Clinical Rounds in Endocrinology

Volume II
Pediatric Endocrinology

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Anuradha Aggarwal
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Springer

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Dedicated
to
My beloved mother late Shrimati Munna Kumari Bhansali,
the inspiring force in my life
My father Shri ML Bhansali, the guiding light
of my life
My wife Sandhya, my pillar of strength who always
stood by me and
My loving children Shipra, Shobhit, and Akanksha

Anil Bhansali

Foreword

I feel humbled to take this opportunity to introduce the text that follows, which I am confident, will prove to be a cerebral feast for the readers. I know Dr. Bhansali as an astute clinician and dedicated academician and have expected his textbook to be a perfect combination of theory and practical medicine. I am glad that this textbook has stood up to the expectation.

This textbook covers all the significant disorders commonly encountered in pediatric endocrinology practice in 12 chapters, which include first two chapters on growth disorders followed by one chapter each on thyroid disorders, Cushing's syndrome, delayed and precocious puberty, Turner syndrome, rickets, congenital adrenal hyperplasia (CAH), disorders of sex development (DSD), and young diabetes and multiple endocrine neoplasia syndromes. Each chapter begins with a clinical case vignette followed by detailed description of the topic, presented as answers to questions of clinical relevance.

I feel the details covered in case vignette represents the proverbial "Well begun is half done." The cases are replete with complete details about clinical features, examination, diagnosis, and management. However, the outstanding feature is the discussion of differential diagnosis, with pertinent arguments for and against each differential, which will immediately make both the practicing endocrinologists and trainees to feel familiar with the essential logical navigation. I am sure; it would definitely enhance their clinical approach to these disorders.

The patients' photographs are well representative and give a lively clinical experience to readers. The discussion of the topic is enriched with well-illustrated diagrams and informative algorithmic flowcharts. Moreover, the underlying physiology is explained at such places that relevance of clinical findings is enhanced. The contrasting features in related disorders are brought out well in tabulated forms for easy understanding. To name a few, there are good tables comparing features of different growth charts, merits and demerits of different GH stimulation tests, and differential features of various DSDs. Most importantly, Indian normative references are given, for example, those on age-specific reference range for testicular volume and stretched penile length to suit the readers in Indian scenario. This text is abreast with updated information on recent developments like discussion on suitability of IAP 2015 growth charts. Practical information on certain topics like that on neonatal screening of CAH and management of CKD-MBD is particularly helpful.

On the whole, I believe this book is a "must have" for endocrine trainees and practicing pediatric endocrinologists alike. It provides a well-abridged quick referral which will certainly enhance clinical approach to pediatric endocrine disorders and benefit the patients at large.

I would like to complement and thank Dr. Bhansali and his colleagues whose relentless efforts have fructified into such a well-written book.

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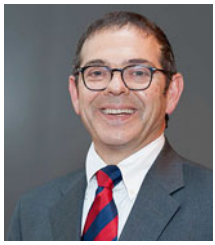
Foreword



It is with great pleasure that I write a foreword to this book on Pediatric Endocrinology as part of the *Clinical Rounds in Endocrinology* series. This book is comprised of an impressive series of chapters covering growth disorders, puberty, thyroid, adrenal, rickets, Turner syndrome, endocrine neoplasia, and diabetes. Adult manifestations of pediatric endocrine disorders are also covered. The structure of the chapters is unusually lively with a case vignette, a detailed stepwise analysis, and a series of short questions/answers covering physiology, pathophysiology, diagnosis, management, and treatment. Illustrative short cases are often presented as part of the chapters. The chapters are richly illustrated by patient photos, imaging, figures, tables, and decision algorithms, helping the reader to rapidly grasp the key messages. Some but not all the chapters also have pros and cons of the various treatment options, for instance management of hypogonadism at puberty. This book will be of interest to all those interested in pediatric endocrinology. For the beginner, this book escapes the traditional textbook format, but its wide series of questions covers all aspects of the topics covered and allow a comprehensive overview. For those who are already acquainted with pediatric endocrinology, this book is up to date with recent references, and I am positive that there will be something for everyone there. Dr. Anil Bhansali and his colleagues are to be commended for achieving such a comprehensive and richly illustrated book that will be of interest not only to the endocrine community in India but also in other areas of the world.

Pr Jean-Claude Carel
March 2016
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Foreword

It is a pleasure to introduce this book on Pediatric Endocrinology to you. Its unique informal question-answer style sets it apart from the routine medical text. The questions asked are full of insight and reflect the years of teaching experience of the authors. Most chapters start off with a case vignette and relevant issues in pathology and physiology are woven around the cases. Management issues are taken up in great depth. Growth, puberty, and disorders of sex development, including congenital adrenal hyperplasia, the core areas of pediatric endocrinology, are covered in minute detail. Other relevant chapters include most issues of importance to those treating not only children and adolescents but also those caring for the young patient in transition to adulthood. The rich collection of patient photographs and flowcharts makes for easy clinical learning. This book will provide useful and refreshing reading for practitioners and teachers of pediatric endocrinology, endocrinology, pediatrics, and also clinical genetics and gynecology.

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Preface

Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, is the premier medical and research institute in India. This tertiary health-care center, right from its days of inception, has always been at the forefront in the field of medical sciences. The concept of endocrinology in India was originated from this institute, and endocrinology as a super-specialty department was established way back in 1964 at this institute by Professor G.K. Rastogi.

This department has an age-old tradition of grand academic rounds. Detailed discussions pertaining to every aspect of patient's care include right from analysis of symptoms, demonstration and interpretation of signs, critical appraisal of differentials, judicious use of investigations and their appropriate analysis, and finally optimal treatment strategies. This legacy of clinical rounds was inherited from my great teacher, Professor R.J. Dash, who had in-depth and enormous knowledge of the subject with a great ability to critically analyze it. Several thought-provoking questions were spontaneously generated during these interactive sessions, with inputs and suggestions by residents and views and counter-views by the faculty members making these clinical rounds a "sea of knowledge." Further, this continuous process of exchange of knowledge helped us in providing the best possible medical care to our patients and growth of this super-specialty in India.

I had a long-cherished dream to compile these clinical rounds in text form with precise information, comprehensive knowledge, and critical analysis of the facts to facilitate the dissemination of the knowledge to endocrinologists, physicians, pediatricians, and gynecologists.

Recently, there is a paradigm shift in the pattern of the books available in endocrinology with a focus on molecular endocrinology rather than on clinical endocrinology. It was decided to write a book in the "question–answer" format as this pattern not only simulates clinical rounds, but will also help to the health-care professionals in dealing with challenges faced by them in day-to-day practice. Incidentally, books in such format are not readily available, particularly in endocrinology.

The idea of writing this book was conceived, conceptualized, and formatted by me, with utmost caution to present the scientific facts in the most comprehensive manner and was amply supported by my team of coauthors: Dr. Girish Parthan, Dr. Anuradha Aggarwal, and Dr. Yashpal Gogate. Dr. Soham Mukherjee and Dr. Mandeep Singla worked untiringly with me for the last 1 year in reviewing the literature, adding clinical images, tables, and illustrations, and finally editing the

text to make the book in its present shape. Further, the decision of adding the case vignette was strongly propounded by Soham; otherwise, this book would have been incomplete. The whole process in itself was a great learning experience.

This book on pediatric endocrinology includes 12 chapters covering disorders of the pituitary, adrenal, thyroid, parathyroid, gonads, and diabetes. Most chapters begin with a case vignette, followed by a stepwise analysis of the case including diagnosis and management, and subsequently a series of question and answers. Another salient feature of this book is a multitude of clinical images, illustrations, tables, and algorithms for better understanding of the clinical problem.

We hope this endeavor will help health-care professionals to conceptualize the subject of endocrinology and will translate into better patient management.

Anil Bhansali
Anuradha Aggarwal
Girish Parthan
Yashpal Gogate

Acknowledgements

We are grateful to all those who have helped us in accomplishment of this endeavor. It is indeed difficult to name all who have contributed to this book, though a few names with a lion's share in the completion of this mammoth task are mentioned.

We are grateful to all of our patients who have helped us in learning clinical endocrinology, for without them this book would have never been written.

I, Dr. Anil Bhansali, thank all my colleagues including Dr. Sanjay Kumar Bhadada, Dr. Pinkai Dutta, Dr. Rama Walia, Dr. Ashu Rastogi, Dr Soham Mukherjee and Dr. Naresh Sachdeva for their valuable suggestions and continuous support throughout this journey. I heartily appreciate the relentless and selfless efforts made by Dr Soham Mukherjee to accomplish this dream and without his support, this would not have been achieved.

I also sincerely appreciate the effort of my coauthors Dr. Girish, Dr. Anuradha, and Dr. Yashpal for their untiring and immense contribution in making this book in the present form. They have indeed inculcated the "soul" into it.

My sincere and heartful thanks to Dr. Mandeep Singla for his relentless support and continuous encouragement during the entire period. I thank all my other residents including Dr. Abhishek Hajela, Dr. Suja P Sukumar, Dr. Kushdev Jariyal, Dr. Vikram Shekhawat, Dr. Pawan, and Dr. Anshita for their help and encouragement. I also thank Prof. B.R. Mittal and Dr. Anish Bhattacharya from the Department of Nuclear Medicine and Dr. Chirag Ahuja from the Department of Radiodiagnosis for their suggestions and worthy contributions.

We are grateful to our family members for their continuous support and perseverance; without that it would have been impossible to fulfill this dream. I, Dr. Anil Bhansali, sincerely express my gratitude and appreciation to my wife Sandhya and my children Shobhit, Shipra, and Akanksha who have supported me throughout this long journey to accomplish this venture. I am also thankful to my all brothers, Sunil, Raj Kumar and Aniruddh, and sisters, Madhu, Manju and Menu for their wholehearted support to accomplish this work. I really admire my friends, justice Hari Pal Verma and Harish Singla, for their continuous encouragement and support. I, Dr. Anuradha, sincerely thank my husband Dr. Vaibhav for his continuous support and cooperation in writing this book. I, Dr. Girish, sincerely thank my wife Dr. Rajlakshmi Iyer who has allowed me to accomplish this work untiringly. I, Dr. Yashpal Gogate, sincerely thank my wife Dr. Ketki for her persistent encouragement.

We are also thankful to Mrs. Anjali Aggarwal and Sanjay Kumar for designing the beautiful diagrams and editing the images. We appreciate the kind help extended by Mr. Abhijeet and Mr. Paramjeet for acquisition of the clinical images. We also thank Mrs. Rama Puri, Mrs. Usha Sharma, Mr. Mahabir Singh, and Mr. Surinder Pandey for their uninterrupted assistance throughout the period of writing this book.

We are also thankful to our publisher Springer and their team members Dr. Naren Aggarwal, Mrs. Teena Bedi, and Mr. Pradeep Kumar. Without them this book would have never been in the present form.

Finally, we are thankful to the Almighty for providing the wisdom, courage, and strength to complete this endeavor and for the fulfillment of this long-cherished dream.

Anil Bhansali
Anuradha Aggarwal
Girish Parthan
Yashpal Gogate

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Disorders of Growth and Development: Clinical Perspectives

1

1.1 Case Vignette

A 9-year-old boy presented with complaint of growth failure since 2 years of age. He was a product of non-consanguineous marriage and was delivered at term by normal vaginal delivery. His birth weight was 3.3 Kg and he had normal Apgar score. However, data of his birth length was not available. He had history of prolonged physiological jaundice, that lasted for 3 weeks and required phototherapy for its resolution. There was no history of any episode of hypoglycemia. His developmental milestones were normal, except delay in walking which was due to congenital dislocation of his left hip. The growth velocity data available, showed his height at 1 year of age was 65 cm, at second year 75 cm, and later at the age of 9 year it was 96 cm with annual growth velocity of approximately 3 cm/year from third year of age onward. He has good scholastic performance and now is studying in fourth standard. There was no history of any systemic illness, chronic diarrhea, drug intake (e.g., steroid), head injury, meningitis/encephalitis, headache, and visual defects. He had no history of fatigue, lethargy, irritability, somnolence, or constipation. His both parents were short and were at $<3^{rd}$ percentile. He has one sibling with normal history of growth and development. On examination, his height was 96 cm (−7 SDS, height age 3 years, target height 164 cm), upper and lower segment ratio 1.2, arm span 92 cm, weight 21.2 Kg (weight age 6 years), and BMI 23 Kg/m^2 ($>95^{th}$ percentile). He had cherubic face with frontal bossing, depressed nasal bridge, midfacial hypoplasia, low-set ears, and poor dentition with crowded teeth. He had no goiter. His blood pressure was 90/60 mmHg. He had bilateral palpable testes with testicular volume of 1 ml and stretch penile length of 2 cm with Tanner staging of A_1P_1. He had bilateral palpable testes with testicular volume of 1 ml and stretched penile length of 2 cm, and he had bilateral lipomastia. Systemic examination was unremarkable except shortening of his left lower limb with restriction of movement at the left hip joint. On investigations, his hemoglobin was 10 g/dl with normal total and differential leukocyte counts. Renal and liver function tests, electrolytes (K^+ and

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HCO_3^-), calcium profile, and IgA tTG were normal. Hormonal profile showed serum T_4 6.7 μg/dl (N 4.8–12.7), TSH 4.6 μIU/ml (N 0.27–4.2), 0800h cortisol 140 nmol/L (N 171–536), LH <0.1 mIU/ml (N 1.7–8.6), FSH 0.52 mIU/ml (N 1.5–12.4), testosterone <0.08 nmol/L (N 9.9–27.8), prolactin 5.07 ng/ml (N 4–15.2), and IGF1 50 ng/ml (N 58–401). GH response to insulin-induced hypoglycemia and clonidine stimulation test, after priming with estrogen, were performed and showed subnormal peak GH response to both these stimuli (<0.03 ng/ml for both). Peak cortisol response to insulin-induced hypoglycemia was also suboptimal (150 nmol/L). His bone age was 7 years. CEMRI sella showed small pituitary with normal midline stalk and eutopic posterior pituitary bright spot. X-ray pelvis showed dislocation of left hip joint. With this profile, a diagnosis of multiple pituitary hormone deficiency (GH and ACTH) was considered, and patient was initiated with rhGH at doses of 0.3 mg/Kg/week and hydrocortisone 2.5 mg twice a day. With this treatment, the growth velocity during first year was 11 cm and during second year was 14 cm, and after 2 years of initiation of rhGH therapy, the height increased from 96 to 121 cm. His weight increased from 21 to 41 Kg, possibly due to immobilization during his surgery for hip dislocation. After 3 months of initiation of rhGH, the serum T_4 level declined to 5.2 μg/dl and he was initiated with 50 μg/day of L-thyroxine. Serum IGF1 levels were 378 ng/ml (N 70–458) and 251 ng/ml (N 82–516) after first and second years of therapy, respectively. No adverse event was noted during the course of therapy (Fig. 1.1).

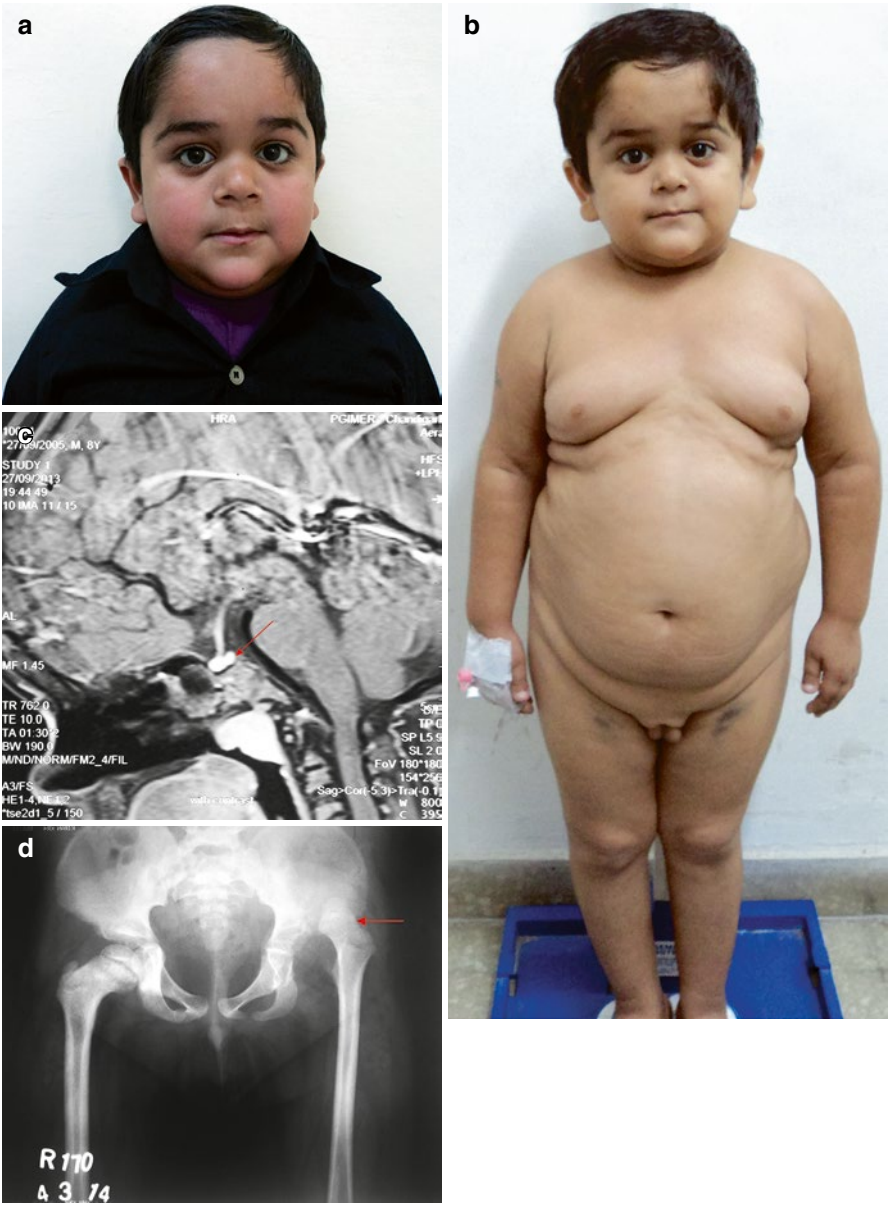

Fig. 1.1 A child with GH deficiency. Note (**a**) cherubic face, frontal bossing and midfacial hypoplasia (**b**) short stature, micropenis, and lipomastia. (**c**) CEMRI sella showing hypoplastic pituitary (*red arrow*). (**d**) X-ray pelvis demonstrating congenital hip dislocation (*red arrow*). (**e**, **f**) Improvement in facial features and height gain after 2 years of rhGH therapy. (**g**) Growth chart showing catch-up growth after initiation of rhGH therapy

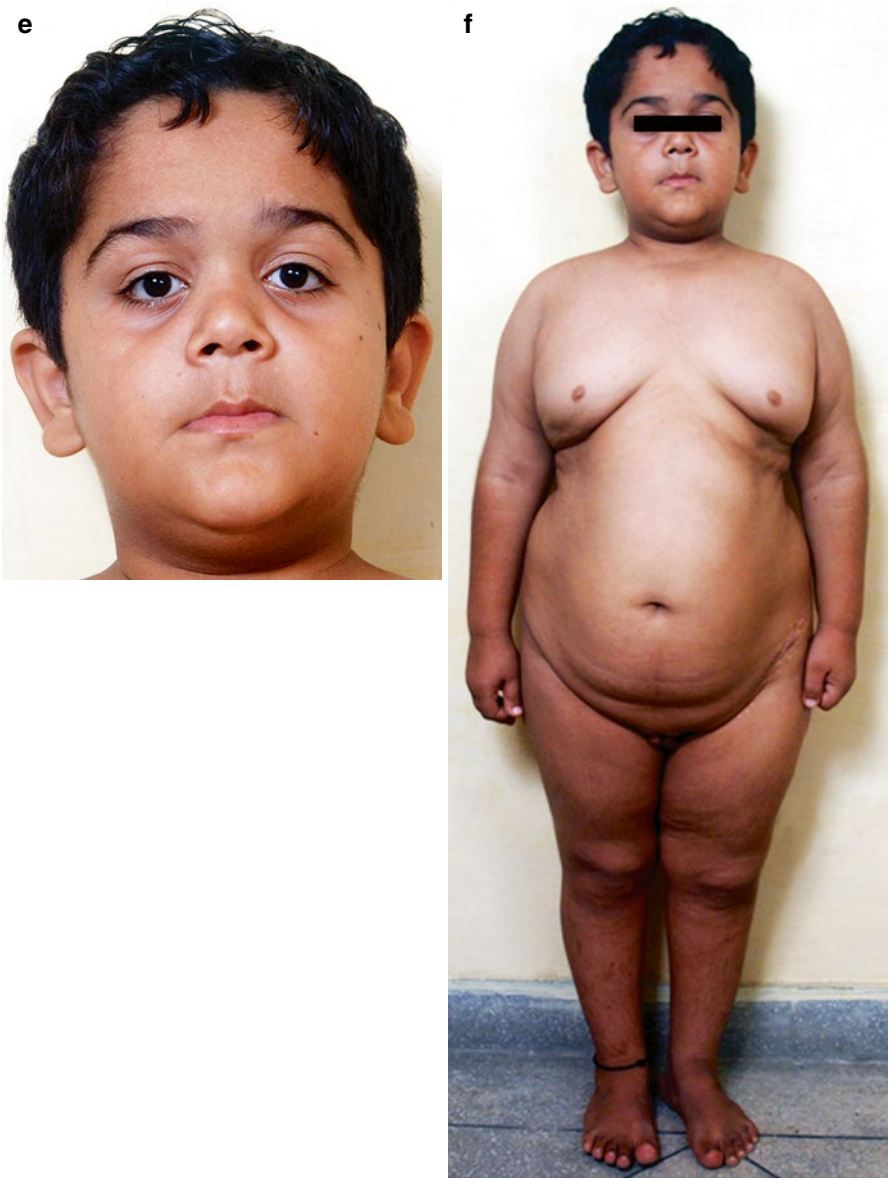

Fig. 1.1 (continued)

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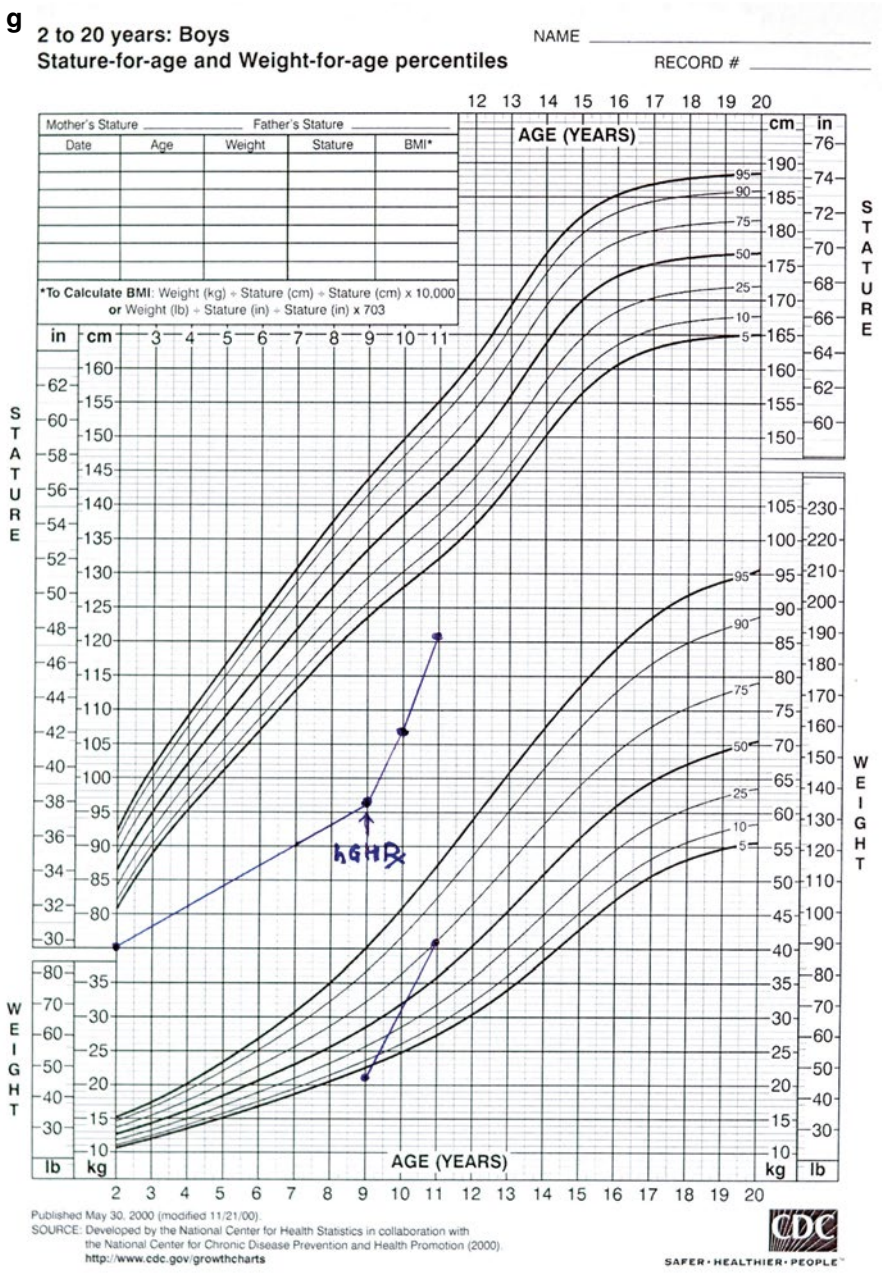

Fig. 1.1 (continued)

