" from the European Ubiquitous Pharmacogenomics Project. The quick response (QR) code is linked to web-based, patient-specific pharmacogenetics dosing recommendations.

clopidogrel, mercaptopurine, simvastatin, carbamazepine, thioguanine, and warfarin, as well as a video library on a range of pharmacogenetics topics. The Pharmacogenomics Knowledgebase (PharmGKB; www.pharmgkb.org), a widely used online resource that curates pharmacogenetics variants and houses a wealth of pharmacogenetics data, also provides general pharmacogenomics information for lay audiences. Lastly, the Genetics Home Reference (https://ghr.nlm.nih.gov/), a website from the U.S. National Library of Medicine, provides information about issues related to precision medicine, including pharmacogenomics [69].

Patient education is, and always will be, a critical component of a successful pharmacogenomics implementation. Educating patients about pharmacogenomics and the implications of their test results facilitates patient-centered care and may also lead to better medication adherence. Many strategies have been developed to aid in this endeavor, including the use of in-person counseling, written materials, and technology.

CONCLUSION

Evidence-based clinical practice guidelines and clinician/patient education are cornerstones of effective pharmacogenomics implementation strategies. CPIC and DPWG guidelines are facilitating the adoption of pharmacogenomics in clinical practice by providing specific prescribing recommendations for clinically actionable gene–drug pairs and resources for integration of this information into EHRs. Lessons learned from current implementations reveal diverse approaches to educating clinicians and patients about pharmacogenetics testing.

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D

PHARMACOGENOMICS

CHALLENGES AND OPPORTUNITIES IN THERAPEUTIC IMPLEMENTATION

SECOND EDITION

Edited by

Y. W. FRANCIS LAM, Pharm.D., FCCP
Professor of Pharmacology, Minnie Stevens Piper Professor (2018), James O'Burke Endowed Centennial Fellow in Pharmacy, University of Texas Health Science Center, San Antonio, TX, USA

STUART A. SCOTT, Ph.D., FACMG
Associate Professor, Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; Division Head, Pharmacogenomics, Sema4, Stamford, CT, USA

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Pharmacogenomics: Challenges and Opportunities in Therapeutic Implementation, Second Edition provides comprehensive coverage of the challenges and opportunities facing the therapeutic implications of pharmacogenomics from academic, regulatory, pharmaceutical, socio-ethical and economic perspectives. While emphasis is on the limitations in moving the science into drug development and direct therapeutic applications, this book also focuses on clinical areas with successful applications and important initiatives that can further advance the discipline. New chapters cover important topics such as pharmacogenomic data technologies, clinical testing strategies, cost-effectiveness, and pharmacogenomic education and practice guidelines. The importance of ethnicity is also discussed, which highlights pharmacogenomic diversity across Latin American populations. With chapters written by interdisciplinary experts and containing insight into the future direction of the field, ***Pharmacogenomics: Challenges and Opportunities in Therapeutic Implementation, Second Edition*** is an indispensable resource for academic and industry scientists, graduate students and clinicians engaged in pharmacogenomics research and therapeutic implementation.

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