1.2 Stepwise Analysis

The index child was born of full-term normal vaginal delivery with birth weight of 3.3 Kg, thereby mitigating the possibility of intrauterine growth retardation as the cause of short stature. The birth length is usually normal in neonates with congenital growth hormone deficiency as intrauterine growth is GH independent and is predominantly dependent upon maternal nutritional status, uteroplacental blood flow, placental IGF1 and IGF2, and fetal insulin. Prolonged physiological jaundice and micropenis (stretched penile length <2.5 cm) are the clues to suspect congenital growth hormone deficiency in the index child. Prolonged physiological jaundice is a result of decreased glucuronyl transferase activity, as this enzyme requires initial activation by GH, thyroxine, and cortisol. Besides GH deficiency, micropenis may be due to intrauterine testosterone deficiency. Other manifestation of congenital GH deficiency is neonatal hypoglycemia, which was not present in our patient. Neonatal hypoglycemia is a result of decreased GH-mediated hepatic gluconeogenesis and glycogenolysis. Further, breech presentation has also been shown to be associated with congenital GH deficiency; however, the cause and effect association between breech presentation and GH deficiency remains conjectural. Optimal nutrition is the key factor in determining the growth during infancy, while GH is essential for growth throughout the childhood, and along with gonadal steroids it results in pubertal growth spurt. The growth faltering in the index patient was evident even at the age of 1 year. The child continued to have growth velocity of 3 cm/year which is subnormal for the prepubertal age (5–6 cm/year). Any child who has growth faltering or has height SDS of <−3 requires urgent evaluation. The index patient had growth faltering as well as height SDS of −7, therefore he required immediate evaluation. Systemic disorders as a cause of short stature was unlikely in our patient, as weight is more severely compromised than height in these disorders, as opposed to endocrine disorders where height is more severely compromised than weight, as was seen in our patient (height age 3 years, weight age 6 years). After exclusion of systemic disorders, common endocrine disorders associated with short stature which should be considered in our patient include growth hormone deficiency, Cushing's syndrome, juvenile primary hypothyroidism, and obesity–hypogonadism syndrome. The probability of Cushing's syndrome was less likely in our patient as his weight was <3rd percentile, and he did not have any stigma of protein catabolism, or moon facies, a characteristic feature of childhood Cushing's syndrome. Juvenile primary hypothyroidism was also less likely in our patient, as he did not have myxoedematous manifestations, and deep tendon reflexes were normal. Obesity–hypogonadism syndrome was also unlikely as these syndromes are usually associated with subnormal mental development, skeletal anomalies, retinitis pigmentosa, and neurodeficits. The possibility of CDGP was also unlikely as he had severe short stature and growth velocity 3 cm/year. Further, the body proportions can also help to define the cause of short stature as proportionate short stature is usually associated with growth hormone deficiency, Cushing's syndrome, and systemic disorders, whereas primary hypothyroidism, rickets–osteomalacia, and skeletal dysplasias are associated with disproportionate short stature. The expected upper segment to lower segment ratio (US/LS) at the age of 9 years is 1:1; however, in our patient it was 1.2 which may not be truly representative in this patient due to concurrent presence of congenital dislocation of hip. Delayed bone age is usually a feature of all endocrine and systemic disorders and

excludes the diagnosis of intrinsic short stature. The index patient had typical facies of growth hormone (GH) deficiency as he had frontal bossing, depressed nasal bridge, midfacial hypoplasia, and micrognathia. These features are attributed to GH deficiency as GH is required for maxillary and mandibular bone growth, and frontal bossing is due to apparent prominence of frontal bone in relation to midfacial hypoplasia. Further, not only GH but thyroxine is also required for the development of nasal bridge. In addition, micropenis and delayed bone age also support the diagnosis of GH deficiency. Further, the patient had congenital dislocation of the hip which has been described in children with growth hormone insensitivity syndrome, and possibly it may be incidental in our patient. Before proceeding to GH dynamic tests, optimal investigations were carried out for exclusion of chronic systemic disorders (chronic kidney disease, chronic liver disease, renal tubular acidosis, and celiac disease), hypothyroidism, and pseudohypoparathyroidism. Serum IGF1 should be estimated as a screening test for growth hormone deficiency; however IGF1 level within age-matched reference range does not exclude the GH deficiency, as the sensitivity of IGF1 to diagnose GH deficiency is only 70 %. The index patient had a low serum IGF1 level. There is plethora of provocative tests to assess the GH reserve; however, two tests are required to establish the diagnosis of GH deficiency as single test lacks the requisite specificity. The provocative tests should be carried out in the fasting state and euthyroidism should be achieved prior to performing the test. In addition, gonadal steroid should be replaced in those children who are in peripubertal age. Insulin-induced hypoglycemia is considered as the "gold standard" test, and the index patient underwent insulin-induced hypoglycemia and clonidine stimulation tests. Further, insulin-induced hypoglycemia test provides an opportunity of simultaneous assessment of hypothalamo–pituitary–adrenal axis. Peak GH response to both these stimuli was undetectable, and cortisol response to insulin-induced hypoglycemia was subnormal in the index patient, thereby substantiating the diagnosis of GH and ACTH deficiency. After confirmation of diagnosis of hypopituitarism, plain and CEMRI sellar–suprasellar region should be performed in these children. MRI findings may include the presence of mass lesion in sellar–suprasellar region (e.g., craniopharyngioma) and classic tetrad (small sella, hypoplastic pituitary, redundant stalk, and ectopic posterior pituitary bright spot) suggestive of pituitary transcription factor defects (e.g., Pit-1 and PROP-1) or may be normal (e.g., idiopathic growth hormone deficiency). The index patient had small sella and hypoplastic pituitary. With this profile the diagnosis of multiple pituitary hormone deficiency was considered as he had GH, ACTH, and prolactin deficiency possibly due to pituitary transcription factor defect. He was initiated with rhGH at a dose of 0.3 mg/Kg/week and hydrocortisone at a dose of 10 mg/m^2. He had height gain of 11 cm in first year and 14 cm in second year of rhGH therapy. The expected gain in the first year after initiating rhGH therapy is approximately 10–12 cm followed by 8–10 cm in the second year and then progressively declines to 5 cm/year. The gradual decline in growth velocity has been attributed to "chondrocyte senescence." The greater height gain during second year as compared to first year of rhGH therapy in our patient can be attributed to corrective surgery for congenital dislocation of hip and replacement with L-thyroxine after unmasking of hypothyroidism due to concurrent TSH deficiency and possibly due to sustained growth response as he had severe GH deficiency. Development of hypothyroidism during rhGH therapy in our patient was either as a result of inhibition of TSH by increased somatostatin tone after rhGH therapy or as

evolution of de novo TSH deficiency. Weight-based dosage is recommended for the initiation of rhGH therapy and monitoring is performed with anthropometric measurement. IGF1-targeted GH therapy is not recommended, but serum IGF1 should be monitored annually to avoid IGF1 levels above the reference range which may be associated with adverse events. The side effects associated with rhGH therapy include slipped capital femoral epiphysis, benign intracranial hypertension, gynecomastia, kyphoscoliosis, and glucose intolerance. The child should be monitored at three to six monthly intervals for auxology, pubertal development, and adverse events. In addition, serum T_4 and cortisol should also be monitored initially at 3 months of rhGH therapy and thereafter annually. Manifestations of hypocortisolism may appear on rhGH therapy either as a result of increased catabolism of cortisol due to inhibition of 11β-hydroxysteroid dehydrogenase type 1 or evolution of underlying ACTH deficiency as a part of multiple pituitary hormone deficiency. Early diagnosis of GH deficiency and timely initiation of rhGH therapy may be rewarding to achieve the adult target height in short children. Regular follow-up and periodic monitoring of evolution of other hormone deficiency are essential for optimal outcome.

1.3 Clinical Rounds

1. *How to define short stature*?

   A child with height two standard deviation below the mean (−2SD or 2.3rd percentile) as compared to children of the same age, gender, and race is considered to have short stature. In a normally distributed population (Gaussian distribution), height of 95% of individuals falls within 2SD from the population mean. The probability of detecting a child with growth disorder is higher in individuals who are 2SD above or below the mean. Therefore, a height 2SD below the mean is used to define short stature.

2. *What is the normal growth pattern during childhood*?

   The normal growth is reflected by the progressive increase in auxological parameters like height, weight, and head circumference in reference to the established standards for that particular age, gender, and race. The mean length of a healthy newborn is 50 cm and grows at height velocity of 25 cm in the first year, 12 cm in the second year, 8 cm in the third year, and 5 cm per year thereafter till the onset of puberty. Therefore, a child doubles his birth length by 4 years of age. In addition, height at the age of 2 years is approximately half of the individual's final adult height. The pubertal growth spurt is approximately 28 cm in boys and 25 cm in girls, which corresponds to a height velocity of 9.5 cm per year in boys and 8.3 cm per year in girls. A newborn looses up to 10% of birth weight during the first week of life and thereafter starts gaining weight. The weight of a child doubles by 4 months of age, triples by 1 year, and quadruples by 2 years of age. The head circumference is 32–35 cm at birth, 43–46 cm by the first year, 49 cm by the second year, and reaches adult value (56 cm for males, 54 cm for females) by 5–6 years of age. Height velocity of a healthy growing child is given in the table below.

Age	Height velocity (per year)
Birth	–
0–1 year	25 cm
1–2 year	12.5 cm
2–3 year	8 cm
3 years – puberty	5 cm
Puberty	
Boys	9.5 cm
Girls	8.3 cm

3. *What are the determinants of fetal growth*?

The determinants of fetal growth include maternal nutritional status, placental sufficiency, placental insulin-like growth factor 2 (IGF2) and IGF1, and fetal insulin. In addition, various growth factors like epidermal growth factor, fibroblast growth factor, nerve growth factor, and parathyroid hormone-related peptide (PTHrP) also play an important role in fetal growth. Although, GH has important role in postnatal growth, it has minimum effect on intrauterine growth as evidenced by GH receptor knockout mice which has normal size at birth.

4. *What is the role of insulin-like growth factors in fetal growth and development*?

Placental insulin-like growth factors (IGF1 and IGF2) play an important role in fetal growth and development. It has been shown in mice that deletion of either IGF1 or IGF2 gene results in low birth weight (60 % of normal), whereas deletion of type 1 IGF receptor (IGF1R) results in greater reduction in birth weight (45 % of normal). This is because both IGF1 and IGF2 act through IGF1R to promote fetal growth. However, type 2 IGF receptor (IGF2R) is important in the regulation of IGF2 action by increasing its turnover. The table given below illustrates the effects of insulin and IGF1/IGF2 on fetal growth and development.

Animal model	Birth weight
IGF1 gene knockout mice	60 % of normal
IGF2 gene knockout mice	60 % of normal
Combined IGF1 and IGF2 gene knockout mice	30 % of normal
IGF1 R knockout mice	45 % of normal
Combined IGF1 gene and IGF1R knockout mice	45 % of normal
Combined IGF2 gene and IGF1R knockout mice	30 % of normal
IGF2 R knockout mice	130 % of normal
Insulin receptor knockout mice	Normal
Insulin gene knockout mice	78 % of normal

5. *What is the role of parathyroid hormone-related peptide in fetal growth and development*?

Parathyroid hormone-related peptide (PTHrP) plays an important role in fetal growth and development by facilitating transplacental transport of calcium and

promoting uteroplacental blood flow. In addition, it also regulates the growth and differentiation of chondrocytes during fetal life. The role of PTHrP in fetal growth is evidenced by short stature and skeletal dysplasia in patients harboring PTHrP receptor mutations; activating mutations cause Blomstrand dysplasia and inactivating mutations cause Jansen chondro-osteodystrophy.

6. *Is growth of a child exclusively GH-dependent*?

   A child with congenital growth hormone deficiency (GHD) has near-normal birth length. However, there is a rapid decline in height velocity by the age of 2 years in these children; thereafter, they continue to grow at a reduced height velocity. If left untreated, the child can attain a final adult height which is approximately 70 % of his/her genetic potential, with a height deficit of 38 cm in males and 33 cm in females. This suggests that growth is not exclusively a GH-dependent phenomenon and other hormones also play a role.

7. *What are the determinants of postnatal growth*?

   Postnatal growth is determined by nutritional factors, hormones, and genetic potential of an individual. During infancy, growth is predominantly influenced by nutritional status of the child. During the prepubertal period, hormones like GH–IGF1, thyroxine, and insulin play an important role, while pubertal growth spurt is caused by progressive increase in gonadal steroids and the consequent GH–IGF1 surge. However, the final height of an individual is determined by his/her genetic potential. This is possibly attributed to predetermined chondrocyte potential for skeletal growth, IGF1 sensitivity, rate of ossification maturation, and ethnicity of an individual.

8. *What are the hormones required for postnatal growth*?

   Growth hormone and IGF1 are the prime mediators of postnatal linear growth. GH-mediated IGF1 generation is facilitated by nutritional status, insulin, thyroxine, gonadal steroids, and, possibly, vitamin D. In an individual, limb growth is predominantly dependent on GH–IGF1, while truncal growth on gonadal steroids. Thyroxine, insulin, and testosterone not only facilitate GH-mediated IGF1 generation but also promote GH-independent IGF1 generation. Further, insulin acts directly on IGF1 R, albeit at a much lower affinity (100-fold less) than IGF1. Estrogen in low concentration promotes GH-mediated IGF1 generation but, in high concentration, inhibits IGF1 generation. Testosterone stimulates GH-mediated IGF1 generation by its direct effect and by aromatization to estrogen. In addition, it also promotes GH-independent IGF1 generation by its direct effect on hepatocytes.

9. *What is the structure of epiphyseal growth plate*?

   The growth plate, also known as physis, is present between the epiphysis and metaphysis at the ends of long bones. It comprises of five zones: resting zone, proliferative zone, hypertrophic zone, calcification zone, and ossification zone,

from epiphysis to metaphysis. The process of linear growth initiates at the epiphyseal end of growth plate and new bone is laid down at the metaphysis (Fig. 1.2a, b).

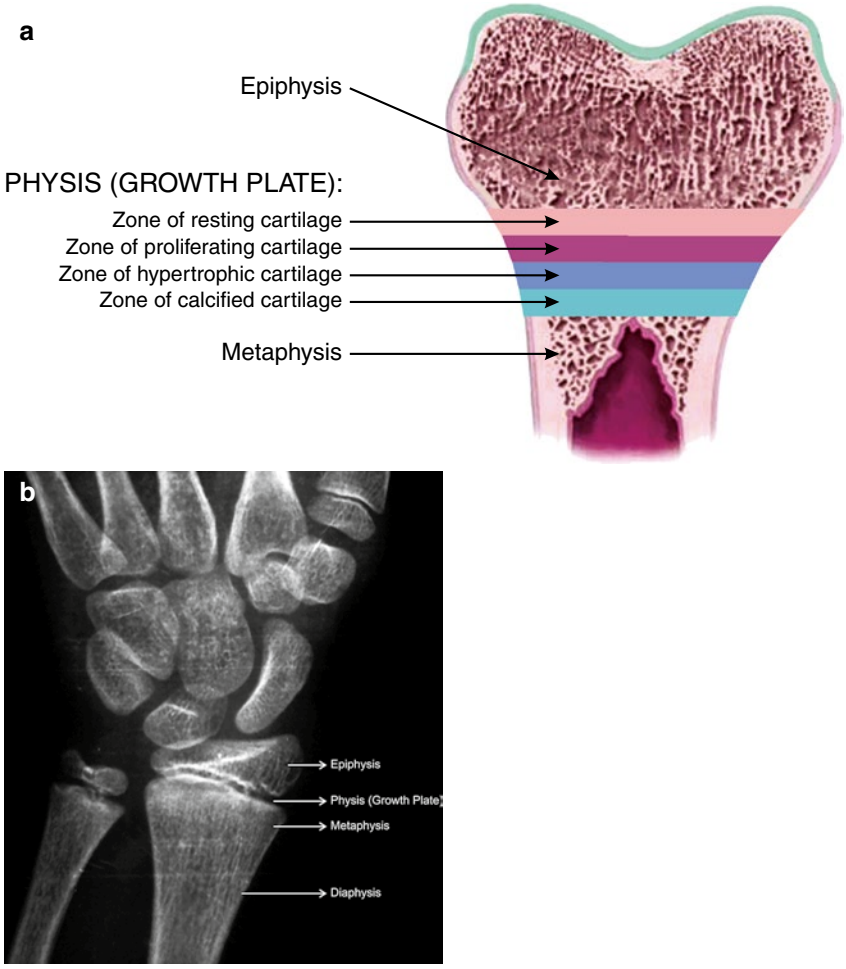

Fig. 1.2 (**a**) Showing different zones of growth plate. (**b**) X-ray wrist AP view showing physis (growth plate) as a radiolucent area between epiphysis and metaphysis

10. *How does linear growth occur in a child*?

Linear growth is a result of a well-regulated and coordinated process called "chondro-osteogenesis," which includes chondrocyte proliferation, differentiation/hypertrophy, apoptosis, and endochondral ossification. Longitudinal bone growth occurs at the epiphyseal growth plate located at the ends of long bones. In the resting zone, there is a reserve of chondrocytes, which proliferate under the influence of GH and IGF1. These proliferating chondrocytes enlarge to hypertrophic chondrocytes in the presence of IGF1, thyroid hormones, and gonadal steroids. The paracrine factors that help in chondrocyte proliferation and hypertrophy include PTHrP, IHH, BMPs, FGFs, RUNX2, and SOX9.

These terminally differentiated cells eventually undergo apoptosis. Later, the growth plate is invaded by blood vessels and bone cell precursors from metaphysis, resulting in remodeling of cartilage into bone, a process termed as endochondral ossification. The various endocrine and paracrine factors that regulate "chondro-osteogenesis" are depicted in the figure given below (Fig. 1.3).

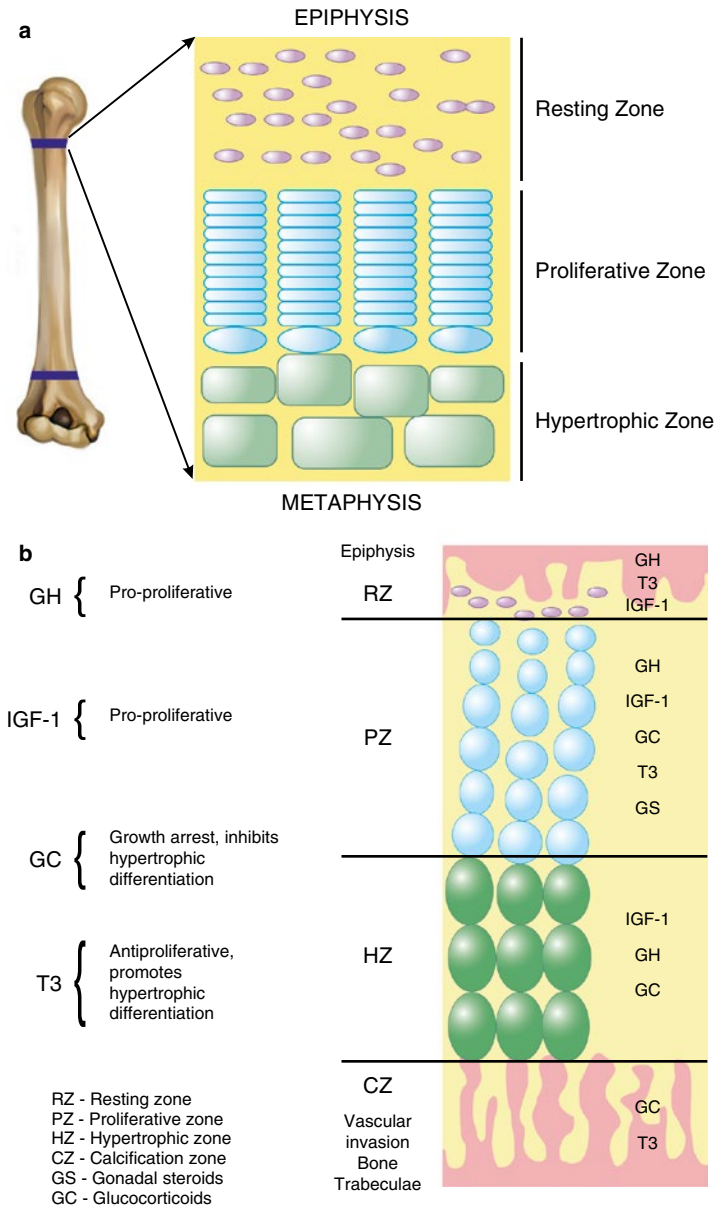

Fig. 1.3 (**a**) Different zones of growth plate. (**b**) Site of action of various endocrine and paracrine factors on growth plate

11. *How does circumferential bone growth occur in a child*?

The linear bone growth occurs at epiphyseal growth plate (at the end of long bones), while circumferential bone growth (appositional bone growth) occurs beneath the periosteum at diaphysis. The appositional bone growth is the result of intramembranous ossification, where osteoblast forms the new bone just beneath the periosteum. Estrogen inhibits, while androgen and GH stimulate appositional bone growth at diaphysis. Periosteal new bone formation is accompanied with endosteal bone resorption as the new bone formation exceeds bone resorption at periosteum and vice versa at endosteum, thereby resulting in increased circumferential bone growth (Fig. 1.4).

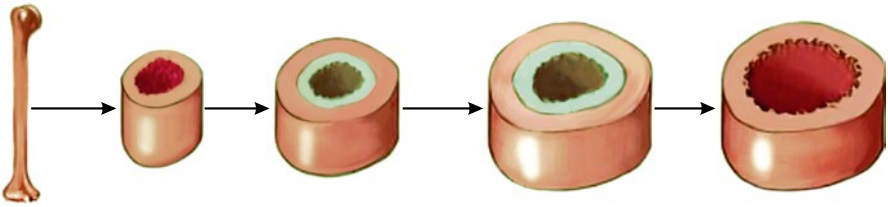

Fig. 1.4 Circumferential growth of a long bone

12. *How does growth hormone promote linear growth*?

Growth hormone promotes linear growth through systemic (liver) and locally derived (growth plate) IGF1. In addition, GH per se has a direct effect on growth plate, independent of IGF1. GH–IGF1 is responsible for the differentiation of pre-chondrocytes to chondrocytes, followed by the proliferation and maturation of chondrocytes in the epiphyseal growth plate. Further, GH–IGF1 also promotes bone collagen synthesis.

13. *What are the hormones responsible for GH-independent IGF1 generation*?

IGF1 generation is predominantly a GH-dependent phenomenon which is facilitated by thyroxine, insulin, and gonadal steroids. However, thyroxine, insulin, and gonadal steroids also promote GH-independent IGF1 generation. This is evidenced by the fact that children with GH deficiency/GH receptor mutation continues to grow, albeit at a lower height velocity, with measurable levels of serum IGF1, which suggest GH-independent IGF1 generation. In addition, these hormones also have a direct effect on epiphyseal growth plate and promote chondrocyte proliferation.

14. *Why are the boys taller than girls*?

Boys are taller than girls because of physiological delay in the initiation of puberty by a period of 2 years (thereby yielding two additional years of cumulative linear growth), more intense pubertal growth spurt, and presence of growth-promoting genes on Y(Yq) chromosome. The average difference in height between adult men and women is 13 cm. This difference is due to growth accumulated during two additional prepubertal years (10 cm) and the greater gain in

height during pubertal growth spurt (3 cm) in boys. This knowledge is important and is used in the calculation of midparental height of an individual.

15. *What are the causes of growth without growth hormone*?

Linear growth is a GH–IGF1-dependent phenomenon; however, there are disorders where growth is GH-independent. Childhood obesity is one of the paradoxical situations where there is an accelerated linear growth with low levels of GH. The other causes of growth without GH include craniopharyngioma (hypothalamic dysfunction-induced adiposity), childhood hyperthyroidism, Beckwith–Wiedemann syndrome (IGF2-mediated growth), and Soto's syndrome (NSD1 mutation).

16. *Why do obese children have higher growth velocity despite low GH*?

Obesity in childhood and adolescence is associated with increased height velocity with low basal as well as stimulated GH levels, normal total IGF1, and increased "free" IGF1. Obesity-induced hyperinsulinemia promotes GH-independent IGF1 generation, increases free IGF1 level by reducing IGFBP1, and directly stimulates IGF1 receptor, thereby resulting in accelerated linear growth. Elevated levels of "free" IGF1 increase somatostatin tone, resulting in decreased GH secretion. In addition, there is an increase in leptin levels in obese children, which also acts as skeletal growth factor. Further, increased aromatization of androgens to estrogens as a result of excess adiposity also contributes to the linear growth. However, the final adult height in obese children does not differ from nonobese children, as a result of early puberty and excess aromatization of androgens leading to premature epiphyseal closure (Fig. 1.5).

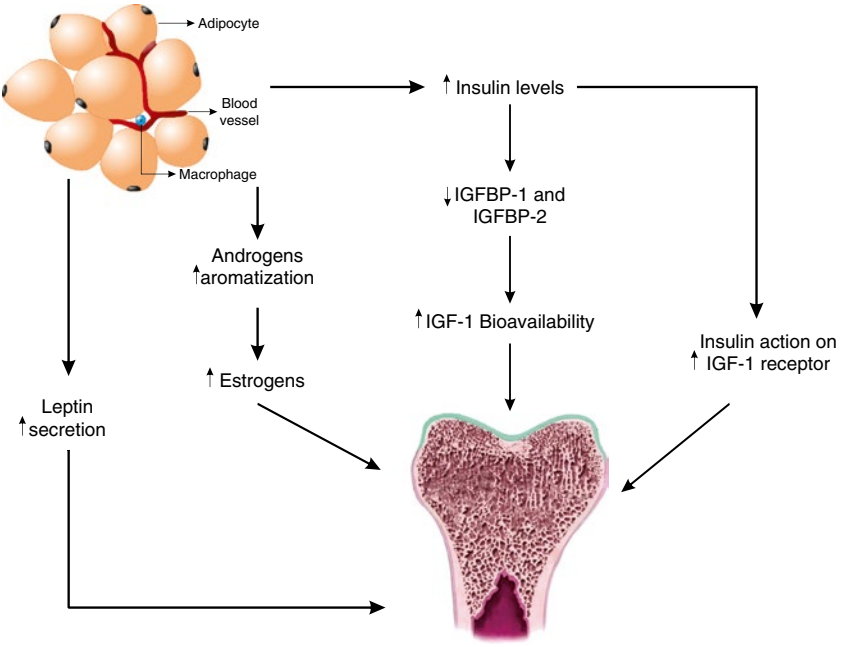

Fig. 1.5 Mechanisms of obesity-related accelerated growth velocity

17. *Why is total IGF1 level normal despite low GH levels in obesity*?

    Obesity is associated with normal IGF1 despite low GH level. This is due to GH-independent, insulin-mediated IGF1 generation and enhanced GH sensitivity because of upregulation of GH receptors, as evidenced by increase in GH-binding proteins (GHBP).

18. *A 6-year-old obese child presented with short stature. Is it of concern*?

    Childhood obesity is associated with normal/accelerated height velocity. Therefore, presence of short stature in an obese child is almost always pathological and should be evaluated further. The common causes of short stature with obesity include Cushing's syndrome, hypothyroidism, isolated growth hormone deficiency, pseudohypoparathyroidism, and Prader–Willi syndrome.

19. *What are the hormones responsible for the development of facial features*?

    Hormones responsible for the development of facial features are thyroxine, GH–IGF1, and gonadal steroids. Thyroxine is responsible for facial bone growth and maturation during prenatal and infantile period. Infants with congenital hypothyroidism therefore have characteristic facial features including immature facies, flat nasal bridge, and pseudohypertelorism. GH–IGF1 is mainly responsible for facial features during prepubertal period. Therefore, patients with congenital growth hormone deficiency manifest with frontal bossing, midfacial hypoplasia, and micrognathia. During peripubertal period, gonadal steroids play an important role in facial maturation and lead to sexual dimorphism in the facial characteristics (Fig. 1.6).

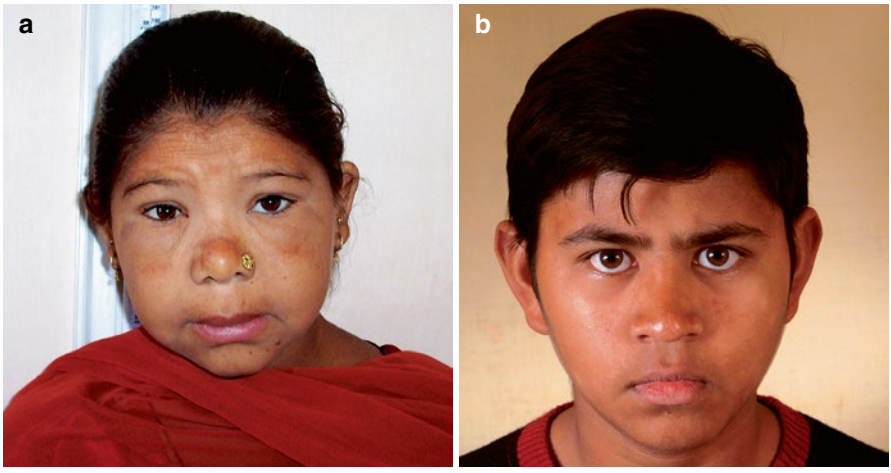

Fig. 1.6 (**a**) Characteristic facial features in a girl with congenital hypothyroidism. (**b**) Immature facies in an adolescent with hypogonadism

20. *What is the importance of measuring body proportions in a short child*?

Measurement of body proportions helps in the differential diagnosis of short stature. The ratio of upper segment (US) to lower segment (LS) is 1.7 at birth, 1.5 at 1 year of age, 1.4 at 2 years, 1.3 at 3 years, 1.1 at 6 years, 1 at 10 years, and 0.92–0.85 in adulthood. A short child is considered to have proportionate short stature when US/LS ratio is in concordance with the chronological age. The causes of proportionate short stature include growth hormone deficiency, familial short stature, CDGP, and chronic systemic disorders. The presence of body proportions which are disproportionate to the chronological age defines disproportionate short stature. This can be due to either short limb or short trunk. The causes include hypothyroidism, rickets, skeletal dysplasias, and mucopolysaccharidosis (Fig. 1.7). An approach to a short child is depicted in the figure given below (Fig. 1.8).

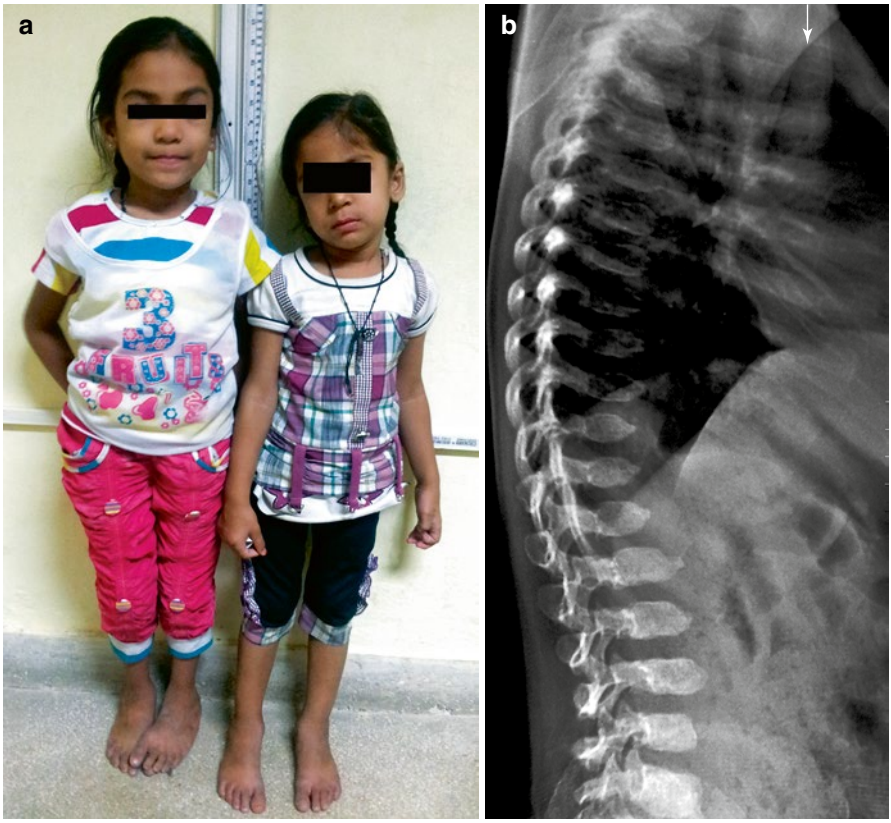

Fig. 1.7 (**a**) Siblings with short stature due to mucopolysaccharidosis. (**b**) Lateral spine radiograph showing reduced vertebral height with anterior beaking of the lower dorsal vertebral bodies in the same patient

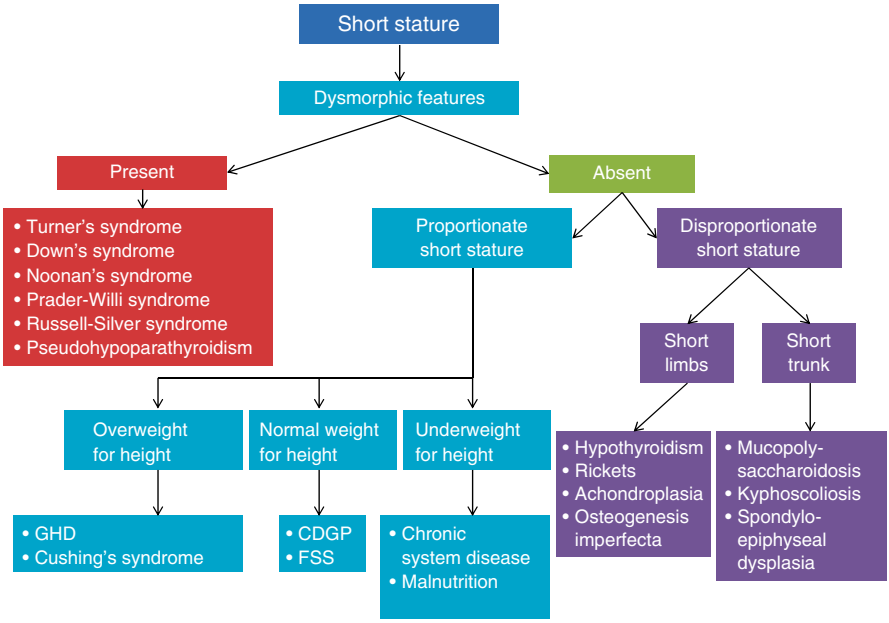

Fig. 1.8 Approach to a child with short stature

21. *What is the importance of measurement of parental height in a child with short stature*?

Genetic factors have a significant contribution to the final adult height of an individual. Therefore, an estimate of the genetic potential for the final adult height of an individual can be predicted on the basis of height of the parents. Midparental height (MPH) is a widely used tool to calculate the genetic potential of an individual. MPH can be calculated as follows:

$$\left(\text{Paternal height} + \text{Maternal height} + 13\right) \div 2 \text{ for boys}$$
$$\left(\text{Paternal height} + \text{Maternal height} - 13\right) \div 2 \text{ for girls}$$

22. *What is the target height of an individual*?

Children of short parents are not as short as their parents, and, similarly, children of tall parents are not as tall as their parents due to the phenomenon of regression to the mean. Therefore, the concept of target height was introduced to predict the final adult height of an individual with allowance for regression to the mean. The phenomenon of regression to the mean indicates that an individual has a tendency to attain a final adult height which is toward mean adult height of that particular population (i.e., 50th percentile). However, an individual can only compensate up to 20% of the difference between midparental height and mean adult height of that particular population, e.g., if the MPH of

a boy is 157 cm and mean adult height of the population is 177 cm for males (50th percentile in CDC growth chart), there is a difference of 20 cm between MPH and mean adult height of the population. An individual can correct 20 % of this difference because of the phenomenon of regression to the mean. Therefore, target height (TH) is calculated as follows:

$$\text{TH} = \text{MPH} + \left[(\text{mean adult height of the population} - \text{MPH}) \times 20\%\right]$$

So, his target height is $157 + \left[(177 - 157) \times 20\%\right]$ i.e. $157 + 4\text{cm} = 161\text{cm}$

95% of the individuals will fall within 10 cm of their target height

23. *How to predict the final adult height of a child*?

The final adult height of children at a particular chronological age can be predicted by doubling the height attained at the age of 2 years, by calculating target height, and by the degree of skeletal maturation (bone age) in relation to chronological age (Bayley and Pinneau method).

24. *What is growth chart*?

Growth chart is a tool to assess the adequacy of growth of a child in reference to healthy children. They are prepared from anthropometric data obtained from large population-based studies. Growth charts for height, weight, body mass index, and head circumference are commonly available and can be longitudinal or cross-sectional. The age of the child is plotted on X-axis of the growth chart, while anthropometric parameter (e.g., height, weight) on the Y-axis. Most growth charts have seven percentile lines: 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles. In addition to standard growth charts, syndrome-specific growth charts are available to define growth pattern in various disorders like Turner, achondroplasia, and Down syndrome.

25. *What is the difference between* "*growth standard chart*" *and* "*growth reference chart*?"

The "growth standard charts" are prescriptive and depict the growth pattern of healthy children who live in ideal conditions for optimal growth (exclusive breastfeeding for initial 4 months, adequate nutrition, favorable psychosocial environment, and good living conditions). These charts represent how children should be growing in an ideal scenario. In comparison, the "growth reference chart" is descriptive and denotes the growth pattern of apparently healthy children in a particular place and time. These charts represent how children are growing in real life (not necessarily in ideal conditions) and provide an opportunity to assess the secular trends of growth in a population.

26. *What are the types of growth charts*?

Growth charts are of two types: cross-sectional and longitudinal. The "cross-sectional growth charts" are constructed by estimation of anthropometric

parameters of healthy children of different age at a given point of time, while the "longitudinal growth charts" are constructed by prospective follow-up of anthropometric parameters of a cohort of healthy children. Cross-sectional growth charts do not take into account height velocity or variations in height during pubertal growth spurt of an individual. These limitations can be overcome by the use of longitudinal growth charts. The cross-sectional growth charts include Centre for Disease Control (CDC), Agarwal and Khadilkar growth charts, whereas Tanner–Whitehouse chart is longitudinal. WHO growth charts are mixed growth chart; it is longitudinal from 0 to 2 years of age and cross-sectional thereafter. The characteristic features of different growth charts are summarized in the table given below.

Parameters	**NCHS/WHO, 1978**	**WHO, 2006**	**CDC, 2000**	**IAP, 2015**
Type of growth chart	Growth reference chart	Growth standard chart	Growth reference chart	Growth reference chart
Region specific	USA	Global (six countries)	USA	India
Age	0–18 years	0–5 years	0–20 years	5–18 years
Growth percentile	5–95	5–95	3–97	3–97
Method of surveillance	Longitudinal	Longitudinal	Cross-sectional	Cross-sectional
	0–2 years	0–2 years		
	Cross-sectional	Cross-sectional		
	2–18 years	2–5 years		

27. *What is the utility of growth chart*?

Growth chart is an important tool for monitoring anthropometric parameters of a child and helps in the objective assessment of adequacy of growth. Accurate plotting of height and weight of a child on a growth chart should be done prior to evaluation of a child for growth abnormalities. Prospective follow-up of height or weight of a child helps in early identification of growth faltering and, thereby, timely evaluation. Growth chart also helps in the differential diagnosis of short stature, prediction of final adult height of an individual, and monitoring the response to therapy. It is imperative to use the same growth chart during follow-up of a child.

28. *What are the merits and demerits of WHO (2006) growth chart*?

WHO (2006) growth chart is unique as it is a "growth standard chart" in comparison to other growth charts which are "growth reference charts." These growth standard charts were constructed by data obtained from healthy children who were predominantly breastfed for at least initial 4 months of life, received optimal nutrition thereafter, and were reared in a good psychosocial environment. WHO (2006) growth chart is also unique that it represents growth standard of children from six different countries. It also provides longitudinal data

for initial 2 years of life. However, WHO growth standards are available for only 0–5 years of age, and later monitoring is recommended with region-specific growth charts. In addition, the use of these growth charts may result in overdiagnosis of short stature, especially in children from developing countries. WHO also derived a cross-sectional growth chart in 2007, which is a "growth reference chart" for children aged 5–19 years.

29. *Which growth chart should be used for Indian children*?

The growth charts based on data from Indian population include Agarwal (1992, 1994), IAP (2007), Khadilkar (2009), Marwaha (2011), and revised IAP (2015) growth charts. Agarwal and Khadilkar charts were derived from school-going children belonging to affluent families, whereas Marwaha chart included school-going children from both upper and lower socioeconomic status. Data obtained from Agarwal et al. was used to derive IAP growth chart (2007), whereas data from nine studies in apparently healthy children from upper and middle socioeconomic class was used to derive revised IAP growth chart (2015). The revised IAP growth chart was constructed after exclusion of overweight children (weight for height above +2 SD score). Agarwal charts are nearly two decades old; hence its use is limited as it does not reflect the secular trend of height over this period. Khadilkar and Marwaha growth charts are relatively recent; however, the use of these charts results in underdiagnosis of obesity. Revised IAP growth chart (2015) addresses most of the limitations of previous growth charts. Indian Academy of Pediatrics recommends WHO growth chart (2006) for children aged 0–5 years and revised IAP growth chart (2015) from 5 to 18 years. It is pertinent to use the same growth chart during follow-up of a child.

30. *What is the importance of simultaneous estimation of height and weight in the evaluation of short stature*?

During the growth of a child, increase in height and weight occurs in concordance with each other. Therefore, the interpretation of linear growth in relation to weight provides clue for the differential diagnosis of short stature. In a growth chart, the age that corresponds to child's height at 50^{th} percentile is height age of the child, whereas the age that corresponds to child's weight at 50^{th} percentile is the weight age. If a child with short stature is "overweight for height" (weight age > height age), then the cause is usually an endocrine disorder like Cushing's syndrome, growth hormone deficiency, hypothyroidism, or Prader–Willi syndrome. If the child with short stature is "underweight for height" (weight age < height age), then systemic causes like nutritional deficiencies, celiac disease, and chronic systemic disorders need to be evaluated (Fig. 1.9a, b).

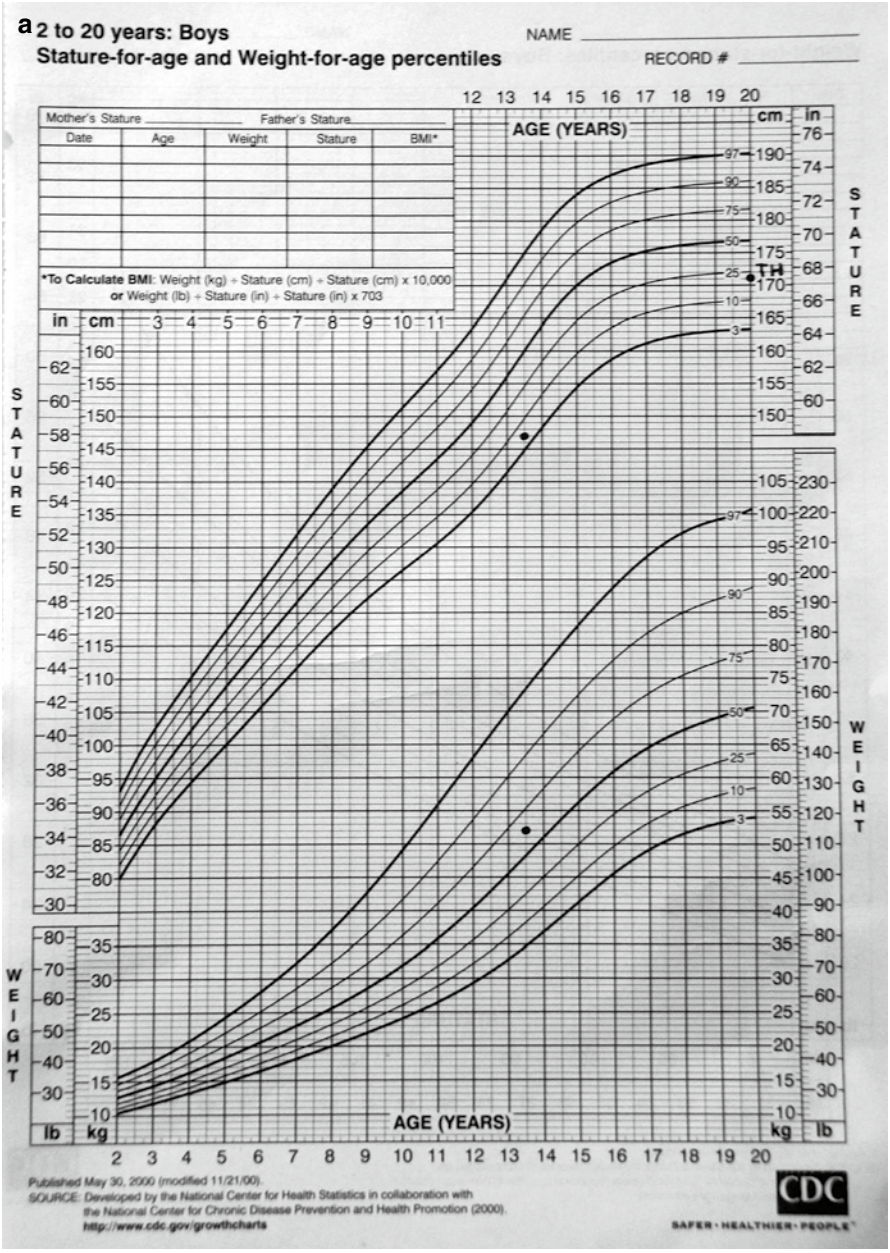

Fig. 1.9 (**a**) Growth chart of a child with Cushing's syndrome showing weight age > height age (14.5 years and 11.5 years, respectvely).

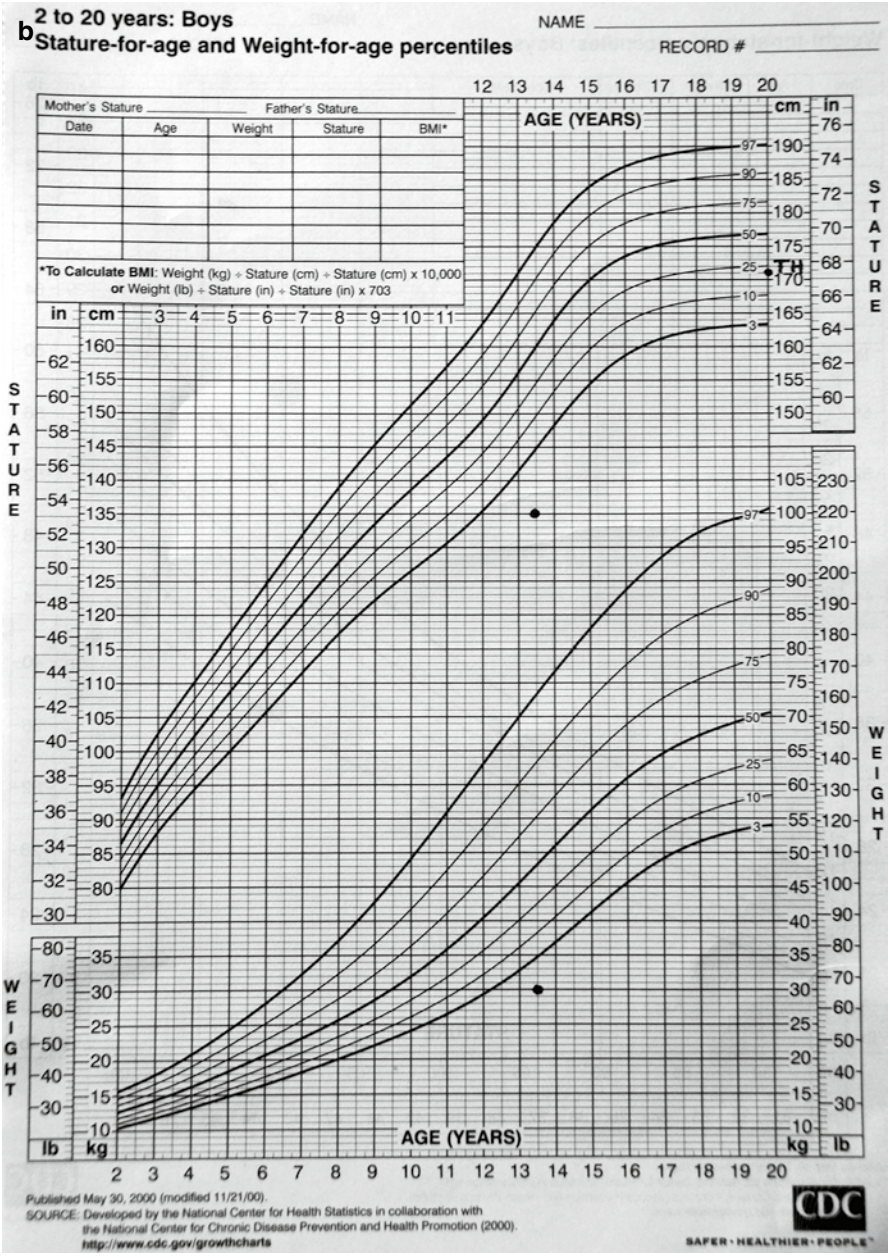

Fig. 1.9 (**b**) Growth chart of a child with celiac disease showing both weight age and height age are proportionally reduced (9.5 year each)

31. *How to calculate standard deviation for height at a particular age*?

    The standard deviation (SD) for height at a particular age can be calculated from a growth chart by subtracting the height at 50th percentile (cm) from the height at 3rd percentile (cm) at that particular age and dividing it by 2. For example, in CDC growth chart for boys at age 10 years, the height at 50th percentile is 138 cm and the height at 3rd percentile is 127 cm. The difference between the two percentiles is 11 cm and dividing it by 2 gives a value of 5.5 cm, which is equivalent to one SD at age 10 years in CDC growth chart for boys.

32. *How to calculate standard deviation score for height at a particular age*?

    Standard deviation (SD) measures the deviation in height of a population from the mean, whereas the standard deviation score (SDS) precisely quantifies the height deficit of a child. The SDS is calculated as follows:

$$\left(\textbf{Height of the child} - \textbf{Height at } 50^{th} \textbf{ percentile for a child of same age}\right) \div \textbf{SD}$$

    For example, if a boy has height of 115 cm at age of 10 years:
    - The mean height (50th percentile) at 10 years is 138 cm in CDC growth chart.
    - The SD at this chronological age is calculated by subtracting height at 50th centile from height at 3rd centile (138–127 = 11) divided by 2, i.e., 5.5 cm.
    - The SDS of the child is calculated as $\left(115-138\right) \div 5.5$, i.e., –4.2SDS. The calculation of SDS in growth chart is illustrated below (Fig. 1.10).

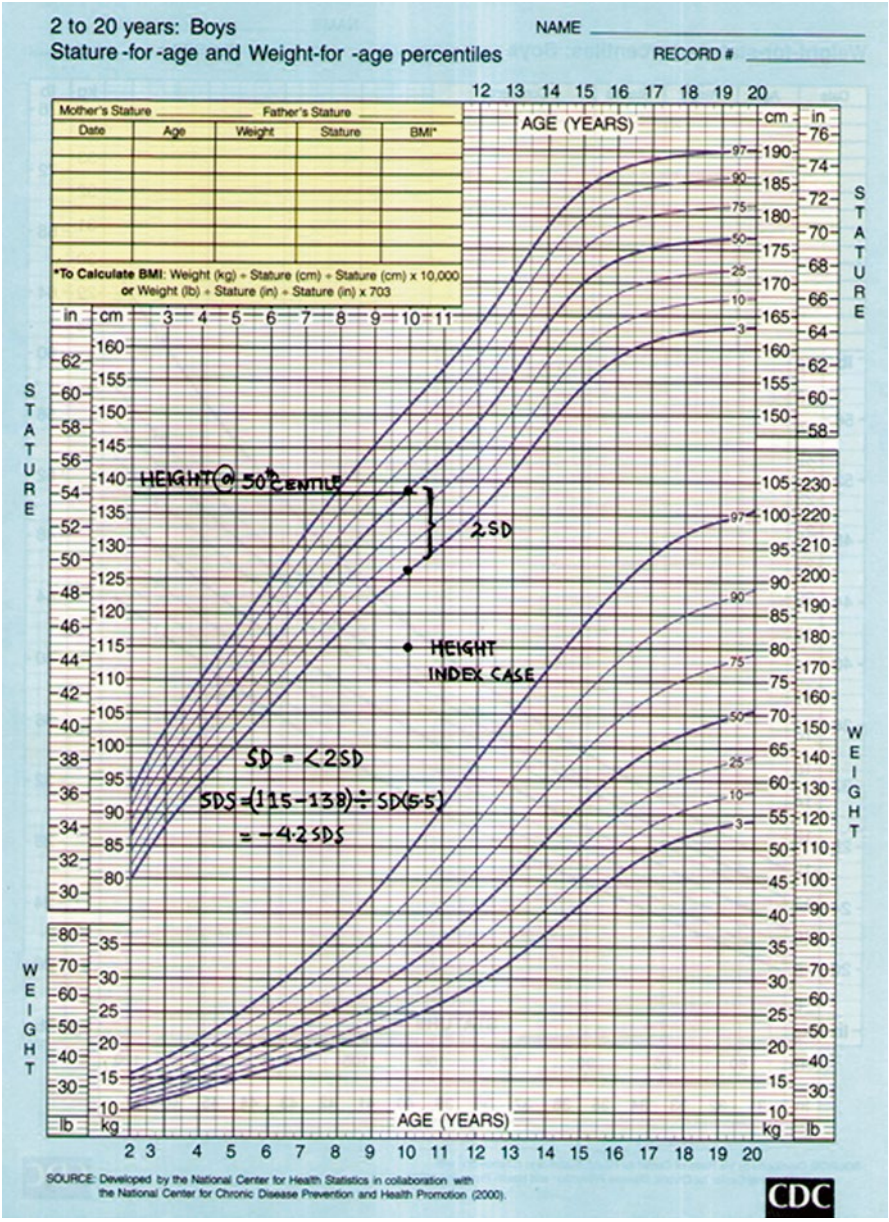

Fig. 1.10 Calculation of height SDS from a growth chart

33. *What are the methods available for the estimation of bone age*?

X-ray of the nondominant hand and wrist AP view is recommended for the assessment of bone age. However, additional X-ray (e.g., knee AP view) may be required in adolescent to determine growth potential. Accurate assessment of bone age can be performed with the help of Greulich and Pyle (gender based) or Tanner–Whitehouse charts; however, the assessment of bone age by these charts is subjective and has marked interindividual variation. These shortcomings can be overcome by the recently introduced software "Bone Xpert" which is an automated method for estimation of bone age from the hand X-ray.

34. *What is the utility of bone age estimation in the evaluation of a short child*?

Bone age (BA) estimation is an essential parameter in the evaluation of a child with short stature as the degree of skeletal maturation in relation to chronological age (CA) and height age (HA) helps in the differential diagnosis of short stature. This is summarized in the table given below.

Parameters	**Differential diagnosis**
CA = BA > HA	Familial short stature
	Intrinsic short stature (e.g., bone dysplasia, Russell–Silver syndrome)
CA > BA = HA	Constitutional delay in growth and puberty
	Growth hormone deficiency
	Cushing's syndrome
	Hypothyroidism
	Chronic systemic disease

35. *How does bone age help in the prediction of adult height*?

The bone age-based methods are more accurate in predicting the adult height potential as compared to the calculated target height. The bone age-based methods include Tanner–Whitehouse II (TW II), Bayley–Pinneau (BP), and Roche–Wainer–Thissen (RWT). The 90 % confidence interval for the prediction is approximately ±6 cm at younger ages. The more advanced the bone age, the greater the accuracy of the adult height prediction. TW II method tends to underpredict and BP method tends to overpredict the adult height, especially in boys. It is important to use the same method to predict adult height during follow-up of a child.

36. *What is the importance of dysmorphic features in a short child*?

The presence of dysmorphic features suggests syndromic short stature and helps to establish an etiological diagnosis. The common causes of short stature with dysmorphic features are summarized in the table given below (Figs. 1.11 and 1.12).

Disease	**Dysmorphic features**
Turner syndrome	Micrognathia, low-set ears, low posterior hairline, short webbed neck, lymphedema, shield chest, cubitus valgus, short fourth and/or fifth metacarpals, cardiac anomalies (left sided), renal anomalies
Noonan syndrome	Hypertelorism, down-slanting eyes, short webbed neck, low posterior hairline, right-sided cardiac anomalies, hypertrophic cardiomyopathy, mental retardation, cryptorchidism
Down syndrome	Mongoloid slant of eyes, developmental delay, umbilical hernia, simian crease, large protruding macroglossia, sandal toe
Prader–Willi syndrome	Obesity, hypotonia, hypogonadism, mental retardation
Russell–Silver syndrome	Small triangular facies, facial asymmetry, clinodactyly, hemihypertrophy, micrognathia
Laurence–Moon–Bardet–Biedl syndrome	Obesity, polydactyly, hypogonadism, retinitis pigmentosa
Seckel syndrome	Microcephaly, beaking of nose, micrognathia, craniosynostosis
GH deficiency due to congenital hypothalamo–pituitary defects	Cleft lip, cleft palate, single central incisor, bifid uvula, nystagmus, rigid cervical spine (short neck)
Pseudohypoparathyroidism	Albright hereditary osteodystrophy phenotype (round face, short fourth and/or fifth metacarpals, obesity, subcutaneous ossification)

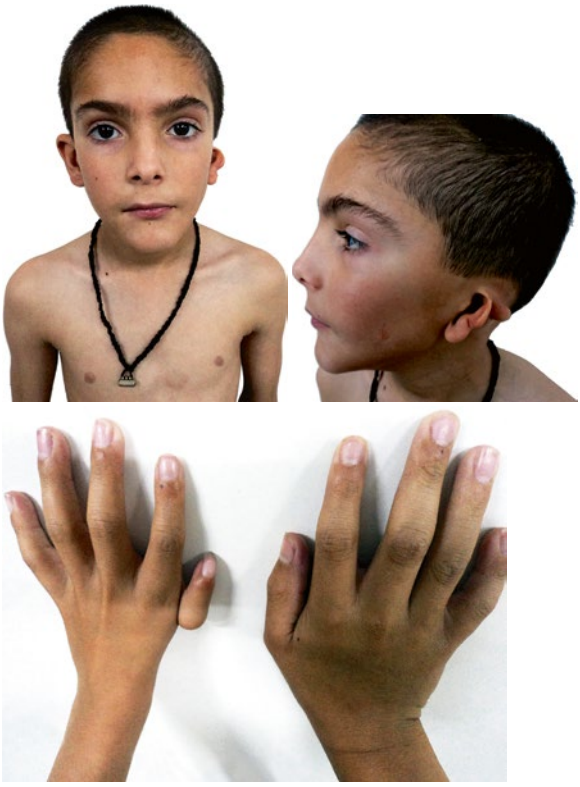

Fig. 1.11 A child with Russell–Silver syndrome. Note the typical triangular facies, dysplastic left ear, and limb asymmetry

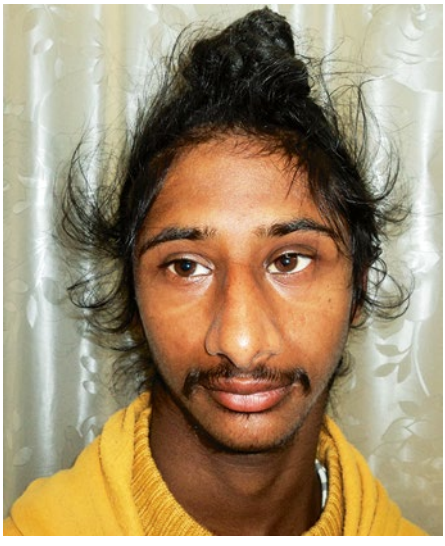

Fig. 1.12 An adolescent with intrinsic short stature due to Seckel syndrome. Note the typical facial features including microcephaly, beaking of nose, and micrognathia

37. *How to define an infant with small for gestational age*?

A neonate with birth length and/or birth weight at least 2SD below the mean for gestational age is defined as small for gestational age (SGA). A birth weight of <2.5 Kg in a full-term newborn is considered as small for gestational age. In clinical practice, the term SGA is interchangeably used with intrauterine growth retardation (IUGR).

38. *What is symmetric SGA*?

The maximum increase in fetal length occurs during early intrauterine period (10–24 weeks), whereas maximum increase in weight occurs during 32–40 weeks. Any insult to the fetus during early pregnancy, either due to chromosomal/syndromic disorders or congenital infections, affects both length and weight, thereby leading to a neonate with symmetric SGA. These neonates do not have catch-up growth because of early insult during gestation and eventually end up with short adult height.

39. *What is asymmetric SGA*?

Any insult to the fetus during late gestation leads to the birth of an infant with compromised birth weight but with normal birth length, i.e., asymmetric SGA. This usually occurs due to fetoplacental insufficiency. Infants with asymmetric SGA usually experience catch-up growth during infancy and finally achieve a normal adult height.

40. *What is intrinsic short stature*?

Intrinsic short stature is associated with disorders which are characterized by inherent limitation of bone growth. The important causes of intrinsic short stature include Turner syndrome, Russell–Silver syndrome, Prader–Willi syndrome, and achondroplasia (Fig. 1.13).

Fig. 1.13 Intrinsic short stature due to achondroplasia in siblings

41. *What is idiopathic short stature*?

Idiopathic short stature (ISS) is defined as height 2SD below the mean for age and gender of corresponding population without any evidence of chronic systemic illness or chromosomal, psychosocial, or endocrine disorders. ISS is a heterogeneous disorder and is predominantly contributed by children with familial short stature (FSS) and constitutional delay in growth and puberty (CDGP). However, some children with ISS neither qualify for the diagnosis of CDGP nor for FSS. GH response to provocative stimuli is essentially normal in children with ISS; however, serum IGF1 levels are variable. The likely abnormalities in children with ISS include reduced integrated GH secretion, defect in GH molecule or its receptor, impaired IGF1 post-receptor signaling, SHOX haploinsufficiency, or defective chondrocyte growth and proliferation (Fig. 1.14).

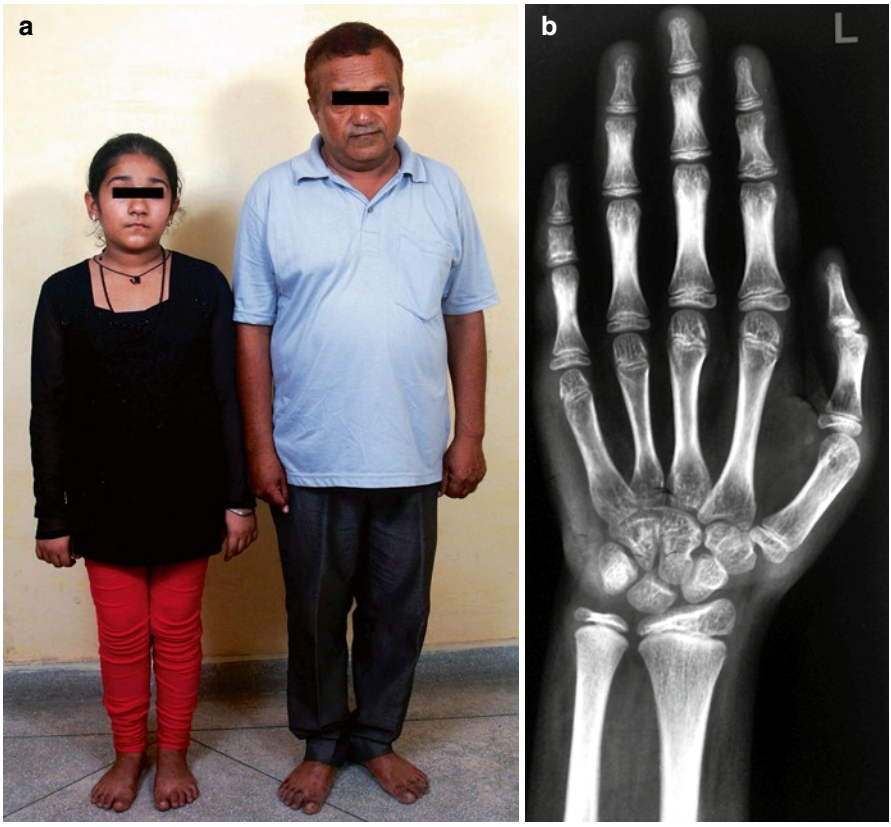

Fig. 1.14 (**a**) A 12-year-old girl with familial short stature along with her father (who is also short). (**b**) X-ray wrist AP view showing bone age of 12 years

42. *How to differentiate between familial short stature and constitutional delay in growth and puberty*?

FSS and CDGP are normal variants of short stature and are associated with a strong family history. Children with both these disorders are born with a normal birth length; however, during infancy they cross the centile curves downward so that the height falls <3rd percentile by the end of second or third year of life ("catch down" growth). Subsequently, these children have normal height velocity till puberty. Children with FSS have normal onset of puberty and eventually have short final adult height (although within their target height range), whereas children with CDGP have a delay in the onset of puberty but eventually attain normal final adult height. GH–IGF1 dynamics are essentially normal in both these variants, except in a few children with CDGP where integrated GH secretion has been shown to be abnormal (low GH pulse frequency and amplitude).

The differences between FSS and CDGP are summarized in the table given below.

Parameters	Familial short stature	Constitutional delay in growth and puberty
Midparental height	Short	Normal
Adrenarche	Normal	Delayed
Onset of puberty	Normal	Delayed
Bone age	Normal	Delayed
Correlation among CA, HA, BA	CA=BA>HA	CA>HA=BA
Final adult height	Short	Almost normal

CA chronological age, *HA* height age, *BA* bone age

43. *What are the treatment options for children with idiopathic short stature*?

Recombinant human growth hormone (rhGH) therapy is recommended in children with ISS whose height is <–2.25 SDS and/or have a predicted adult height <–2.0 SDS. The recommended dose of rhGH in children with ISS is 0.3–0.375 mg/Kg/week, which is higher than the dose recommended in children with GHD, as children with ISS are relatively GH resistant. Therapy with rhGH results in a height gain of 3.5–7.5 cm, with mean duration of treatment of 4–7 years. Children with CDGP do not require therapy with rhGH as the final adult height is normal in these individuals. Monotherapy with GnRH agonists is not recommended in children with ISS. Combination therapy with GnRH agonists and rhGH is a possible therapeutic option in these children; however, there is insufficient data to routinely recommend the combination therapy. Aromatase inhibitors, either alone or in combination with rhGH, have been shown to increase predicted adult height in boys with ISS; however, data regarding the final adult height with these agents is lacking. In addition, aromatase inhibitors are not recommended in girls with ISS as there is limited data regarding the use of these agents in girls.

44. *What are the treatment options in children with CDGP*?

The treatment options for boys with CDGP include oxandrolone, low-dose testosterone, or aromatase inhibitors, whereas in girls, low-dose estradiol is preferred. Oxandrolone (0.05–0.1 mg/Kg/day) is indicated in boys with CDGP aged 10–14 years where short stature is a predominant concern and low-dose testosterone (50–100 mg testosterone esters intramuscularly monthly for a period of 3–6 months) after the age of 14 years where delayed puberty is of significant concern. Although these therapies increase growth rate, final adult height is not altered. Aromatase inhibitors alone and in combination with testosterone have been shown to increase predicted adult height in boys with CDGP; however, data regarding the final adult height with these agents is lacking. Low-dose estradiol is recommended in girls with CDGP, although there is limited literature regarding its use.

45. *What is* "*catch-up*" *growth*?

"Catch-up" growth is defined as accelerated height velocity above statistical limits for the corresponding age and gender during a particular time interval, following a transient period of growth inhibition. "Catch-up" growth helps the child to attain pre-retardation growth curve. The exact mechanisms implicated in "catch-up" growth are not well explicited, but two hypotheses have been proposed: "neuroendocrine" hypothesis and "growth plate" hypothesis. The neuroendocrine hypothesis proposes that there is a set point in the brain for an individual's growth termed as "sizo-stat," and this allows the "catch-up" growth after a period of growth inhibition to achieve the age specified "preset" height of an individual. However, it was refuted later. The "growth plate" hypothesis reveals that during the period of growth inhibition, there is a slowing of chondrocyte senescence, followed by increased chondrocyte growth and proliferation during subsequent "catch-up" growth.

46. *Can* "*catch-up*" *growth occur despite decreased height velocity*?

A unique type of "catch-up" growth is described in patients with precocious puberty who are on therapy with gonadotropin-releasing hormone (GnRH) agonists. In children with precocious puberty, height age is more than chronological age but lesser than bone age. On treatment with GnRH agonist, the height velocity actually decreases, but this is without advancement in bone age thereby "height age catches up with bone age," allowing them to attain the target height. This is an example of "catch-up growth with decreased height velocity."

47. *What is* "*catch down*" *growth*?

The "catch down" growth is defined as deceleration in height velocity below statistical limits for the corresponding age and gender during a particular time interval. The term is commonly used to describe the growth pattern during initial period of life in children with FSS and CDGP, who rapidly crosses centile downward during first 3 years of age. The exact cause for this phenomenon is unknown; however, it is attributed to transient and subnormal functioning of GHRH–GH–IGF1 axis ("lazy pituitary syndrome").

48. *What are skeletal dysplasias*?

Skeletal dysplasias (osteochondrodysplasias) are heterogeneous group of inherited disorders which are characterized by intrinsic abnormality of chondro-osteogenesis. Skeletal dysplasias are commonly classified on the basis of site of involvement as epiphyseal, metaphyseal, diaphyseal, or spondylo (spine) dysplasia. These disorders may be associated with short stature and/or short arm/thighs (rhizomelia), short forearm/legs (mesomelia), or generalized shortening of the entire limb (micromelia/phocomelia). Achondroplasia is the prototype of skeletal dysplasia and is characterized by disproportionate short stature with rhizomelia (shoulder to elbow length < elbow to metacarpal length) and metaphyseal dysplasia. Children with achondroplasia are born with normal birth length but experience progressive decline in growth velocity by 1–2 years of age, and the final adult height is severely

impaired (132 cm in males and 125 cm in females). The other abnormalities include disproportionately enlarged head, characteristic facies, trident hand, brachydactyly, genu varum, exaggerated lumbar lordosis, and limitation of elbow extension and rotation. Serum IGF1 and GH dynamics are normal, and bone age corresponds to chronological age in these individuals. Achondroplasia is due to activating mutations of FGF receptor type 3 (FGFR3). It is considered as a GH-resistant state and high doses of rhGH have been used with limited benefits (Fig. 1.15).

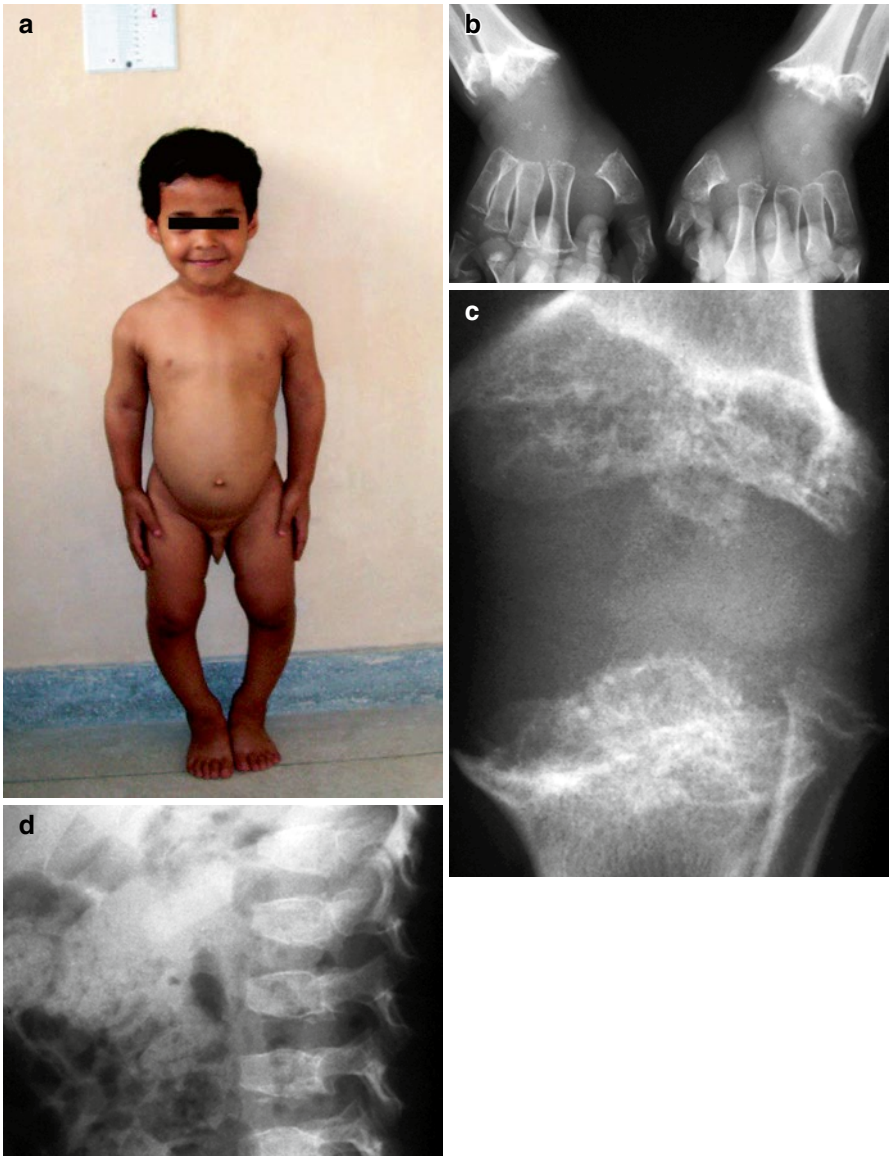

Fig. 1.15 (**a**) A child with short stature due to spondylo-epi-metaphyseal dysplasia, (**b**–**d**) plain radiographs of the wrist with proximal hands, knee, and spine show extensive epi- and metaphyseal dysplasia, juxta-articular osteopenia, and anterior beaking involving the vertebral bodies

49. *When to suspect celiac disease in a short child*?

Celiac disease is an immune-mediated enteropathy triggered by gluten and occurs in genetically susceptible individuals (HLA-DQ2 and DQ8). Celiac disease should be suspected in a short child with gastrointestinal manifestations, anemia, delayed puberty, or rickets–osteomalacia. However, monosymptomatic presentation of celiac disease with short stature is not uncommon. Gastrointestinal manifestations are present only in 30 % of patients with celiac disease and include anorexia, abdominal pain, bloating, diarrhea, steatorrhoea, and weight loss. Every short child should undergo screening for celiac disease if weight for height is impaired, especially in individuals who consume gluten-based diet.

50. *How to diagnose celiac disease*?

IgA tissue transglutaminase (IgA tTG) is a good screening test with a sensitivity of 98 % and specificity of 95 %, and if found positive diagnosis should be confirmed with duodenal biopsy before subjecting to gluten-free diet. However, if IgA tTG is negative in the presence of strong clinical suspicion of celiac disease, serum total IgA should be measured, as serum IgA levels are lower in 2–5 % of patients with celiac disease. Patients with a low serum IgA levels should be subjected to IgG-tTG estimation (with or without anti-deamidated gliadin peptide IgG antibody), and if any of these antibodies is found to be elevated, the patient should be subjected to duodenal biopsy. However, if clinical suspicion is strong and all antibody titers are negative, even then the patient should be subjected to duodenal biopsy.

51. *How does celiac disease cause short stature*?

Celiac disease is a cause of growth failure in 2.9–8.3 % of children with short stature. The decreased linear growth in celiac disease is attributed to nutrient deficiency, altered GH–IGF1 axis, abnormal cytokine milieu, and delayed puberty. The degree of growth impairment is similar irrespective of presence or absence of gastrointestinal manifestations. Both macronutrient and micronutrient deficiency contribute to reduced anabolism and linear growth. Basal and stimulated GH, IGF1, and IGFBP3 are low in celiac disease. Demonstration of anti-pituitary antibodies in few patients with celiac disease points toward involvement of somatotropes by the autoimmune process. In addition, partial growth hormone insensitivity has also been reported, as exogenous GH therapy in children with untreated celiac disease fails to increase serum IGF1 levels. Increased inflammatory cytokines like TNF-α, IL-1, and IL-6 also contribute to impaired growth as they inhibit IGF1 generation. Delayed puberty is not

uncommon in children with celiac disease and also adds to growth impairment.

52. *What is the growth response in children with celiac disease on gluten-free diet*?

Gluten-free diet (GFD) is highly effective in the management of celiac disease. The catch-up growth usually occurs by 6–12 months after introduction of GFD; however, the gain in weight precedes height gain. Failure to attain catch-up growth in children with celiac disease who are on GFD for at least 1 year should raise a suspicion of refractory celiac disease, concurrent autoimmune thyroid disease, growth hormone deficiency, or SHOX haploinsufficiency.

53. *Why is there growth failure in renal tubular acidosis*?

Children with renal tubular acidosis (RTA) may manifest with growth failure. The impairment in linear growth is a result of chronic metabolic acidosis which leads to alterations in GH–IGF1 axis, decreased 1α-hydroxylase activity, and has detrimental effects on epiphyseal growth plate. Metabolic acidosis is associated with decreased GH and IGF1 secretion and action and reduced expression of IGF1 receptor on growth plate. Decreased 1α-hydroxylase activity results in reduced $1,25(OH)_2D$ levels, which lead to impaired endochondral ossification. In addition, metabolic acidosis per se has deleterious effects on hypertrophic chondrocytes at growth plate.

54. *How to treat a child with short stature due to renal tubular acidosis*?

The aim of therapy in a child with RTA is to correct metabolic acidosis in order to promote growth and prevent further progression of skeletal deformities. Therefore, early diagnosis and optimal treatment with oral alkali, either as bicarbonate or citrate, is recommended. However, citrate is preferred over bicarbonate, as it is better tolerated. The recommended dose of oral alkali (bicarbonate or potassium citrate) is 10–20 mmol/Kg daily in divided doses in proximal RTA (higher doses are required due to massive urinary loss of bicarbonate), while 1–2 mmol/Kg daily in distal RTA. Short-term administration of potassium, calcitriol, and phosphate may be required in these patients. Overcorrection with alkali should be avoided as it may worsen hypokalemia and hypercalciuria. During follow-up, serum potassium, pH, bicarbonate, and urinary calcium should be periodically monitored. In addition, renal ultrasonography should be performed at regular interval for the detection of nephrocalcinosis.

55. *What are the causes of growth failure in children with chronic kidney disease*?

Growth failure in a child with CKD is attributed to poor intake, malnutrition, metabolic acidosis, proteinuria, and renal osteodystrophy. In addition, there is alteration in GH–IGF1 axis which is characterized by increased GH, normal total serum IGF1, and reduced free IGF1 levels. Increased GH is due to GH resistance at hepatocytes and growth plate (as a result of uremia) and decreased renal clearance of GH. Serum total IGF1 is normal due to decreased clearance of IGF-binding proteins (IGFBPs), thereby resulting in reduced free IGF1 level. Further, IGF1 resistance at growth plate and use of glucocorticoids contribute to poor linear growth.

56. *How to optimize linear growth in a child with chronic kidney disease*?

Optimal nutrition, correction of anemia and metabolic acidosis, near-normalization of serum calcium, and phosphorus with maintenance of serum PTH appropriate for CKD stage result in improvement of linear growth in children with CKD. However, those children who fail to grow despite these measures should be considered for rhGH therapy. Prior to the initiation of rhGH therapy, serum phosphate (age specific) and PTH (CKD stage specific) should be normalized as high PTH levels are detrimental for chondro-osteogenesis. The higher doses of rhGH, i.e., 0.35 mg/Kg/week are recommended as CKD is a GH-resistant state. Serum phosphate and PTH levels should be monitored, as therapy with rhGH is associated with phosphate retention and consequent further rise in PTH. There are no adverse effects of rhGH on renal function; however, increased risk of chronic graft rejection remains a concern.

57. *How does hypercortisolemia cause short stature*?

Hypercortisolemia, whether endogenous or exogenous, in a child is associated with poor linear growth. The impaired growth is attributed to cortisol-mediated inhibition of GHRH–GH axis, decreased pre-chondrocyte to chondrocyte differentiation, increased chondrocyte apoptosis, impaired local IGF1 generation and action, and increased bone collagen breakdown. Furthermore, cortisol-mediated suppression of hypothalamo–pituitary–gonadal axis and alteration in calcium–vitamin D homeostasis (decreased calcium absorption and hypercalciuria) also contribute to poor linear growth (Fig. 1.16).

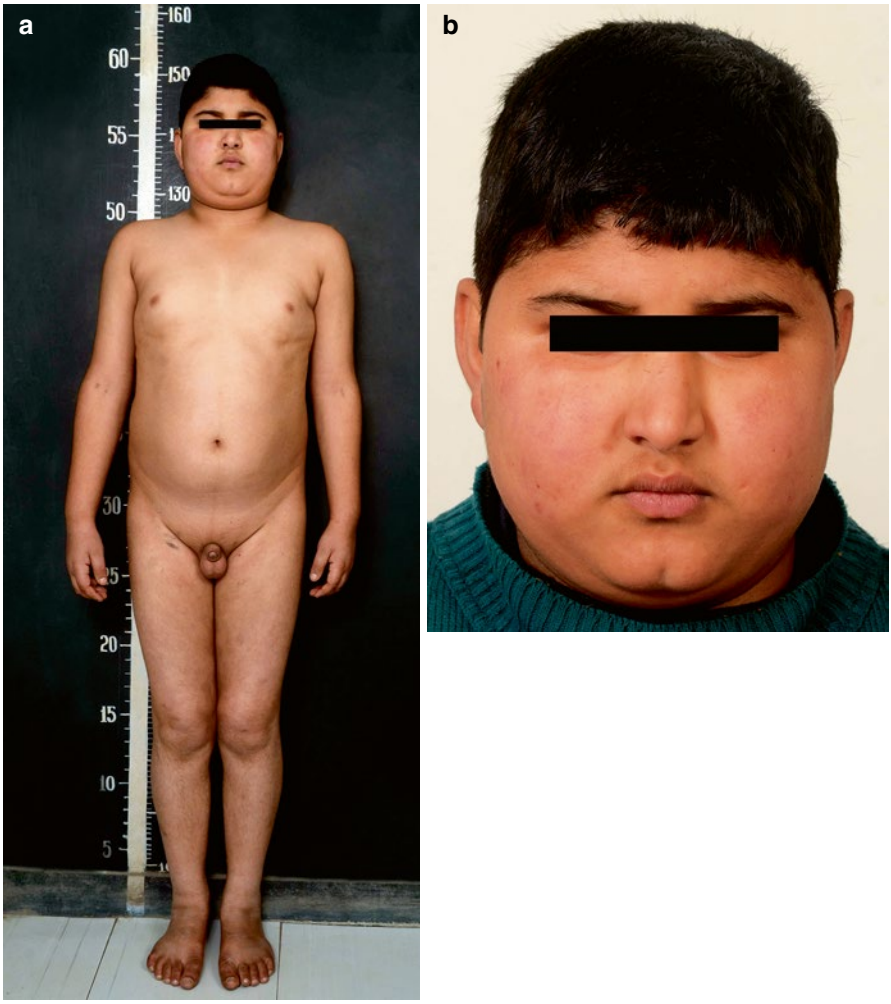

Fig. 1.16 (**a**) A 12-year-old child with short stature, obesity, and (**b**) moon facies due to Cushing's syndrome. Note lack of striae and bruise in the child

58. *What are the causes of childhood Cushing's syndrome with normal growth velocity*?

Normal growth velocity in a child with Cushing's syndrome can occur in the presence of mild hypercortisolemia [as associated with primary pigmented nodular adrenal hyperplasia (PPNAD)], intermittent hypercortisolemia (cyclical Cushing's syndrome), and cortisol and androgen co-secreting adrenal tumors. In addition, children with Cushing's syndrome due to McCune–Albright

syndrome may have normal growth velocity, if associated with concurrent hypersecretion of GH or thyroxine.

59. *Why is short stature a common manifestation of juvenile hypothyroidism*?

Short stature is a common manifestation of juvenile hypothyroidism and is invariably associated with retarded bone age (CA > HA = BA). The causes of poor linear growth in a child with hypothyroidism include reduced basal and stimulated GH secretion, decreased IGF1 generation, and impaired proliferation and maturation of chondrocytes at growth plate. Moreover, pubertal delay in children with long-standing untreated hypothyroidism also contributes to short stature (Fig. 1.17).

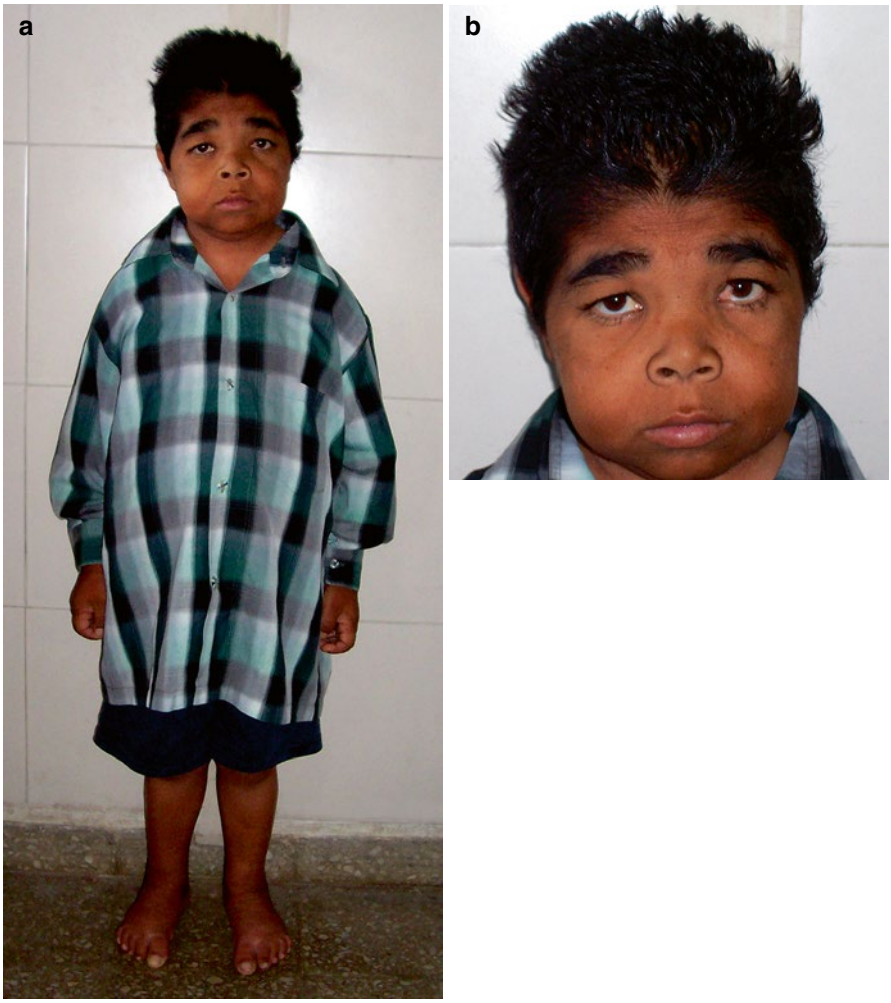

Fig. 1.17 (**a**) A 16-year-old child with short stature due to long-standing untreated congenital hypothyroidism. (**b**) Myxedematous facial features in the same child

60. *How to optimize growth and development in children with juvenile hypothyroidism*?

Early diagnosis and optimal replacement therapy result in attainment of normal final adult height in children with juvenile hypothyroidism. Children with long-standing hypothyroidism will experience catch-up growth after initiation of levothyroxine; however, catch-up growth is often incomplete in these patients due to diminished chondrocyte reserve. Children in the peripubertal age and those who are overzealously treated with levothyroxine may experience rapid skeletal maturation and, hence, compromised final adult height. There are a few reports of use of GnRH analogue with or without rhGH in peripubertal children with juvenile hypothyroidism, who had incomplete catch-up growth. However, the results were variable. Failure to have a catch-up growth despite optimal levothyroxine replacement in children with juvenile hypothyroidism should raise a suspicion of coexisting disorders like celiac disease, Turner syndrome, or growth hormone deficiency.

61. *What are the causes of short stature in children with T1DM*?

Children with poorly controlled type 1 diabetes have impaired linear growth. Uncontrolled diabetes is a catabolic state associated with elevated pro-inflammatory cytokines (TNF-α, 1L-6). In addition, type 1 diabetes is associated with low IGF1 despite high GH levels. This is because optimal concentration of portal vein insulin is required for the expression of GH receptor at hepatocytes and, consequently, GH-mediated IGF1 generation. Further, hypoinsulinemia also results in increased levels of IGFBP1, leading to decreased "free" IGF1. Early and intensive insulin therapy in children with T1DM can normalize these abnormalities in GH–IGF1 axis and may even result in accelerated growth velocity. The other causes of poor linear growth in children with T1DM include associated celiac disease, hypothyroidism, and delayed puberty.

62. *How to suspect growth hormone deficiency in a neonate*?

The clinical clues that suggest the presence of congenital growth hormone deficiency (GHD) in a neonate include midline defects, micropenis, neonatal hypoglycemia, prolonged physiological jaundice, and breech presentation. The birth length is usually normal in neonates with congenital GHD as prenatal growth is GH-independent.

63. *Why is congenital growth hormone deficiency associated with prolonged physiological jaundice*?

Glucuronyl transferase is a key enzyme involved in bilirubin metabolism, and its activity is regulated by thyroxine and growth hormone. Therefore, neonates with congenital hypothyroidism or growth hormone deficiency present with prolonged physiological jaundice (>2 weeks in term and >3 weeks in preterm baby).

64. *How to suspect growth hormone deficiency in a prepubertal child*?

GHD should be suspected in a short child with severe short stature (<−3SD), height <−2SD with height velocity <−1SD over 1 year, or height velocity <−2SD over 1 year. The phenotypic features which suggest a diagnosis of GHD in a short child include prominent forehead, depressed nasal bridge, midfacial hypoplasia, facial immaturity, chubby face, delayed dentition, tardy nail growth, micropenis, and overweight for height. History of breech presentation, presence of nystagmus, or midline abnormalities like cleft lip, cleft palate, or single central incisor and bifid uvula in a short child should also raise a suspicion of GHD. In addition, a child with known sellar–suprasellar pathology or having other anterior pituitary hormone deficiencies should also be evaluated for GHD (Fig. 1.18a, b).

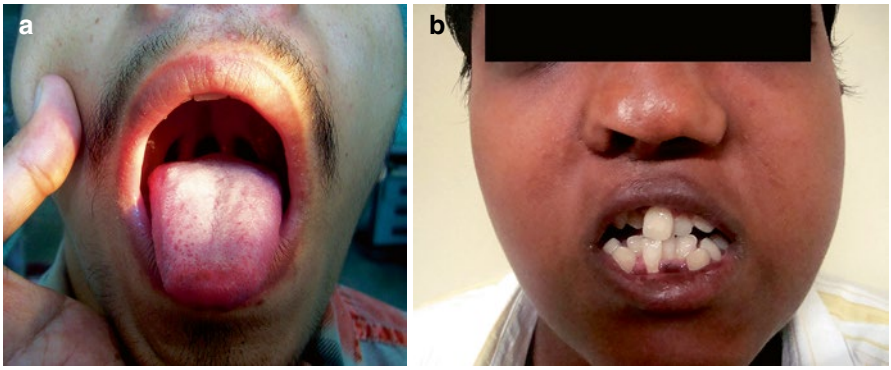

Fig. 1.18 Midline defects in patients with hypopituitarism, (**a**) bifid uvula, (**b**) single central incisor

65. *What are the endocrine complications associated with breech delivery*?

Breech delivery is associated with a risk of injury to pituitary, adrenal, and testes. Injury to the pituitary stalk during breech delivery may manifest later as isolated growth hormone deficiency or multiple pituitary hormone deficiency. However, the cause and effect relationship between breech delivery and hypopituitarism is not well established. In addition, breech delivery may be associated with adrenal hemorrhage (with adrenal insufficiency) and torsion testes.

66. *What are the causes of GHD*?

The most common cause of short stature is familial. GHD accounts for approximately 15 % of children with short stature and may be isolated or associated with multiple pituitary hormone deficiencies. However, isolated GHD in majority of children is idiopathic. The causes of GHD are shown in the figure given below (Figs. 1.19, 1.20, and 1.21).

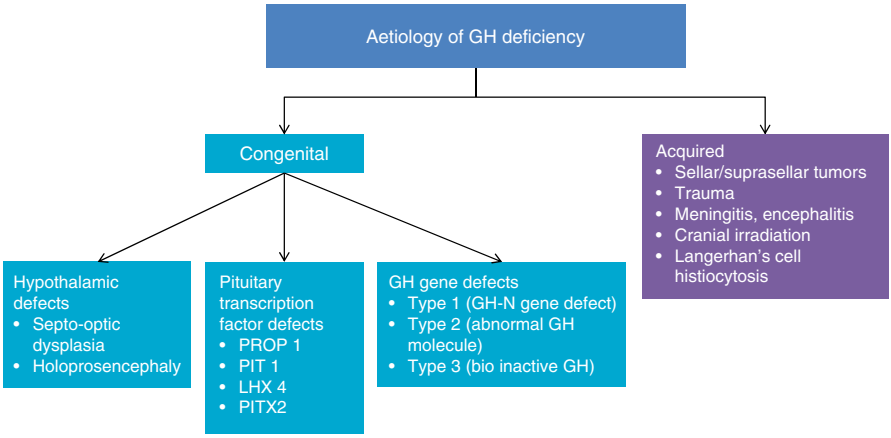

Fig. 1.19 Aetiology of growth hormone deficiency

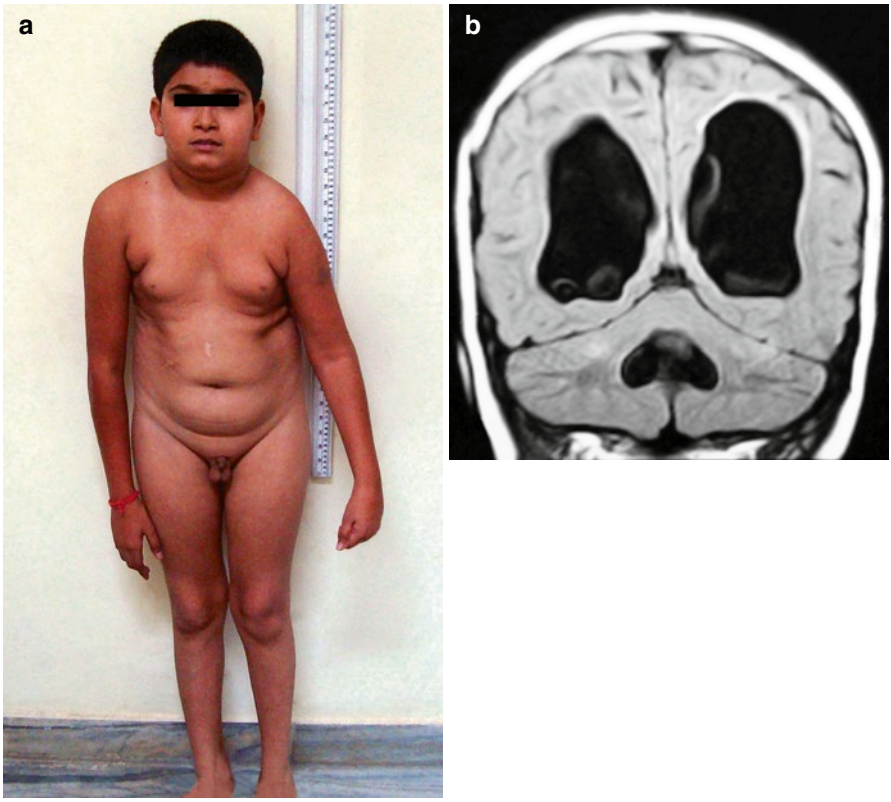

Fig. 1.20 (**a**) Short stature as a result of panhypopituitarism consequent to post-meningitis hydrocephalus. (**b**) Coronal FLAIR MRI showing enlarged ventricles in the same patient

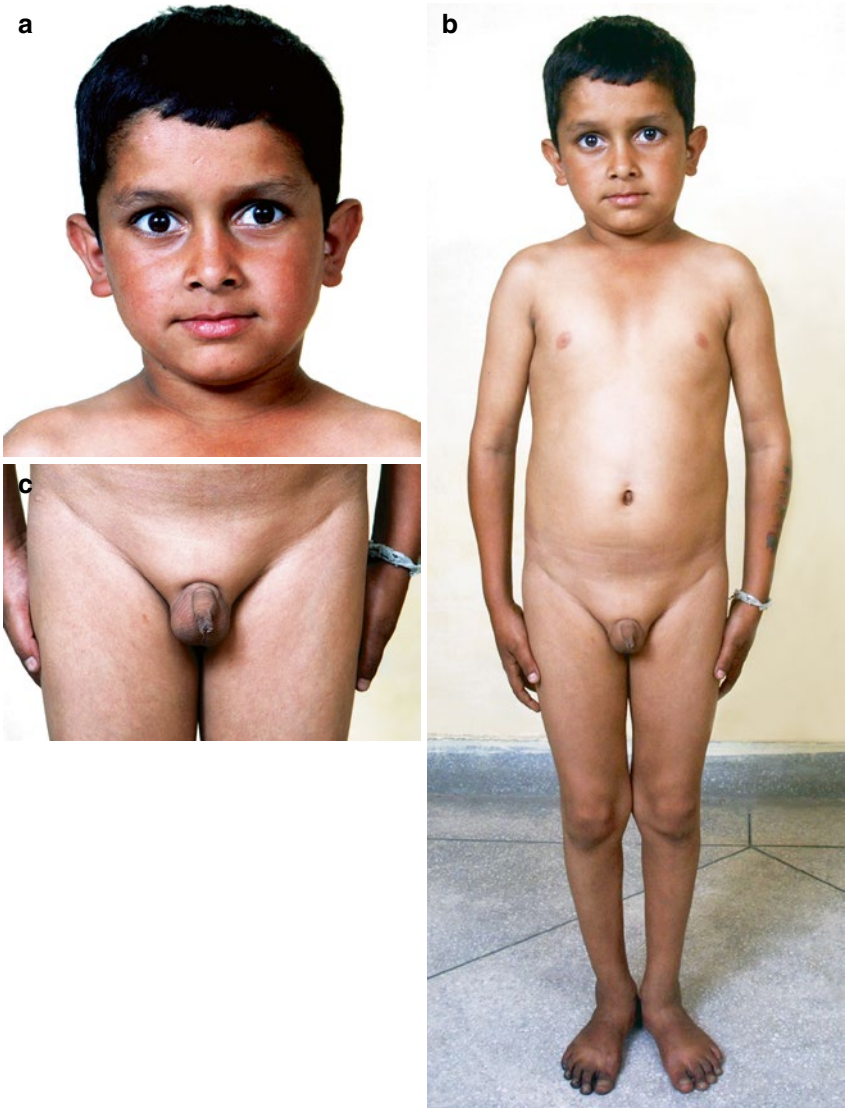

Fig. 1.21 (**a**) A 13-year-old short child with isolated GH deficiency. (**b**) Note the cherubic face, blue sclera, and (**c**) micropenis

67. *What is pseudohypoparathyroidism*?

Pseudohypoparathyroidism is a metabolic bone disease characterised by typical features of Albright hereditary osteodystrophy including round facies, short stature, obesity and short metacarpals and metatarsals. Biochemiocally, high PTH with hypocalcemia and hyperphosphatemia are unique as opposed to idiopathic hypoparathyroidism which is characterised by low PTH. Further, basal ganglia calcification on plain CT helps in predicting the duration of the disease. The defect is confined to either PTH receptor or post-receptor signaling pathway. The photrograph given below depicts a child with pseudohypoparathyroidism (Fig. 1.22).

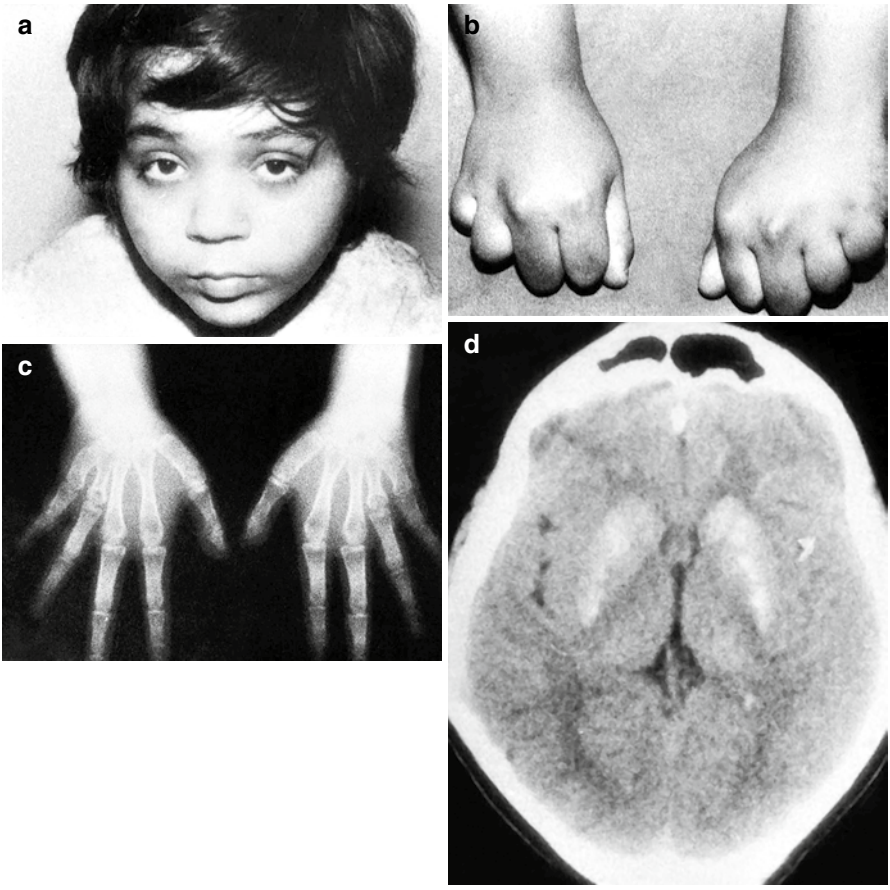

Fig. 1.22 A 12-year-old girl presented with hypocalcemic seizures. Note the (**a**) typical round facies (**b**) brachydactyly (**c**) X-ray hand confirms the presence of short 4th and 5th metacarpals (**d**) CT head depicting bilateral basal ganglia calcification

68. *When to consider subnormal height velocity in a child*?

The mean length of a healthy newborn is 50 cm and grows at height velocity of 25 cm in the first year, 12 cm in the second year, 8 cm in the third year, and 5 cm per year thereafter till the onset of puberty. The pubertal growth spurt is approximately 28 cm in boys and 25 cm in girls. Deviation from this normal pattern should be considered as pathological. In addition, crossing a centile curve downward in growth chart or height velocity <−2 SD for 12 months is subnormal and merits evaluation.

69. *What are the causes of short stature with normal height velocity*?

Majority of children with short stature have subnormal height velocity; however, the children with FSS and CDGP have normal growth velocity despite short stature.

70. *What are the causes of accelerated height velocity with short final adult height*?

The causes of accelerated height velocity with short final adult height are precocious puberty and thyrotoxicosis. These children are usually taller as compared to their peers during childhood but eventually they are short adults. Children with precocious puberty have an initial growth spurt due to gonadal steroid-mediated GH–IGF1 surge followed by early epiphyseal closure due to estrogen excess. Children with thyrotoxicosis have an increased height velocity due to the direct effect of thyroxine on epiphyseal growth plate and thyroxine-mediated GH–IGF1 surge. This is followed by premature epiphyseal closure as a result of the action of thyroxine on growth plate and due to induction of aromatase activity by thyroxine.

71. *What are the causes of accelerated height velocity with normal adult height*?

The causes of accelerated height velocity with normal adult height include childhood obesity, Beckwith–Wiedemann syndrome, and Soto's syndrome.

Further Readings

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Disorders of Growth and Development: Diagnosis and Treatment

2

2.1 Clinical Rounds

1. *Who does need to be evaluated for short stature*?

   Evaluation for short stature is indicated in a child whose:

- Height >3SD below the mean for age and gender
  Or
- Height <−2SD with a height velocity <−1SD over a period of 12 months
  Or
- Height velocity SDS <−2SD over a period of 12 months
  Or
- Predicted adult height >1.5SD below the target height
  Or
- Growth curve crosses downward by ≥1 centile curve in the growth chart over a period of 12 months

2. *Why is height SDS of –3 recommended for the evaluation of short stature*?

   Although a height >2SD below the mean for age and gender is used to define short stature, evaluation of short children based on this criteria yields organic etiology only in 14 % of these children. However, when a height SD <−3 is considered for the evaluation of short stature, the proportion of children with organic causes increases to 58 %. Nevertheless, children with height between −2SD and −3SD need careful monitoring for growth velocity, and if they show faltering, they need further evaluation.

3. *Does a single measurement of height suggest growth failure*?

   A single measurement of height does not suggest growth failure, unless the child is extremely short (<−3SD). The serial measurement of height determined

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over a period of time, preferably for 6–12 months, helps to calculate the height velocity. Short stature when compounded with decreased height velocity suggests growth failure and demands further evaluation.

4. *Who does need an urgent evaluation for GHD*?

The auxological criteria which demands urgent evaluation for GHD include severe short stature (height <−3SD) or height <−2SD with height velocity <−1SD over 1 year or height velocity <−2SD over 1 year. Any neonate with symptoms and signs of GHD/MPHD or any child with sellar–suprasellar mass should be urgently evaluated for GHD, irrespective of auxological criteria. In addition, any short child with signs and symptoms of an intracranial lesion should also be urgently evaluated.

5. *What are the investigations required for evaluation of a short child*?

A detailed history and physical examination usually provides clues to the differential diagnosis of short stature and guide further investigations. The minimum investigations to be done in a short child are complete blood count, creatinine, urine analysis, bicarbonate, calcium, phosphorous, alkaline phosphatase, SGOT, SGPT, albumin, TSH, T_4, and celiac serology (IgA tTG). Karyotype should be done in all girls with unexplained short stature and/or delayed puberty. X-ray for bone age predicts child's growth potential and also may give clues to certain differential diagnosis (rickets/dysplasia). These investigations are mandatory before proceeding for GH-IGF1 dynamics.

6. *What is the* "*ternary complex*" *in GH-IGF axis*?

The "ternary complex" comprises of IGF1, IGFBP3, and acid labile subunit (ALS) in equimolar ratio. Serum IGF1 and ALS are synthesized in hepatocytes, while IGFBP3 in the Kupffer cells of the liver. All the components of ternary complex including IGF1, IGFBP3, and ALS are GH-dependent. The ternary complex prolongs the half-life of IGF1 (from 10 min to 12–15 h) and regulates the bioavailability of IGF1 to target sites. Therefore, patients with mutation of ALS gene have reduced serum levels of IGF1 and IGFBP3. However, growth impairment in these children is modest due to preserved local IGF1 generation at the growth plate.

7. *What are the merits and demerits of serum IGF1 in the diagnosis of growth hormone deficiency*?

Serum IGF1 is a measure of integrated growth hormone secretion. In addition, the circulating levels of IGF1 are stable with minimal diurnal variation. Therefore, estimation of serum IGF1 is a useful tool for the assessment of GH–IGF1 axis. It has a sensitivity and specificity of 70 % each, for the diagnosis of GHD in older children, but the sensitivity is lesser (50 %) in younger children (<6 years) because

of higher degree of overlap between normal and abnormal values in this age group. Therefore, IGF1 is considered as a good screening test for the diagnosis of GHD in older children. However, serum IGF1 levels are influenced by age and nutritional status, and its generation is dependent on optimal levels of insulin, thyroid hormones, and gonadal steroids. Further, IGF1 assays are technically challenging as it requires separation of IGF1 from IGFBP3.

8. *What is the utility of serum IGFBP3 in the diagnosis of growth hormone deficiency*?

   Serum IGFBP3 is GH-dependent and is a measure of integrated GH secretion. Its circulating levels are stable with no diurnal variation. It is not influenced by age, nutritional status, or other endocrine factors. Further, assays for IGFBP3 are relatively technically less demanding. Therefore, IGFBP3 serves as a useful measure for the assessment of GH–IGF1 axis and has a sensitivity and specificity of 60 % and 80–90 %, respectively, for the diagnosis of GHD. It is preferred in the diagnosis of GHD in younger children (<6 years), because of its higher discriminatory value as compared to IGF1. Limited data is available regarding the use of combination of IGF1 and IGFBP3 for the diagnosis of GHD in children.

9. *What is the utility of random GH estimation in the diagnosis of GHD*?

   Growth hormone secretion is pulsatile with four to six pulses at night during non-rapid eye movement (NREM) sleep and three to four pulses during daytime in the postabsorptive phase. Therefore, random estimation of GH may not be useful for the diagnosis of GHD. However, random GH sampling is useful in the evaluation of patients with suspected neonatal GHD and growth hormone insensitivity. A random serum GH value <7 ng/ml in the first week of life (along with clinical signs or symptoms) suggest the diagnosis of neonatal GHD. Random GH >5 ng/ml in the presence of low IGF1 is diagnostic of growth hormone insensitivity.

10. *What is the role of integrated GH sampling in the evaluation of GHD*?

    There are conflicting reports regarding the utility of integrated GH sampling for the evaluation of GHD. Although it is a measure of 24h spontaneous GH secretion, it is seldom used in clinical practice as it is labor intensive, expensive, and has poor sensitivity.

11. *What is neurosecretory dwarfism*?

    Some children with short stature have low serum IGF1 despite normal GH response to provocative stimulation tests. However, on 24h integrated GH sampling, these children have abnormalities in GH secretory profile including

decreased pulse amplitude or frequency (or both) or reduced mean 24h GH concentration. These children are termed as "neurosecretory dwarfs" and are thought to have functional defects in the neuroendocrine regulation of GH secretion. However, the term neurosecretory dwarfism is obsolete and these children are classified under the category of idiopathic short stature (ISS). Children with neurosecretory dysfunction show excellent response to rhGH therapy.

12. *What is the need for GH dynamic tests in the evaluation of GHD*?

Although auxology is the best index for the assessment of GHD, biochemical assessment of GH–IGF1 axis is required for the confirmation of the same. Random GH estimation has limited value in the diagnosis of GHD as GH secretion is pulsatile, and the GH levels can range from <0.1 ng/ml during the nadir to >30 ng/ml during the peak, even in normal individuals. Serum IGF1 is a good screening test; however, there is a great degree of overlap between the levels found in normal children and those with GHD (i.e., low sensitivity). In addition, serum IGF1 is influenced by many factors other than GH. Because of these limitations, GH dynamic tests are employed for the evaluation of GHD.

13. *When should a short child be evaluated for GHD*?

Since GHD is a rare cause of short stature, evaluation of GH–IGF1 axis is recommended only after careful exclusion of common causes of growth failure including chronic systemic diseases (e.g., celiac disease, renal tubular acidosis), hypothyroidism, rickets, Turner's syndrome, pseudohypoparathyroidism, and skeletal dysplasias.

14. *What are the various GH dynamic tests employed for the evaluation of GHD*?

Dynamic tests for the evaluation of GHD can be physiological or pharmacological. The physiological stimuli used are exercise and sleep, whereas pharmacological stimuli include insulin-induced hypoglycemia, clonidine, glucagon, L-dopa, arginine, GHRH, and combined GHRH–arginine.

15. *What are the prerequisites for GH dynamic testing*?

GH secretion is regulated by various factors which include diet, sleep, exercise, thyroid hormones, cortisol, and gonadal steroids. Dietary constituents like amino acids stimulate GH secretion, while glucose and free fatty acids inhibit GH secretion. Therefore, GH dynamic tests are recommended to be performed in the fasting state. Thyroxine has a permissive role in GH–IGF1 secretion;

therefore, euthyroidism should be ensured prior to GH dynamic tests. In addition, gonadal steroids also influence GH–IGF1 secretion; hence, priming with estrogen/testosterone should be considered in children above 8 years of age having Tanner stage ≤2. GH dynamic tests should not be performed in children receiving >15 mg/m^2/day of hydrocortisone or its equivalents, as this may lead to more false-positive results.

16. *What is the rationale of "priming" prior to GH dynamic testing*?

Gonadal steroids potentiate GH secretion and this results in GH–IGF1 surge during puberty. Hence, priming with gonadal steroids is required to optimize GH response to provocative stimuli in children of peripubertal age. However, there are controversies regarding the need for priming, the age at which priming should be done and which agent should be used for priming. Priming with gonadal steroids is suggested in children above 8 years of age having Tanner stage ≤2.

17. *How to prime with gonadal steroids before GH dynamic testing*?

The various protocols for priming are summarized in the table given below.

Drugs	Protocol	Comments
Conjugated equine estrogen	5 mg PO in the previous night and in the morning of test	Less preferred
		Can be used in both boys and girls
Ethinyl estradiol	0.1 mg PO for 3 days, prior to test	Can be used in both boys and girls
Estradiol valerate	2 mg PO for 3 days (>20 Kg), prior to test	Can be used in both boys and girls
	1 mg PO for 3 days (<20 Kg), prior to test	
Testosterone enanthate	100 mg IM injection 3 days prior to test	Can be used only in boys

18. *What are the mechanisms of growth hormone release in response to GH dynamic tests*?

GH secretion is a consequence of interplay between growth hormone-releasing hormone (GHRH) and somatostatin at the level of hypothalamus and pituitary. GHRH stimulates the synthesis and release of GH, while somatostatin exerts an inhibitory effect on GH secretion. Alterations in the somatostatin tone determine the pulsatility of GH secretion. The mechanism of action of various GH secretagogues is summarized in the table given below.

Tests	Mediator	Mechanism
Insulin-induced hypoglycemia	Low plasma glucose (gluco-receptors at hypothalamus)	↓ Somatostatin via cholinergic receptors
		↑ GHRH via α_2-adrenergic receptors
Clonidine	Norepinephrine	↑ GHRH via α_2-adrenergic receptors
Glucagon stimulation test	Glucagon	↑ GHRH via α_2-adrenergic receptors
		↓ Somatostatin via cholinergic receptors
	Peptidyl fragments of glucagon	Direct stimulatory effect of peptidyl fragments on somatotropes
L-Dopa	Dopamine	↑ GHRH via α_2-adrenergic receptors
		↓ Somatostatin via cholinergic receptors
Arginine	Arginine	↓ Somatostatin
GHRH	GHRH	Direct effect
Ghrelin and analogues	Ghrelin	Increases GHRH release
		Potentiates the effect of GHRH

19. *What are the merits and demerits of different GH dynamic tests*?

The merits and demerits of different GH dynamic tests are summarized in the table given below.

Tests	Merits	Demerits
Insulin-induced hypoglycemia (IIH)	Gold standard	Risk of severe hypoglycemia which can cause seizure and arrhythmia
	Assess multiple pituitary hormones (GH, ACTH, prolactin, and ADH)	
Clonidine	Reproducible	Less effective in older children
	Oral route	Causes sedation and hypotension
Glucagon	Preferred in children	Causes nausea and vomiting
	Also assess ACTH reserve	
L-Dopa	Oral route	Causes sedation
		Less potent
Arginine–GHRH	Most potent	Expensive
	Safe	Limited availability
	Reproducible	Falsely negative in those with hypothalamic causes of GHD
Ghrelin and analogues	Potent	Limited data

20. *Which is the preferred GH dynamic test*?

Insulin-induced hypoglycemia is considered as the gold standard for the diagnosis of GHD. However, the test is associated with adverse events; hence, clonidine and glucagon stimulation tests are commonly performed in clinical practice. Considering the merits and demerits of different GH dynamic tests, arginine–GHRH seems to be an alternative to these tests; however, cost and limited availability precludes its routine use in clinical practice.

21. *Why is there a need for two dynamic tests in the diagnosis of GHD*?

At least two GH dynamic tests are required for the confirmation of the diagnosis of GHD due to low specificity of these tests. Both the tests should be abnormal to make a definitive diagnosis of GHD. However, a single dynamic test is sufficient to diagnose GHD in those with structural pituitary defects or multiple pituitary hormone deficiencies.

22. *How to define GH deficiency after GH dynamic tests*?

For the diagnosis of childhood GHD, a peak serum GH level <10 ng/ml after provocative stimuli is considered as subnormal, whereas a cutoff <7 ng/ml is considered as severe GHD. In spite of the differences in relative potency and mechanism of action, the stimulated levels of GH differ modestly between the different dynamic tests. Hence, the same GH cutoffs are used to define GHD while using various tests. However, when using GHRH–arginine a cutoff ≤19 ng/ml is suggested because of its high potency.

23. *What are the limitations of GH dynamic tests in childhood GHD*?

GH dynamic tests are nonphysiological and have poor specificity and reproducibility. These tests assess pituitary GH reserve but do not provide information regarding pulsatility of GH secretion and bioactivity. Hence, a normal peak GH response to a dynamic test may not necessarily translate into optimal linear growth. In addition, the cutoffs for the diagnosis of GHD are arbitrary and do not take into account the effect of age and BMI on GH dynamics. There is also controversy regarding priming with sex steroids. Lastly, the risks associated with GH dynamic tests like hypoglycemia, seizure, and hypotension limit their use in clinical practice.

24. *What is the importance of CT/MR imaging in the evaluation of children with GHD*?

All patients with documented GHD should be subjected to MR imaging of the sellar region to exclude the possibility of sellar–suprasellar mass lesions, structural defects of pituitary gland and stalk or midline defects. In addition, CT head may be required to detect calcification in patients with craniopharyngioma (Figs. 2.1, 2.2, and 2.3).

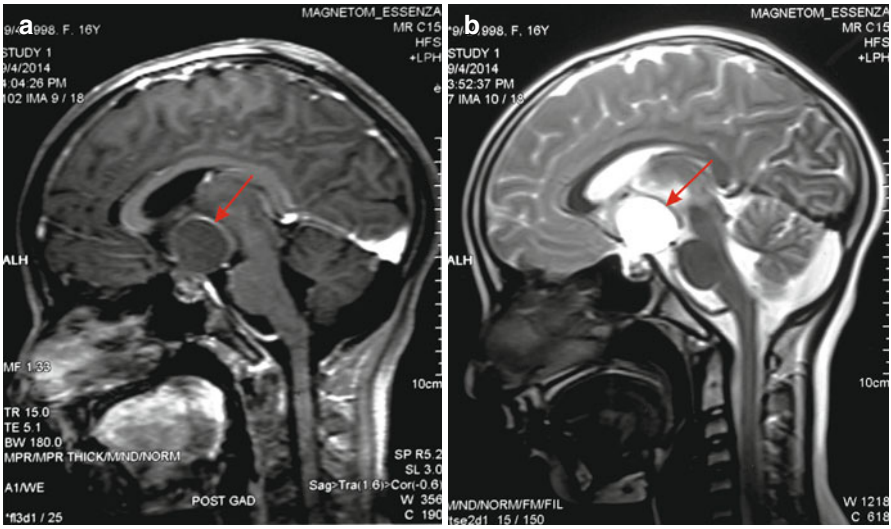


Fig. 2.1 (**a**) Sagittal CEMRI and (**b**) sagittal T2W MR image showing a predominantly cystic suprasellar mass lesion (*red arrow*) suggestive of craniopharyngioma

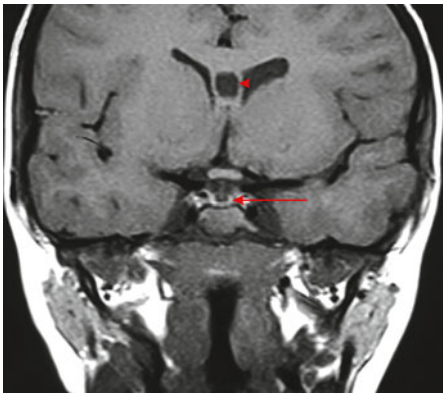


Fig. 2.2 Coronal CEMRI demonstrates a hypoplastic pituitary gland (*red arrow*) in a child with panhypopituitarism. Also note cavum septum pellucidum (*arrow head*)

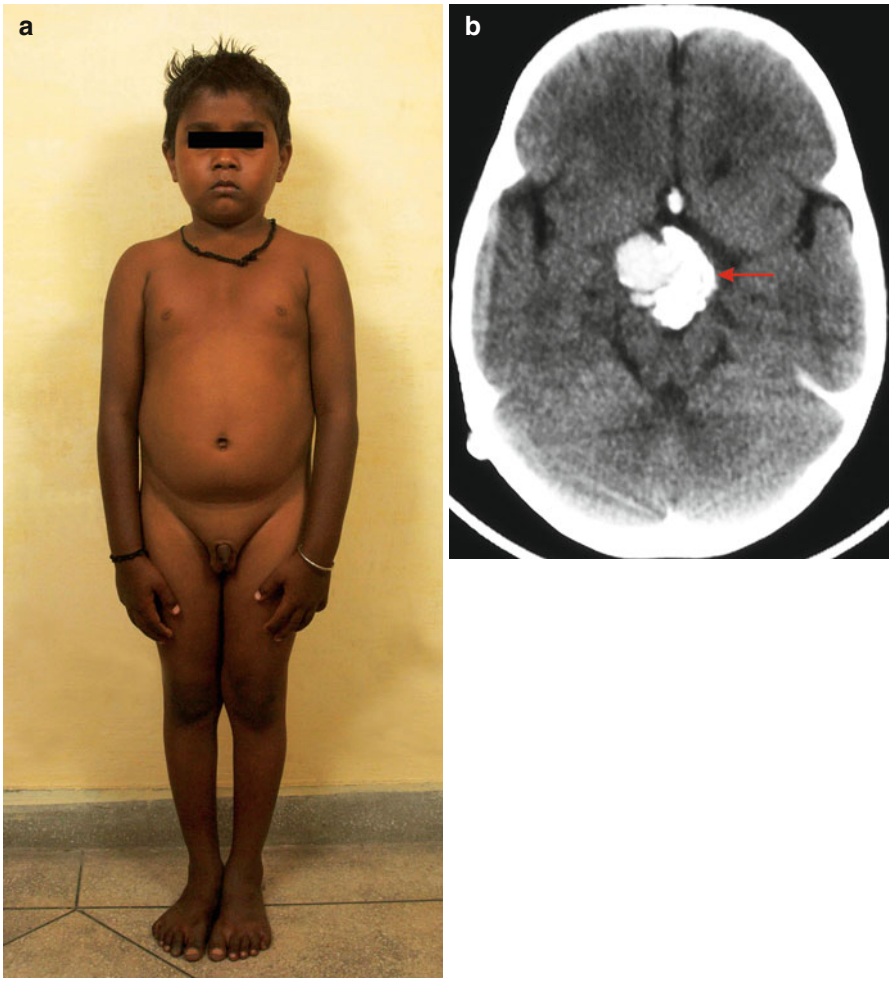


Fig. 2.3 (**a**) A 6-year-old boy with short stature (**b**) axial plain CT scan showing dense suprasellar chunky calcification (*red arrow*) in the same patient suggestive of craniopharyngioma

25. *What is MRI "tetrad" associated with GHD/MPHD?*

The MRI tetrad includes small sella, hypoplastic/absent anterior pituitary, redundant/absent pituitary stalk, and ectopic/absent posterior pituitary bright spot. The presence of these structural defects in a short child is highly predictive of multiple pituitary hormone deficiency; hence, only a single GH dynamic test is required to establish the diagnosis of GHD in these patients. The presence of these abnormalities is also suggestive of irreversible GHD; therefore, GH dynamic tests may not be required during transition to adulthood in these patients (Fig. 2.4).

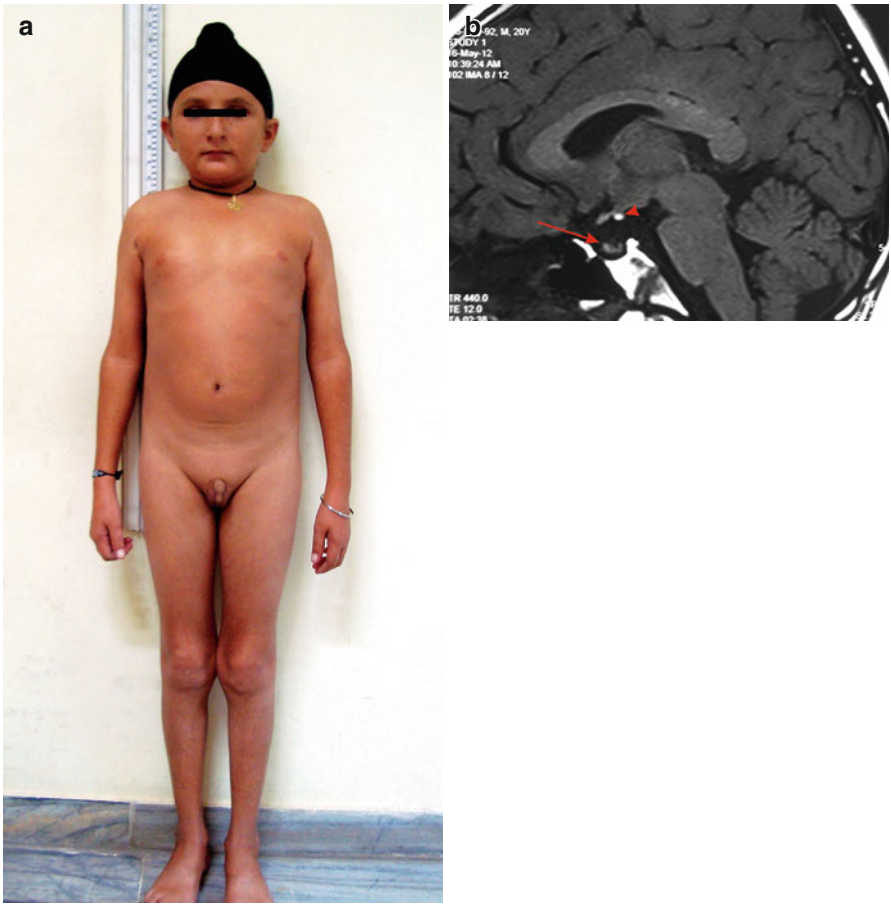


Fig. 2.4 (**a**) A 20-year-old male with short stature and delayed puberty due to multiple pituitary hormone deficiency. (**b**) Sagittal CEMRI showing small pituitary (*red arrow*), redundant stalk, and ectopic posterior pituitary bright spot (*arrow head*) suggestive of pituitary transcription factor defect

26. *What is septo-optic dysplasia*?

Septo-optic dysplasia (SOD) includes midline defects of brain (absence of septum pellucidum, corpus callosum agenesis), optic nerve dysplasia, and pituitary hypoplasia. Two out of these three abnormalities are required for the diagnosis of SOD. Familial forms of SOD are caused by HESX1 transcription factor defect and are associated with multiple pituitary hormone deficiency including vasopressin (Fig. 2.5).

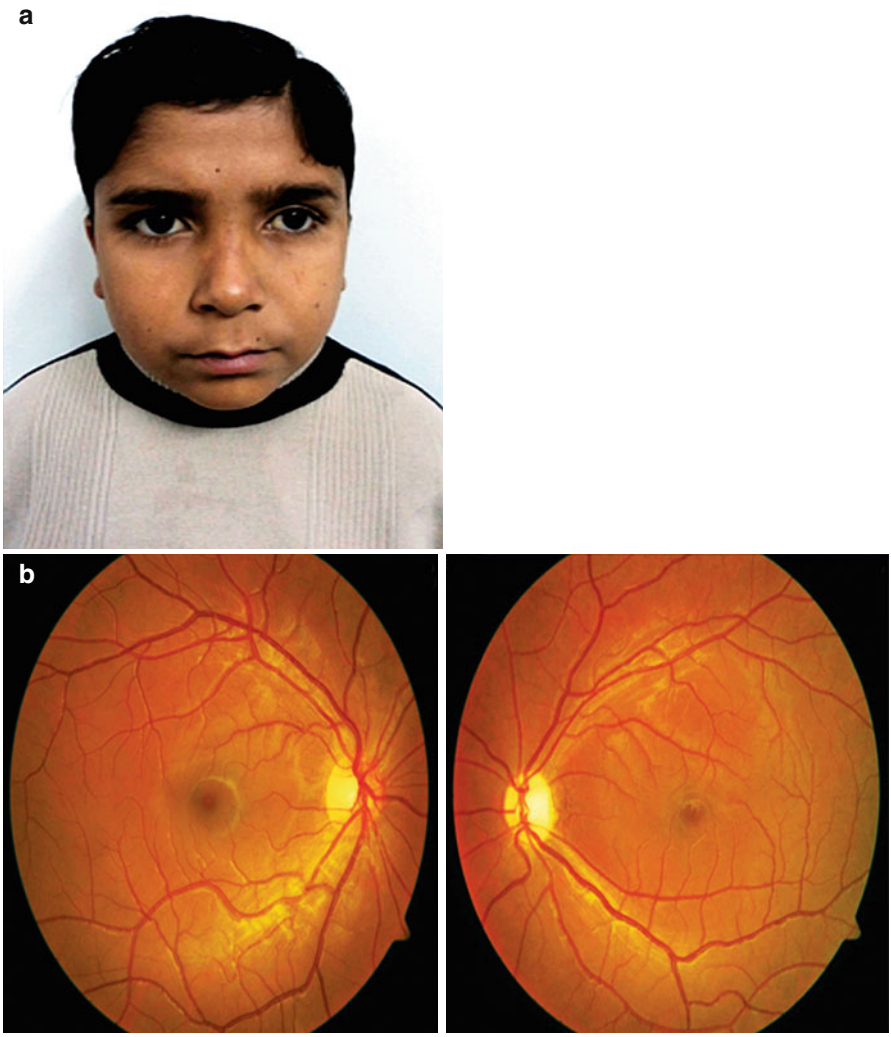
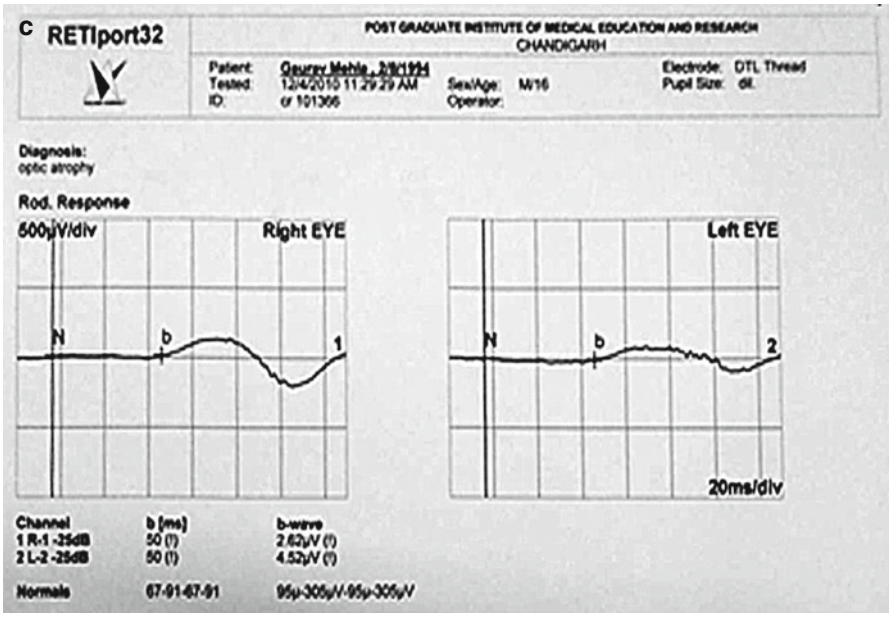
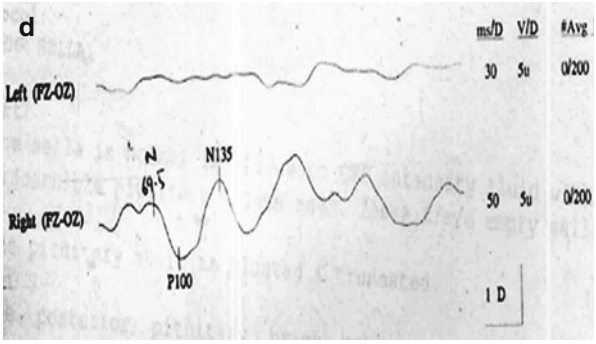
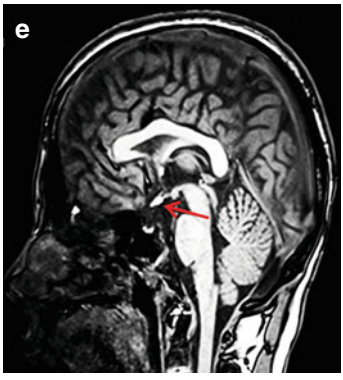


Fig. 2.5 (**a**) A 21-year-old boy with panhypopituitarism and septo-optic dysplasia. (**b**) Fundus examination showing temporal pallor of left optic disc. (**c**) Electroretinogram depicting absence of *a* and *b* wave in the left eye. (**d**) Visual evoked responses revealed absence of waveforms in the left eye. (**e**) CEMRI sella demonstrating classical tetrad (ectopic posterior pituitary bright spot, *red arrow*) of pituitary transcription factor defect. (**f**) Coronal MR image depicting left optic nerve hypoplasia as compared to right optic nerve (*red arrow*)







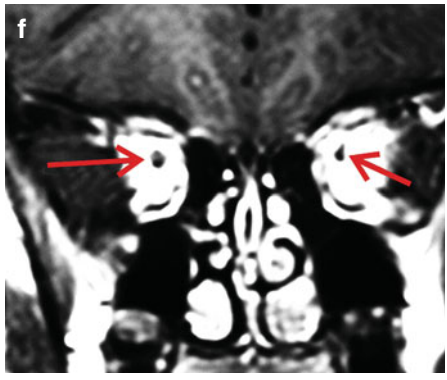


Fig. 2.5 (continued)

27. *What is waxing and waning pituitary*?

Alterations in the size of the pituitary gland in children with congenital GH or multiple pituitary hormone deficiency due to PROP1 transcription factor defects have been described during the course of disease. "Waxing" is probably due to physiological compensatory hyperplasia of the residual pituitary cells due to increase in the expression of transcription factors like HESX1 which is normally repressed by PROP1 or due to hypertrophy of the intermediate lobe and "waning" is due to ongoing pituitary damage and fibrosis.

28. *How to treat a child with growth hormone deficiency*?

A child with documented growth hormone deficiency should be treated with rhGH in a dose of 0.18–0.35 mg/Kg/week (1 mg = 3 IU). The rhGH is administered subcutaneously, daily between 8 and 9 pm to mimic the physiology of GH secretion. Optimal response to GH requires adequate nutrition and regular physical activity. In addition, regular surveillance for the development of thyroid hormone and cortisol deficiency is required to optimize the response to rhGH therapy.

29. *What are the predictors of response to rhGH therapy*?

The predictors of response to rhGH therapy include optimal doses, daily administration, initiation of therapy at an early age, severity of GH deficiency, pretreatment growth velocity, and genetic potential of an individual. In addition, individuals with GH receptor polymorphism (GHRd3) have been shown to respond better to rhGH therapy. However, children with previous spinal irradiation exhibit suboptimal response to rhGH therapy.

30. *How to adjust the doses of rhGH in a child with GHD*?

Optimal growth response is the best index to assess the adequacy of rhGH therapy in a child with GHD. The dose of rhGH should be periodically adjusted on the basis of body weight. If the growth response is not adequate, dose of rhGH should be increased to 0.35 mg/Kg/week after ensuring compliance to therapy and exclusion of hypothyroidism. If the growth response is suboptimal even after adequate doses of rhGH, serum IGF1 should be measured and if low, a possibility of resistance to GH should be considered. In addition, estimation of serum IGF1 is recommended at periodic intervals to ensure compliance and safety. However, serum IGF1 level alone should not be used to increase the dose of rhGH therapy as IGF-based dosing schedules are associated with higher requirement of rhGH (about 2.5 times) as compared to weight-based regimens. The higher doses of rhGH are associated with more adverse events and do not translate into increased linear growth. Persistently (>2 years) high serum IGF1 (>2SD) should be avoided as it may be associated with an increased risk of malignancy. It has been shown that increasing the doses of rhGH during puberty (up to 0.7 mg/Kg/week) results in increased final adult height by approximately 4.6 cm (Fig. 2.6).

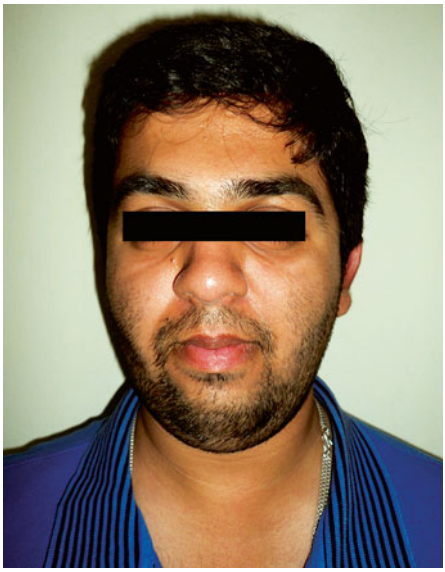


Fig. 2.6 Acromegaloid features in an 18-year-old boy with GH deficiency following IGF1-targeted rhGH therapy

31. *How to monitor a child on rhGH therapy*?

After initiation of GH therapy, auxological parameters including height, weight, body proportions, and waist circumference should be monitored at three monthly intervals. Height velocity should be monitored six monthly and bone age annually. In addition, children should be evaluated for the development of hypothyroidism, hypocortisolism, and dysglycemia. Serum IGF1 should be estimated after 3 months of initiation of therapy and yearly, thereafter. Serum IGF1 should not exceed the normal reference range for age and gender (>2SD), as it may be associated with adverse events. The child also needs to be under regular surveillance for the development of side effects like edema, gynecomastia, papilloedema (pseudotumor cerebri), slipped capital femoral epiphysis, pancreatitis, and worsening of preexisting scoliosis (Figs. 2.7 and 2.8).

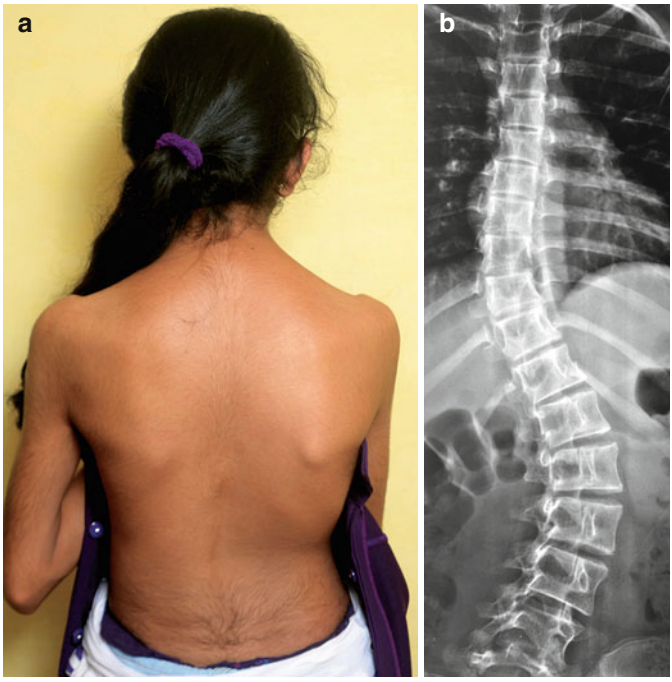


Fig. 2.7 (**a**, **b**) Development of scoliosis in a child after rhGH therapy

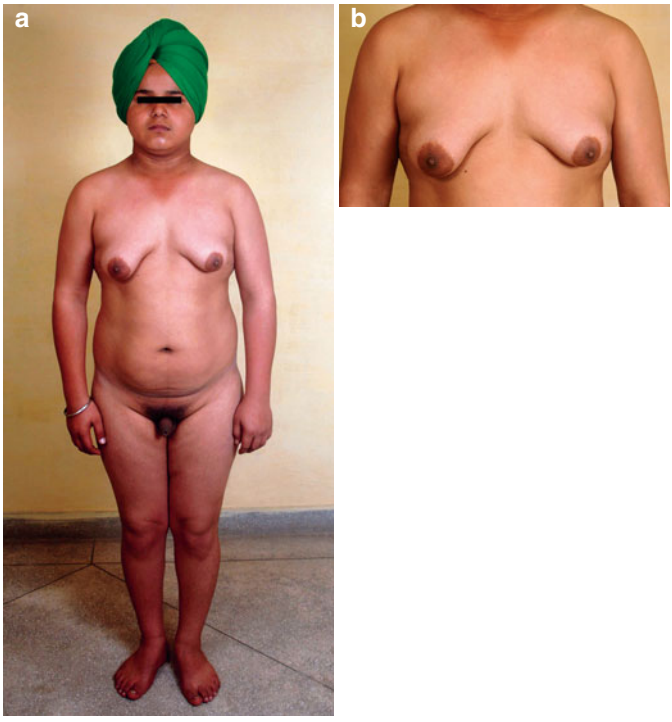


Fig. 2.8 (**a**, **b**) Development of gynecomastia in an 18-year-old boy with multiple pituitary hormone deficiency after rhGH therapy. Patient did not receive prior testosterone therapy

32. *What is the expected growth response to rhGH therapy in children with GHD*?

Treatment with rhGH results in a brisk growth response of 10–12 cm in the first year and 7–9 cm in the second and third years, followed by 5 cm/year thereafter. The height SDS should increase by at least 0.25 SDS in the first year of treatment. Suboptimal response to rhGH therapy should raise a suspicion of poor compliance, malnutrition, coexisting celiac disease, and hypothyroidism. After exclusion of these causes, further titration of rhGH doses should be based on body weight, growth velocity, and pubertal development.

33. *Why is there a decline in growth velocity with the continued use of rhGH*?

Initiation of rhGH therapy is associated with a rapid increase in growth velocity in the first year, followed by progressive decline in efficacy over the next few years. This phenomenon was initially thought to be associated with the development of anti-GH antibodies; however, the antibody titers were not sufficient to interfere with the action of GH. Increased chondrocyte recruitment and proliferation from the resting zone of epiphyseal growth plate is responsible for catch up growth after the initiation of GH therapy. The subsequent reduction in the efficacy of rhGH over the years is due to the progressive decline in the chondrocyte reserve, and increased "chondrocyte senescence" has been suggested as possible mechanisms.

34. *What are the causes of suboptimal response to rhGH therapy*?

Besides technical reasons (optimal dose, daily administration, and injection techniques), the important causes of suboptimal response to rhGH therapy include development or unmasking of secondary hypothyroidism, concurrent presence of celiac disease, malnutrition, previous spinal irradiation, and development of anti-GH antibodies particularly in those with GH gene (GH-N) deletion.

35. *Why is it necessary to monitor serum T_4 in patients on rhGH therapy*?

Therapy with rhGH results in increased peripheral conversion of T_4 to T_3, leading to decrease in T_4 and increased T_3. These changes usually occur within first 3 months after initiation of therapy and resolves spontaneously by 6–12 months. Some studies have shown a decrease in TSH after rhGH therapy; this may be due to inhibition of thyrotropes as a consequence of increased T_3 and augmented somatostatin tone. The development of hypothyroidism is uncommon in majority of patients; however, rhGH therapy may unmask central hypothyroidism in individuals with multiple pituitary hormone deficiency. Hence, it is recommended that thyroid function tests should be performed after 3 months of initiation of rhGH therapy and annually thereafter.

36. *When to induce puberty in children with multiple pituitary hormone deficiency those who are on rhGH therapy*?

In children with multiple pituitary hormone deficiency, those who are on rhGH therapy from childhood, and those who have a normal growth, puberty should be induced with gonadal steroids at a chronological age of 11–12 years in girls and 12–13 years in boys, if there is evidence of gonadotropin deficiency (e.g., micropenis, cryptorchidism). However, in children with multiple pituitary hormone deficiency, who were initiated on rhGH at a later age and have subnormal prepubertal height, puberty should be induced at the age of 13 years in girls and 14 years in boys. Further delaying the induction of puberty may have a negative impact on psychosocial development and bone health. For induction of puberty, gonadal steroids should be initiated at low doses and the doses are progressively increased over a period of 3–4 years. However, prior to initiation of gonadal steroids, a child with MPHD should be optimally replaced with L-thyroxine, as under or over replacement may interfere with growth and puberty. Overtreatment with glucocorticoids should be avoided as it adversely affects growth and puberty. It has been shown that increasing the doses of rhGH during puberty (up to 0.7 mg/Kg/week) results in increased final adult height by approximately 4.6 cm.

37. *What is the role of combination therapy with rhGH and GnRH agonists in children with isolated GHD*?

Delaying the onset of puberty may improve the final adult height in children with short stature at the onset of puberty. Combination therapy with rhGH and GnRH agonists may be considered in children with GHD and having short stature at the onset of puberty. However, studies of combined use of rhGH and GnRH agonists have shown variable results. Hence, the routine use of rhGH and GnRH agonists in children with isolated GHD is not recommended.

38. *When to discontinue rhGH therapy in a short child*?

The primary aim of treatment with rhGH in a short child is to achieve a final adult height as close to the target height as possible. The end points for discontinuation of rhGH therapy include achievement of the target height, decrease in growth velocity to <2 cm/year or closure of epiphysis. However, patients with persistent GHD due to underlying genetic or structural defects and some patients with idiopathic GHD require continuation of rhGH therapy during adulthood.

39. *What are the long-term risks associated with rhGH therapy in children with GHD*?

It has been shown that long-term rhGH therapy in children with GHD is associated with higher risk of mortality and increased risk of neoplasms. In a recent study, Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE), it was demonstrated that rhGH therapy for children with short stature was associated with an increase in all-cause mortality with a standardized mortality ratio (SMR) of 1.33. Patients who received higher doses of rhGH (>50 μg/Kg/day) had an increased incidence of bone tumors, subarachnoid/intracerebral hemorrhage, cardiovascular events, and an increased all-cause mortality with a SMR of 2.94. The risk of second neoplasms (i.e., benign meningioma) is higher in children treated with rhGH who were childhood survivors of primary brain tumors and had exposure to cranial irradiation. However, the risk of tumor recurrence is not increased in children with craniopharyngioma, nonfunctional pituitary tumors, medulloblastoma, and germ cell tumors after rhGH therapy.

40. *Is there a need for continuation of rhGH therapy during adulthood*?

Growth hormone is not only required for linear growth but is also essential for the maintenance of normal body composition, cardiovascular health, skeletal integrity, and quality of life. Adult GHD is associated with altered body composition (decreased lean mass and increased fat mass), visceral adiposity, adverse lipid profile (high LDL-C and low HDL-C), insulin resistance, hypertension, increased procoagulant activity, systolic dysfunction, and increased cardiovascular mortality. In addition, GHD is also associated with poor quality of life, low bone mineral density, and increased risk of fracture. Therapy with rhGH results in improvement in body composition, lipid profile, inflammatory markers, bone mineral density, and quality of life. However, rhGH therapy has not been shown to improve cardiovascular mortality in adults with GHD.

41. *Do all children with GHD require reassessment during transition to adulthood*?

Approximately 50 % of children with isolated idiopathic GHD do not have persistent disease on retesting during adulthood, whereas 96 % of children with multiple pituitary hormone deficiency have persistent GHD. Hence, reassessment of GH–IGF1 axis during transition to adulthood is done on the basis of presence or absence of structural abnormality or multiple pituitary hormone deficiency. An approach to a child with GHD during transition to adulthood is shown in the figure given below (Fig. 2.9).

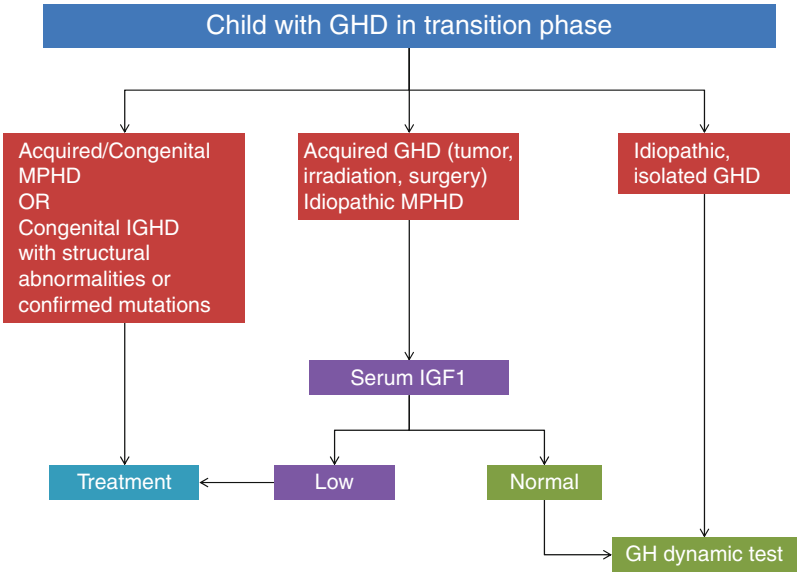


Fig. 2.9 Approach to a child with GHD during transition to adulthood

42. *Who are the children likely to have reversible GHD during transition to adulthood*?

Approximately 50 % of children with idiopathic isolated GHD have reversible growth hormone deficiency, as evidenced by normal GH dynamic studies during adulthood. The cause of this reversibility is not well explicited. It may be possibly due to recovery from transient disruption of neuroendocrine alteration in GH–IGF1 axis. Alternatively, reversibility may be due to change in diagnostic cutoffs of GHD for adults.

43. *Who should be tested for adult-onset GHD*?

As idiopathic isolated GHD is rare in adults, only those with a high probability of having GHD are to be tested for adult-onset GHD. Hence, adults with hypothalamic–pituitary disorders, traumatic brain injury, subarachnoid hemorrhage, and those with multiple pituitary hormone deficiency should be screened. In addition, those with history of cranial irradiation and surgery in hypothalamic–pituitary region should also be tested. In adults with organic disease and having MPHD ($\geq$3 hormone deficiency), a low IGF1 is highly suggestive of GHD and GH dynamic tests may not be necessary. However, in those with organic disease and having <3 hormone deficiency, serum IGF1 and a single GH dynamic test should be done to establish a diagnosis of GHD. Two GH dynamic tests are recommended before making a diagnosis of idiopathic isolated GHD in adults.

44. *What are the GH dynamic tests recommended for the diagnosis of GHD in adults*?

Insulin-induced hypoglycemia (IIH) and GHRH–arginine test are the recommended GH dynamic tests for the diagnosis of GHD in adults. However, if IIH is contraindicated (seizure disorder, coronary artery disease) or GHRH–arginine is not available, glucagon stimulation test can be performed. In those with hypothalamic causes of GHD, GHRH test is not preferred as the test is falsely normal in these individuals. The cutoffs to define GHD in adults are summarized in the table given below.

Tests	**Peak GH (ng/ml)**
IIH	<5.1
GHRH+arginine	
BMI <25 Kg/m^2	<11.5
BMI 25–30 Kg/m^2	<8
BMI >30 Kg/m^2	<4.2
Glucagon	<3

45. *Why are cutoffs for the diagnosis of GHD lower in adults*?

Serum GH cutoffs are lower for the diagnosis of GHD in adults as there is a progressive decline in function of somatotropes after puberty. In addition, lower levels of GH are required in adults (for metabolic effects) as compared to children (for linear growth).

46. *Why is the dose of rhGH lower in adults as compared to children with GHD*?

GH secretion is higher in children and adolescents as compared to adults. There is a progressive decline in GH secretion and hepatic sensitivity to GH with advancing age; hence, serum IGF1 levels progressively declines with aging. Therefore, the dose of rhGH (for promoting linear growth) in children is much higher than in adults with GHD (for the improvement in metabolic profile and quality of life). The initiating doses of rhGH are 0.2 mg/day and 0.3 mg/day in adult men and women, respectively, and 0.1 mg/day in older individuals. The doses are increased by 0.1–0.2 mg after 1–2 months to maintain serum IGF1 in the upper normal range.

47. *Why is the dose of rhGH in adults IGF1-based rather than weight-based*?

As opposed to children where weight-based GH regimens are used, IGF1-based regimens are recommended in adults with GHD. In adults, weight-based GH regimens are associated with higher doses and, hence, more adverse effects like pares-

thesia, joint stiffness, peripheral edema, and arthralgia. Higher doses of rhGH do not translate into any additional benefits in metabolic profile as compared to lower doses. Further, there is also high inter-individual variability in GH absorption kinetics from subcutaneous sites in adults as compared to children which favors IGF1-based regimen and precludes the use of weight-based regimen.

48. *Why are the doses of rhGH higher in women than in men with GHD*?

The circulating levels of GH have been shown to be twofold higher in adult women as compared to men, and this is thought to be due to the direct effects of estrogen on GH secretion. However, the circulating levels of IGF1 are almost similar or rather lower in women during adulthood, which is due to the antagonistic effects of estrogen on GH-mediated IGF1 generation at hepatocytes. Hence, women require higher doses of rhGH for the treatment of GHD. Interestingly, during prepubertal and peripubertal period, serum IGF1 levels are higher in girls as compared to boys, probably due to stimulatory effects of low concentration of estrogen on GH-mediated IGF1 generation.

49. *What are the FDA-approved indications of rhGH therapy besides GHD*?

The FDA-approved indications of rhGH therapy in children with short stature without GHD include Turner syndrome, Noonan syndrome, chronic kidney disease, Prader–Willi syndrome, idiopathic short stature (height <−2.25 SD), small for gestational age, and SHOX haploinsufficiency (Leri–Weill syndrome). In addition, rhGH therapy is also approved in patients with AIDS-associated cachexia and short bowel syndrome on total parenteral nutrition.

50. *Is GH dynamics mandatory before initiating rhGH therapy in a short child*?

GH dynamics are mandatory to establish the diagnosis of GHD in a short child. However, in children with short stature due to Turner syndrome, Noonan syndrome, Prader–Willi syndrome, small for gestational age, and chronic kidney disease, GH dynamic tests are not required prior to initiation of rhGH therapy. Recombinant hGH therapy should be initiated in children with these disorders when the child starts faltering on standard growth chart and should be followed up on the same growth chart.

51. *Which growth chart is recommended for children with intrinsic short stature*?

The height of children with intrinsic short stature should be monitored on standard growth chart as well as syndrome-specific growth chart. Monitoring of height of a child on standard growth chart allows the early recognition of growth failure (crossing of one centile curve downward) and timely initiation of

rhGH therapy, if indicated. Further, children who are treated with rhGH should be monitored on standard growth chart. A child who falters on syndrome-specific growth chart should be evaluated for secondary causes of growth failure like hypothyroidism, celiac disease, or coexisting GHD.

52. *What are the causes of GH-sufficient short stature*?

Children with systemic disorders, idiopathic short stature, intrinsic short stature, small for gestational age, and GH insensitivity syndrome are short despite GH sufficiency.

53. *Why is higher dose of rhGH recommended in the treatment of children with non-GHD short stature*?

The dose of rhGH used in children with non-GHD short stature is higher (0.375 mg/Kg/week) as compared to that used in children with GHD (0.3 mg/Kg/week). This is because these disorders are GH-resistant states and need supraphysiological doses for optimal growth.

54. *What is Laron syndrome*?

Laron syndrome is an autosomal recessive disorder, which is a prototype of growth hormone insensitivity syndromes (GHIS). The defects in GHIS include defective GH receptor dimerization or post-receptor signaling events resulting in decreased IGF1 generation (e.g., STAT5b mutation) or defective IGF1 stabilization and rarely mutations in IGF1 gene. The characteristic features of Laron syndrome include severe short stature (−4 to −10SDS), midfacial hypoplasia, blue sclera, delayed motor milestones, and hip dysplasia. Despite severe short stature, birth length and birth weight are usually normal. Biochemically, it is characterized by high/normal GH levels along with low IGF1. Disorders like malnutrition, uncontrolled diabetes, and chronic renal failure can also have a similar biochemical profile. Treatment with recombinant human IGF1 is effective in children with Laron syndrome (Fig. 2.10).

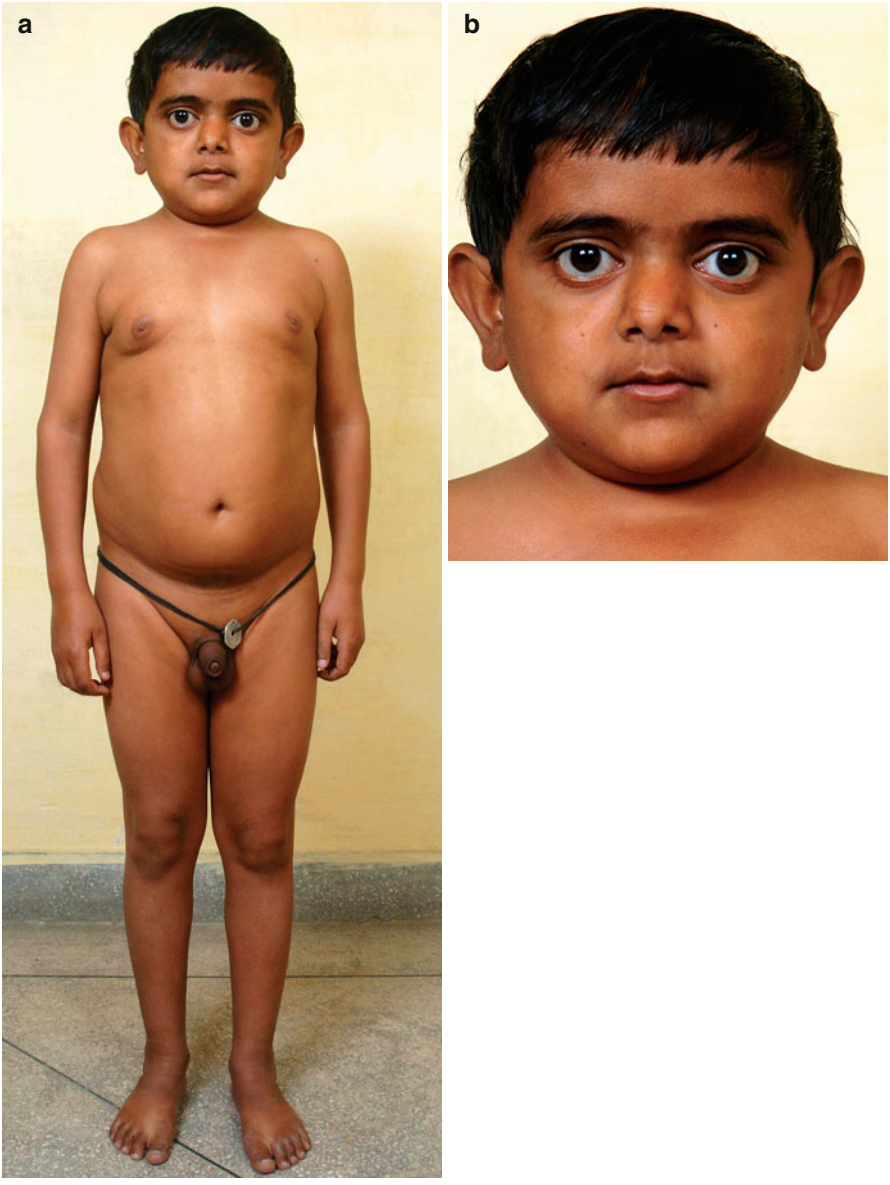


Fig. 2.10 (**a**) A 14-year-old child with Laron syndrome. (**b**) Note the typical facial features in the same child

55. *How to diagnose GH insensitivity syndrome*?

The presence of low IGF1 along with peak GH >15 ng/ml during GH dynamic test in a short child should raise a suspicion of GHIS. The diagnostic criteria (Savage and Blum) for GHIS include height SDS <–3, basal GH >5 ng/ml, serum IGF1 <50 ng/ml, GH binding with GHBP <10 %, and subnormal response to IGF1 generation test. In this test, rhGH is administered daily for four consecutive days at a dose of 0.033 mg/Kg/d subcutaneously at 2000h and serum IGF1 is estimated at baseline and on day 5 at 0800h. An increment in IGF1 of <15 ng/ml is considered subnormal. Three out of these five criteria are required to make a diagnosis of GHIS. In addition, a low serum basal IGFBP3 (<0.1 centile for age) and an increment in IGFBP3 <0.4 mg/L after IGF1 generation test also supports the diagnosis.

56. *Why are the pygmies short*?

Pygmies are an aboriginal group of people endemic to Africa and characteristically have severe short stature. The severe growth failure in these individuals was thought to be due to IGF1 receptor mutation or defects in downstream IGF1 signaling pathway. However, the recent studies have demonstrated that they have normal levels of GH, low levels of GHBP, and normal to low levels of IGF1. The exact cause of short stature in these individuals is not known; however, defects in GH receptor, IGF1 gene, and IGF1 receptor are the proposed mechanisms. Treatment with recombinant human GH or IGF1 does not improve height in pygmies.

57. *What are the causes of tall stature*?

An individual with height two standard deviation (>97th percentile) or more above the mean as compared to children of the same age, gender, and ethnicity is considered to have tall stature. The causes include familial tall stature, acrogigantism, Marfan syndrome, homocystinuria, and XYY syndrome. In addition, individuals with hypogonadotropic hypogonadism, Klinefelter's syndrome, estrogen receptor mutations, and aromatase deficiency also have tall stature in adulthood (Fig. 2.11).

Fig. 2.11 Acrogigantism due to microsomatotropinoma

58. *How to treat adolescents with familial tall stature*?

Increased GH pulse amplitude and relatively higher IGF1 level have been demonstrated in children with familial tall stature. The treatment modalities for adolescents with familial tall stature include testosterone in boys and estrogen in girls. Bromocriptine and somatostatin analogues have been tried in both sexes with limited success.

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Thyroid Disorders in Children

3

3.1 Case Vignette

A 14-year-old girl presented with complaints of growth failure and poor development of secondary sexual characteristics. She also complained of occasional headache and for that a brain imaging was performed. The MRI of the brain showed a sellar–suprasellar mass and she was referred to the department of neurosurgery for surgical intervention. An endocrine consultation was sought prior to subjecting the patient to surgery. A detailed history was elucidated, which revealed that she had linear growth failure for the last 7–8 years. She also complained of lethargy, weakness, constipation, cold intolerance, dry skin, and decreased appetite. She studied till class seventh; however, later she dropped out because of progressive decline in her scholastic performance. There was history of poor development of secondary sexual characteristics; however the mother gave a history that the patient had a single episode of vaginal bleed at the age of 12 years. She had no history of visual field defect or diminution of visual acuity. She was residing in iodine-sufficient area and had no family history of autoimmune disorders. On examination, her height was 116 cm (−7 SDS, height age 6 years and target height 164 cm), weight 35 Kg (weight age 10.5 years), pulse rate 64/min, regular and blood pressure 90/60 mmHg. Her facial features revealed pallor with yellowish hue, periorbital puffiness, and depressed nasal bridge. She did not have goiter. Her skin was dry and coarse with papillomatous eruptions (toad's skin) and scalp hair was dry, thin, and brittle. Deep tendon reflexes were grossly delayed particularly the relaxation phase. She also had myoedema which was elicitable on flicking the biceps belly with thumb and index finger and showed a post-flicker mounding phase. She did not have a pseudohypertrophy of calf muscle. Other systemic examination was unremarkable. On investigations, hemoglobin was 8 g/dl with microcytic hypochromic anemia. Liver and renal function tests were normal. Serum cholesterol was 220 mg/dl, LDL-C 160 mg/dl, HDL-C 30 mg/dl, and triglyceride 220 mg/dl. Hormonal profile revealed, serum T_3 0.3 ng/ml (N 0.8–2), T_4 0.3 μg/dl (N 4.8–12.7), TSH 1024 μIU/ml (0.27–4.2), TPO

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>1200 IU/ml ($N<34$), prolactin 50 ng/ml (N 4.7–23.3), LH 0.8 mIU/ml (N 2.4–12.6), FSH 8.6 mIU/ml (N 3.5–12.5), estradiol 15 pg/ml (N 12.5–166), and 0800h cortisol 170 nmol/L (N 171–536). Bone age was 6 years and there was no epiphyseal dysgenesis. CEMRI sella revealed a 1.5×1.8 cm homogeneously enhancing sellar–suprasellar mass, while the rest of the brain parenchyma was unremarkable. Ultrasound pelvis showed bilateral enlarged multicystic ovaries with small uterus and endometrial thickness of 1 mm. With this clinical and biochemical profile, she was diagnosed as a case of long-standing, untreated juvenile primary hypothyroidism of autoimmune origin (Hashimoto's thyroiditis) with thyro-lactotrope hyperplasia and multicystic ovaries. She was initiated with L-thyroxine at a dose of 25 µg/day, with a weekly increase by 25 µg till a dose of 100 µg/day was attained. In addition, hydrocortisone was also added at a dose of 10 mg/day in divided doses. At 6 weeks of follow-up, serum T_4 was 6.6 µg/dl and TSH 15 µIU/ml. With initiation of treatment, she had polyuria which abated later. The dose of L-thyroxine was increased to 125 µg/day and hydrocortisone was withdrawn, and the repeat serum 0800h cortisol after 24h of omission of hydrocortisone was 390 nmol/L (Figs. 3.1 and 3.2).

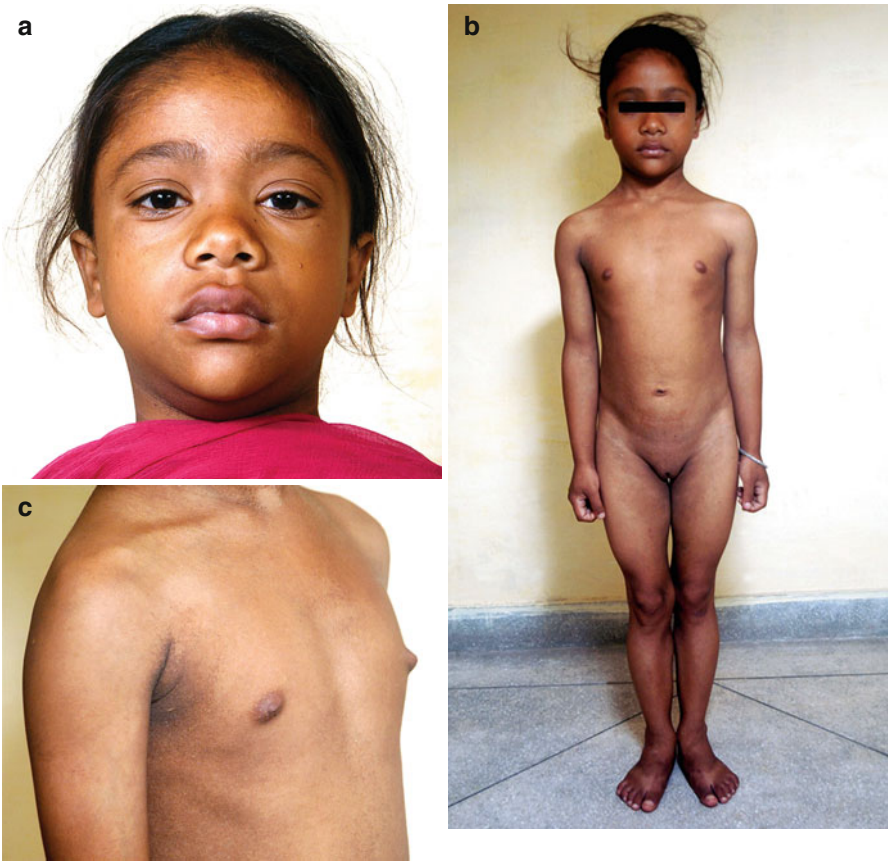

Fig. 3.1 (**a**) A 14-year-old child with myxoedematous features, (**b**) short stature with poor secondary sexual characteristics (**c**). Note the breast budding (B_2) in the same child

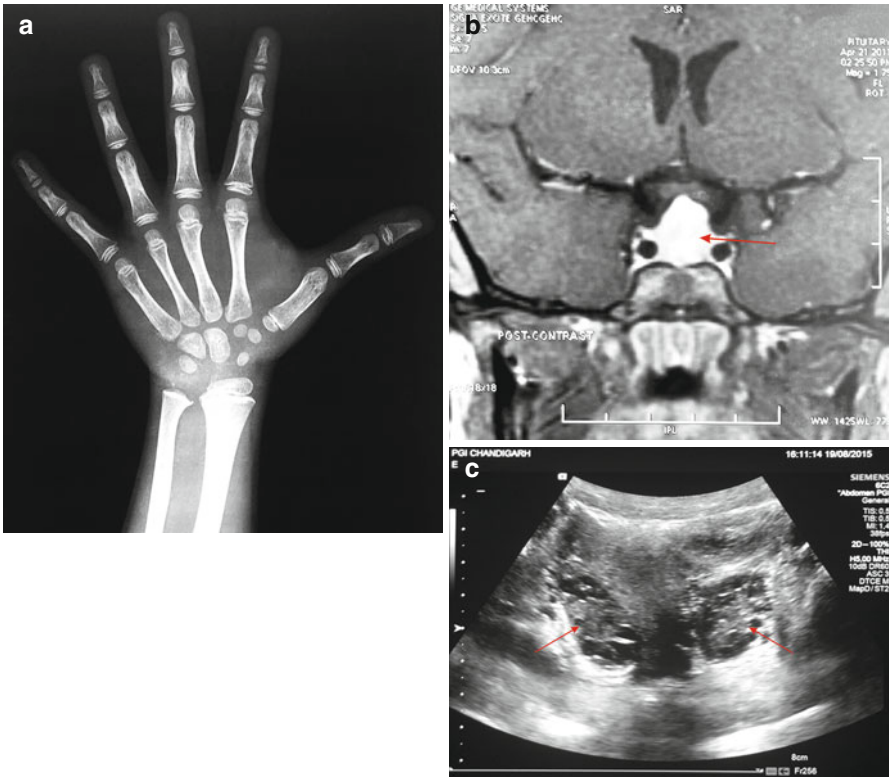

Fig. 3.2 (**a**) X-ray showing bone age of 6 years. (**b**) CEMRI sella showing diffuse pituitary enlargement (thyro-lactotrope hyperplasia, *red arrow*). (**c**) Ultrasonography of the pelvis depicting bilateral enlarged multicystic ovaries (*red arrows*)

3.2 Stepwise Analysis

The index patient presented with growth failure and poor development of secondary sexual characteristics. With these presenting complaints, the possibilities in a girl child include Turner syndrome, chronic systemic illness, panhypopituitarism, Cushing's syndrome, and juvenile hypothyroidism. As her height was more compromised than weight (height age < weight age < chronological age; 6 <10.5 <14 years), chronic systemic illness is unlikely. Severely retarded bone age (BA < CA) and absence of Turner stigma decline the possibility of Turner syndrome. In the index patient as height and weight are both severely compromised, the probability of Cushing's syndrome is unlikely, where the children are usually short but obese. The overt features of myxoedematous hypothyroidism and severe

retardation of linear growth support the diagnosis of juvenile primary hypothyroidism. However, patients with panhypopituitarism may also have severe retardation of linear growth (due to combined deficiency of GH and TSH), but lack myxoedematous manifestations. This is because increased TSH is required for the accumulation of glycosaminoglycans in interstitial tissues which is conspicuously low in patients with secondary hypothyroidism. In addition, TSH-independent T_4 synthesis still continues in patients with secondary hypothyroidism; therefore, T_4 deficiency is less severe in these patients as compared to patients with primary hypothyroidism. The thyroid profile in the index patient confirmed the diagnosis of juvenile primary hypothyroidism due to Hashimoto's thyroiditis as she had very high TSH and TPO antibody titer. Short stature and delayed puberty are the usual presenting manifestations of juvenile primary hypothyroidism. Growth failure in patients with juvenile hypothyroidism is a result of reduced GH secretion and GH-mediated IGF1 generation as thyroxine is required for both GH secretion and IGF1 generation. In addition, thyroid hormone has a direct effect on epiphyseal growth plate and promotes differentiation of chondrocytes to hypertrophic chondrocytes which is required for growth of long bones. The mechanism of delayed puberty in adolescents with hypothyroidism remains elusive; however, optimal concentration of thyroxine may be required for initiation and maturation of GnRH pulse generator activity and appropriate gonadal function. Further, elevated level of prolactin in these patients may interfere with the development and maturation of hypothalamo–pituitary–gonadal axis. Nevertheless, few patients with juvenile hypothyroidism may present with gonadotropin-independent precocious puberty. The index patient had one episode of vaginal bleed at the age of 12 years with inappropriate development of secondary sexual characteristics (A_-, P_1, B_2) which suggest aberrant production of estrogen by ovarian follicle resulting in estrogen breakthrough bleed. This is usually seen in children with gonadotropin-independent precocious puberty where the dichotomy between the breast development and menarche can be observed as slow, and progressive priming with estrogen is required for the breast development as opposed to the uterus which may respond to sudden rise in estrogen. However, in the index case, this should not be termed gonadotropin-independent precocious puberty, as she had menarche at the age of 12 years. The presence of multicystic ovaries in our patient can be explained by increased TRH-mediated FSH secretion and decreased FSH clearance, thereby resulting in increased FSH, and consequent follicular cyst formation. In addition, high circulating levels of TSH also act on FSH receptor on ovarian follicle due to specificity-spillover, resulting in follicular growth and development. Presence of thyro-lactotrope hyperplasia in our patient is a manifestation of long-standing, untreated, severe primary hypothyroidism which occurs as a result of TRH-mediated stimulation of thyro-lactotropes due to decreased negative feedback by low levels of circulating thyroxine. Children and adolescents with long-standing,

severe hypothyroidism should be initiated with low doses of L-thyroxine, and the dose should be increased gradually. The reasons for initiating low doses in such scenario include potential risk of adrenal crisis (lazy adrenal syndrome), seizures (water and electrolyte imbalance), and hyperkinetic manifestations (upregulation of thyroid hormone receptors). Hydrocortisone should be added along with L-thyroxine in patients suspected to have adrenal insufficiency either primary (due to polyglandular endocrinopathy) or secondary (due to pituitary pathology) or in patients with long-standing hypothyroidism (lazy adrenal syndrome). Our patient was initiated on hydrocortisone and low dose of L-thyroxine and later L-thyroxine dose was gradually increased to 125 μg/day. She had polyuria after initiation of therapy which occurs as a result of passage of glycosaminoglycans through urine. Others symptoms of hypothyroidism progressively abate within 4–8 weeks except hoarse voice and skin changes which take longer time to resolve. Children usually experience "catch-up" growth after initiation of treatment with L-thyroxine; however, they may not be able to attain the target adult height particularly in those who have been diagnosed late (as growth is a cumulative phenomenon) and in those who are in peripubertal age. This occurs because skeletal maturation is much faster than statural growth after initiation of L-thyroxine therapy. Adolescents in peripubertal age may soon enter into puberty after initiation of L-thyroxine therapy, which may further compromise their final adult height. Other untoward consequences of long-standing hypothyroidism like multicystic ovaries and thyro-lactotrope hyperplasia resolve by 6–12 months with optimum L-thyroxine replacement and do not require surgical intervention.

3.3 Clinical Rounds

1. *What is congenital hypothyroidism?*

   Congenital hypothyroidism (CH) is a disorder characterized by thyroid hormone deficiency at birth. Congenital hypothyroidism can be sporadic or endemic. Sporadic CH is usually due to thyroid dysplasia or dyshormonogenesis and neonates with sporadic CH have less severe manifestations of hypothyroidism due to transplacental transfer of maternal thyroxine to the fetus. Timely initiation of L-thyroxine therapy is associated with almost near-normal neurocognitive outcome. Endemic CH is due to severe maternal and fetal iodine deficiency with consequent severe thyroid hormone insufficiency resulting in neurocognitive dysfunction at birth. Even timely initiation of L-thyroxine therapy does not improve the neurocognitive outcome but only improves myxoedematous manifestations. Infants with neurological cretinism should not be considered to have congenital hypothyroidism as their thyroid functions are normal at birth.

2. *What is the importance of maternal thyroid hormones in fetal brain development?*

   Thyroid hormones are essential for the fetal brain development, and they help in neuronal cell differentiation, migration, synaptogenesis, and myelination. During embryogenesis (first trimester) maternal T_4 is responsible for neuronal development. During second trimester (12^{th} week) and onward, fetal thyroid gland starts functioning and gets mature by 20^{th} week of gestation and becomes the exclusive source of thyroid hormones. In neonates with sporadic congenital hypothyroidism, maternal T_4 is the exclusive source for thyroid hormones during the entire intrauterine period, and hence, these newborns have near-normal brain development at birth. However, they should be soon replaced (within 6 weeks of life) with L-thyroxine to avoid the decline in cognitive score as the brain development continues till the age of 3 years. However in neonates with endemic cretinism, severe maternal T_4 deficiency (due to severe iodine deficiency) during the first trimester or throughout the entire gestation results in neurological and myxoedematous cretin, respectively.

3. *What are the physiological alterations in thyroid function during the neonatal period?*

   Immediately after birth of a term baby, there is a physiological TSH surge, which can be as high as 80 μIU/ml. The elevation of TSH occurs in response to exposure to cold environment after birth. The elevated TSH increases free T_4 within 24–48h, which results in induction of non-shivering thermogenesis. Thereafter, there is a decline in TSH (which starts after 60 min of birth) and free T_4 levels, and by second week free T_4 normalizes and TSH falls to <10 μIU/ml.

4. *What is the rationale of neonatal screening for congenital hypothyroidism?*

   Congenital hypothyroidism (CH) is the most common preventable cause of intellectual disability. It is a common disorder with a prevalence of about 1 in 2500 live-births, and many newborns are asymptomatic, even with severe T_4 deficiency (T_4 <3 μg/dl). Delay in diagnosis and initiation of therapy results in irreversible neurocognitive dysfunction. Further, relatively simple biochemical test is available for screening, and therapy is inexpensive and highly rewarding. Neonatal screening for congenital hypothyroidism has also been shown to be cost effective.

5. *When to screen for congenital hypothyroidism?*

   Screening for congenital hypothyroidism is recommended in a newborn between the second and third day of life. This is because, immediately after birth, there is a neonatal TSH surge, followed by rapid decline in serum TSH levels during the first 24h of life (serum TSH levels falls to 50 % of peak value by 2h and to 20 % by 24h). Thereafter, there is a gradual fall in serum TSH. Hence, neonatal screening performed within the first 24h of life frequently yields false-positive results and is not preferred. However, sampling from cord blood is indicated in

those neonates whose mother is receiving antithyroid drugs or with history of previous baby with congenital hypothyroidism.

6. *How to screen for congenital hypothyroidism?*

Sample obtained by heel prick is preferred for neonatal screening of congenital hypothyroidism. Following heel prick, blood drop is placed on specially designed filter paper (Guthrie's card), is allowed to dry (for 3h), and is sent to the laboratory. The common strategies employed for neonatal screening include "primary TSH" or "primary T_4–backup TSH." However, the ideal screening strategy is combined estimation of both T_4 and TSH. All newborns with abnormal screening results should have a confirmatory venous sample for FT_4 and TSH (Fig. 3.3).

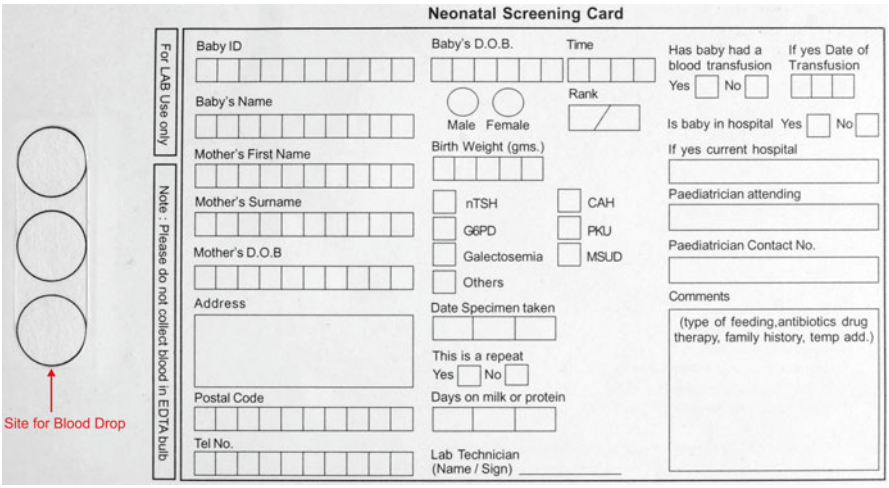

Fig. 3.3 Neonatal screening card (Guthrie's card). Note the filter paper marked with three circles, where blood drop is to be placed for common screening of congenital hypothyroidism, congenital adrenal hyperplasia, and phenylketonuria

7. *Can cord blood be used for screening of congenital hypothyroidism?*

Sample from umbilical cord can be used for screening of congenital hypothyroidism. Cord blood contains mixed blood from both umbilical artery and veins and can be smeared on filter paper (dried blood spot, DBS) or can be used after separation of serum. Cord blood sampling is performed immediately after birth (prior to neonatal surge which occurs after 30 min of birth), thereby reducing the number of false-positive screening tests as a result of neonatal TSH surge. This also allows for early discharge of healthy newborns and reduces the recall rate for confirmation of thyroid dysfunction. However, cord blood is not recommended for neonatal screening for phenylketonuria (PKU) and congenital adrenal hyperplasia (CAH). This is because sampling immediately after birth will result in underdiagnosis of phenylketonuria and overdiagnosis (false positive) of congenital adrenal hyperplasia in newborns.

Hence, cord blood sampling is not preferred for neonatal screening of congenital hypothyroidism in regions where neonatal screening for PKU and CAH is routinely performed, as screening for all these disorders (PKU, CAH, and CH) can be done from a single sample obtained from heel prick after 2–3 days of life. However, cord blood is an alternative to heel prick sampling for neonatal screening of congenital hypothyroidism in regions with low prevalence of phenylketonuria and CAH.

8. *What are the merits and demerits of different strategies in neonatal screening program for congenital hypothyroidism?*

The commonly used strategies for neonatal screening of congenital hypothyroidism include "primary TSH" and "primary T_4–backup TSH." TSH is the most sensitive test for the diagnosis of primary congenital hypothyroidism; however, a primary TSH strategy will miss central hypothyroidism and neonates with hypothyroxinemia with delayed TSH rise (which is common in newborns with low birth weight). In addition, congenital thyroxine-binding globulin deficiency will also be missed which may not be of clinical relevance. The primary T_4 approach can diagnose secondary hypothyroidism and thyroxine-binding globulin deficiency; however the primary T_4 strategy will miss compensated hypothyroidism (e.g., in ectopic thyroid tissue) and subclinical hypothyroidism. Both these approaches require a recall rate of approximately 0.05 % and may miss 3–5 % patients with congenital hypothyroidism. This may be due to improper sample collection, technical difficulties with assays, and immaturity of hypothalamo–pituitary–thyroid axis. "Simultaneous T_4 and TSH"-based neonatal screening is the ideal screening strategy; however, the cost-effectiveness of this approach has not been proven. The table given below shows the incidence of various etiologies of congenital hypothyroidism and merits and demerits of screening with various approaches.

Cause	Incidence	Primary TSH	Primary T_4–backup TSH	Simultaneous T_4 and TSH
Primary hypothyroidism	1 in 2500	Good	Good	Excellent
Secondary hypothyroidism	1 in 16,000–1 in 100,000	No	Few cases[a]	Few cases[a]
Subclinical hypothyroidism	1 in 30,000	Yes	No	Yes

[a]Free T_4 is required for diagnosis as total T_4 can be normal in newborn with secondary hypothyroidism because of increased TBG as a result of transplacental transfer of estradiol

9. *How to interpret the results of neonatal screening program?*

The primary TSH-based approach for neonatal screening program and further management are depicted in the figure given below (Fig. 3.4).

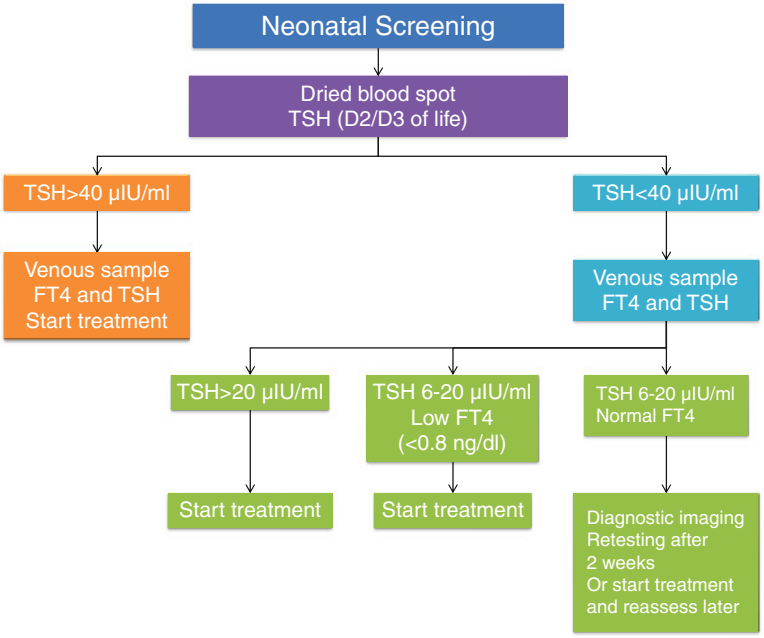

Fig. 3.4 Screening and management strategies for congenital hypothyroidism

10. *What is transient congenital hypothyroidism?*

Transient congenital hypothyroidism is defined as abnormal thyroid profile on neonatal screening (low T_4 and high TSH), with normalization of thyroid function after 1–2 months of age. The causes of transient congenital hypothyroidism include maternal/fetal iodine deficiency or excess, fetal exposure to maternal antithyroid drugs, transplacental passage of maternal thyrotropin receptor-blocking antibodies (TRAbs), heterozygous mutations of DUOX2/DUOXA2, and congenital hepatic hemangioma.

11. *Why do patients with hepatic hemangioma have hypothyroidism?*

Patients with hepatic hemangioma express type 3 deiodinase enzyme, which converts T_4 to reverse T_3. This results in reduced levels of T_4 and T_3 and consequently "consumptive" hypothyroidism. This is a rare cause of hypothyroidism and is considered as a paraneoplastic manifestation of hemangiomas. Consumptive hypothyroidism is usually seen in infants, although a few cases have also been described in adults. These patients require therapy with large doses of L-thyroxine for normalization of serum T_4. The disorder is self-limiting and usually abates by the age of 5–6 years.

12. *What are the causes of permanent congenital hypothyroidism?*

The most common cause of permanent congenital hypothyroidism is thyroid dysgenesis (85 %) followed by dyshormonogenesis (10–15 %) and rarely, central hypothyroidism. Thyroid dysgenesis is commonly sporadic, although 2 % are familial. However, thyroid dyshormonogenesis is inherited as an autosomal recessive disorder.

Disorders	Etiology	Pathogenesis
Thyroid dysgenesis	TTF-1, TTF-2, PAX-8, FOXE1, NKX2.1, and TSH receptor gene mutations	Defect in thyroid transcription factors which are required for growth, development, and migration of thyroid gland
–Thyroid agenesis		
–Thyroid hypoplasia		
–Ectopic thyroid		
Dyshormonogenesis	Sodium–iodide symporter, thyroid peroxidase (TPO), pendrin, DUOX1/DUOX2 and thyroglobulin, iodotyrosine deiodinase gene (*DEHAL1*) mutations	Defective thyroid hormone synthesis
Central hypothyroidism	HESX1, LHX3, LHX4, PIT1, PROP1 gene mutations	Defective TRH–TSH axis
	TSH-β gene mutations	
	TRH receptor gene mutations	

13. *What are the clinical features of congenital hypothyroidism in a newborn?*

The clinical manifestations are commonly subtle in newborns with congenital hypothyroidism (CH). Newborns with CH may be asymptomatic because of presence of maternal T_4 (which contributes to approximately one-third of circulating T_4 in a newborn) and residual functioning thyroid tissue. The classical symptoms of congenital hypothyroidism include lethargy, hoarse cry, feeding difficulty, constipation, and increased somnolence. The characteristic signs of CH include prolonged neonatal jaundice, macroglossia, umbilical hernia, wide posterior fontanelle, hypotonia, dry skin, and hypothermia. Twenty percent of neonates with CH have history of postmaturity (>42 weeks). Birth length is normal, while birth weight may be >90th percentile. Presence of goiter points to a clinical diagnosis of thyroid dyshormonogenesis, whereas absence of goiter suggests thyroid dysgenesis; however it does not rule out dyshormonogenesis. In addition, oral cavity should also be carefully examined for lingual thyroid. Deafness may be present in newborns with CH as an association with congenital hypothyroidism or as a manifestation of Pendred syndrome. Delayed bone age is a characteristic feature of CH, as evidenced by the absence of distal femoral and proximal tibial epiphysis in approximately 54 % of neonates at birth (femoral epiphysis appears by 34 weeks and tibial epiphysis by 39 weeks of intrauterine life).

14. *Are congenital malformations common in newborns with congenital hypothyroidism?*

    There is a four-fold higher prevalence of congenital malformations in newborns with congenital hypothyroidism as compared to general population (8.4 % vs. 2 %). Congenital cardiac malformations are most common, followed by malformations of nervous system and eyes and cleft lip/palate. The most common congenital cardiac malformation is ostium secundum atrial septal defect, followed by tetralogy of Fallot and pulmonary stenosis.

15. *What are the radiological manifestations of congenital hypothyroidism?*

    Thyroid hormones play an important role in epiphyseal growth and development. Thyroid hormone deficiency during intrauterine period may result in absent distal femoral and upper tibial epiphyses (in a term newborn) and wide posterior fontanelle. Epiphyseal dysgenesis, short long bones, anterior beaking of 12th thoracic, first and second lumbar vertebra, enlarged sella, and delayed bone age are other manifestations of untreated congenital hypothyroidism in a child.

16. *What is Kocher–Debre–Semelaigne syndrome?*

    Kocher–Debre–Semelaigne syndrome refers to pseudohypertrophy of calf muscles as a result of long-standing untreated congenital/juvenile hypothyroidism. Pseudohypertrophy is a result of accumulation of glycogen, glycosaminoglycans, and connective tissue in the muscle as a result of hypothyroidism. The level of creatine phosphokinase is mildly elevated and electromyogram shows a myopathic pattern. The counterpart of Kocher–Debre–Semelaigne syndrome in adults is Hoffman's syndrome. Both these disorders are reversible with thyroxine therapy (Fig. 3.5).

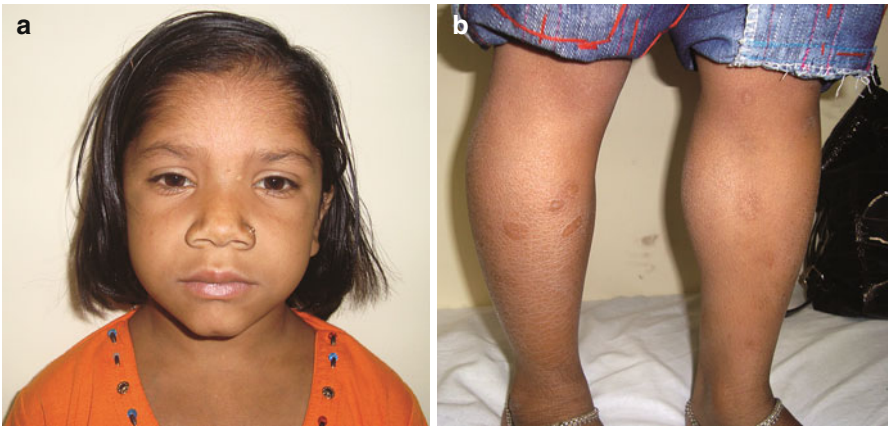

Fig. 3.5 (**a**) Myxedematous features in a girl with long-standing untreated primary hypothyroidism. (**b**) Pseudohypertrophy of the calf muscles in the same patient

17. *What are the investigations required to establish an etiological diagnosis in a newborn with confirmed congenital hypothyroidism?*

    Once a biochemical diagnosis of congenital hypothyroidism is established, newborns should be evaluated for the etiological diagnosis. In newborns with primary hypothyroidism, these include ultrasonography of neck, radionuclide thyroid scan (^{99m}Tc pertechnetate/^{123}I scan), serum thyroglobulin, TRAbs, and urinary iodine levels. In newborns with secondary hypothyroidism, MRI sella, other pituitary hormones, and ophthalmological evaluation for optic nerve hypoplasia should be performed. However, initiation of L-thyroxine should not be delayed in neonates with congenital hypothyroidism awaiting these investigations (Fig. 3.6).

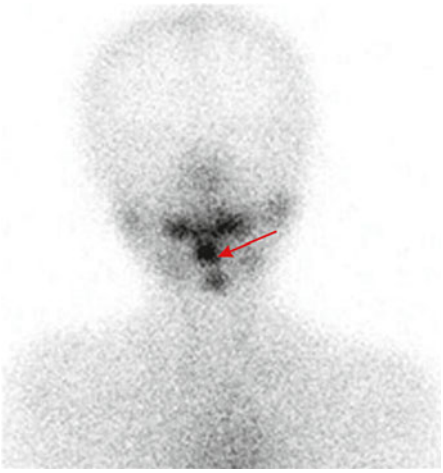

Fig. 3.6 ^{99m}Tc pertechnetate scan in a child with congenital hypothyroidism showing tracer uptake in the lingual region (*red arrow*) suggestive of ectopic thyroid

18. *What are the causes of negative 99mTc pertechnetate scan in a neonate with congenital hypothyroidism?*

    Absence of tracer uptake in a neonate with congenital hypothyroidism usually suggests a diagnosis of thyroid agenesis. However, thyroid uptake may be absent even in the presence of eutopic thyroid gland in conditions like antenatal maternal iodine exposure, transplacental transfer of TSH receptor-blocking antibodies, TSH suppression from L-thyroxine treatment, TSH receptor-inactivating mutations, and dyshormonogenesis due to sodium–iodide symporter (NIS) defect. Therefore, ultrasonography of the thyroid and estimation of thyroglobulin should be performed to establish the diagnosis of thyroid aplasia (Fig. 3.7).

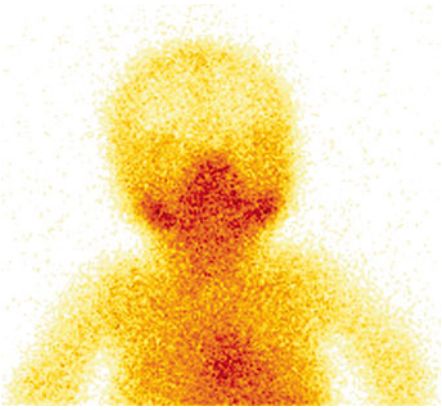

Fig. 3.7 ^{99m}Tc pertechnetate scan demonstrating no tracer uptake in the thyroid region or along the line of descent of the gland in a child with congenital hypothyroidism due to thyroid aplasia

19. *How to approach a newborn with congenital hypothyroidism?*

Thyroid radionuclide uptake and scan (^{99m}Tc pertechnetate/^{123}I scan) is the preferred first-line investigation in a newborn with primary CH, and this can be done even within a week after initiation of L-thyroxine therapy. An approach to newborn with congenital hypothyroidism is shown in the figure given below. If a child was not evaluated for the etiology of congenital hypothyroidism before initiation of therapy, L-thyroxine should not be discontinued, and the child may be reevaluated after 3 years of age (Fig. 3.8).

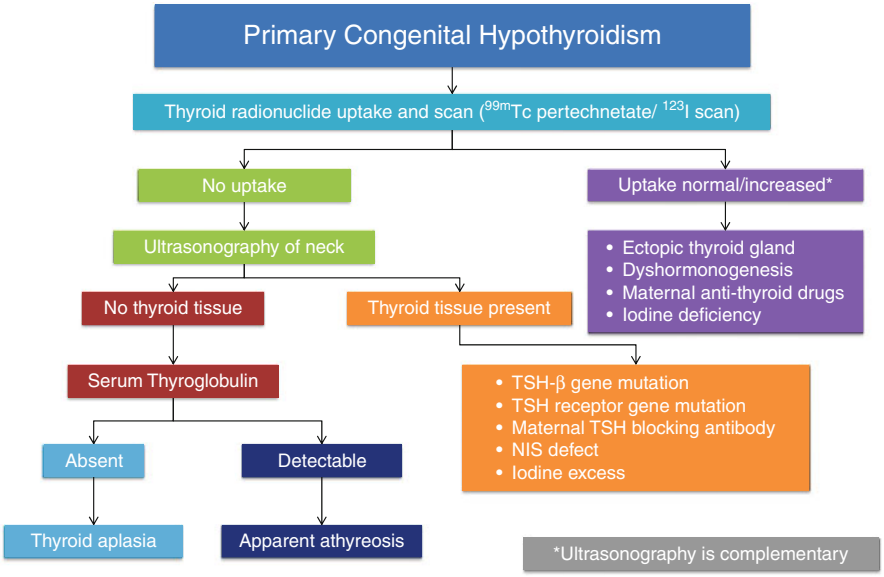

Fig. 3.8 Diagnostic approach to a child with primary congenital hypothyroidism

20. *How to treat congenital hypothyroidism?*

The goal of therapy in congenital hypothyroidism is normalization of T_4/FT_4 and TSH as rapidly as possible (T_4/FT_4 within 2 weeks and TSH within a month) for normal growth and development. Oral L-thyroxine (L-T_4) is the therapy of choice for congenital hypothyroidism. Although T_3 is required for neuronal growth and development, T_3 in brain is predominantly derived from local deiodination of circulating T_4; hence therapy with T_3 is not recommended. L-thyroxine should be initiated at a dose of 10–15 μg/Kg/day for infants aged 0–3 months, 8–10 μg/Kg/day for 4–6 months, and 6–8 μg/Kg/day for infants of 7–12 months. The tablet must be crushed and mixed with breast milk/formula feed/water before administration. L-thyroxine tablet should not be mixed with preparations containing iron/calcium/soya as they interfere with the absorption of L-T_4.

21. *How to monitor a newborn with congenital hypothyroidism on L-thyroxine therapy?*

After initiation of therapy, infants should be closely followed up with estimation of T_4/FT_4 and TSH every fortnightly till T_4/FT_4 and TSH are normalized and, thereafter, one to three monthly till 12 months of age. After infancy, child can be followed up every 2–3 monthly till 3 years of life. Sample for thyroid profile can be taken either before the administration of next dose or at least 4h after intake of L-thyroxine. TSH should be maintained in the age-specific normal range and T_4/FT_4 in the upper half of age-specific normal range. The recommended cutoffs are TSH 0.5–2.5 μIU/ml and T_4 between 10 and 16 μg/dl (or FT_4 1.4–2.3 ng/dl) during first 3 years of life; thereafter TSH alone can be monitored every 3–6 months and maintained between 0.5 and 2.5 μIU/ml. However, it should be noted that some infants may have TSH above the reference range, despite having T_4/FT_4 above age-specific normal range. This is possibly because of resetting of hypothalamo–pituitary–thyroid axis as a result of long-standing hypothyroidism during intrauterine life. Linear growth and milestones should be regularly monitored in children with congenital hypothyroidism on therapy. In addition, periodic assessment for hearing is also essential in these children.

22. *What is the neurological outcome of newborns with congenital hypothyroidism?*

It is important to initiate therapy as early as possible (preferably within 2 weeks of birth) in newborns with congenital hypothyroidism for optimal neurocognitive development. It has been shown that early therapy is associated with near-normal intellectual outcome. However, newborns with severe congenital hypothyroidism (as evidenced by athyreosis, absent distal femoral epiphysis, FT_4 <0.38 ng/dl, and significantly elevated TSH), delay in initiation of L-T_4

therapy (>6 weeks) and those with poor compliance to therapy may have subnormal IQ and cognitive score (Fig. 3.9).

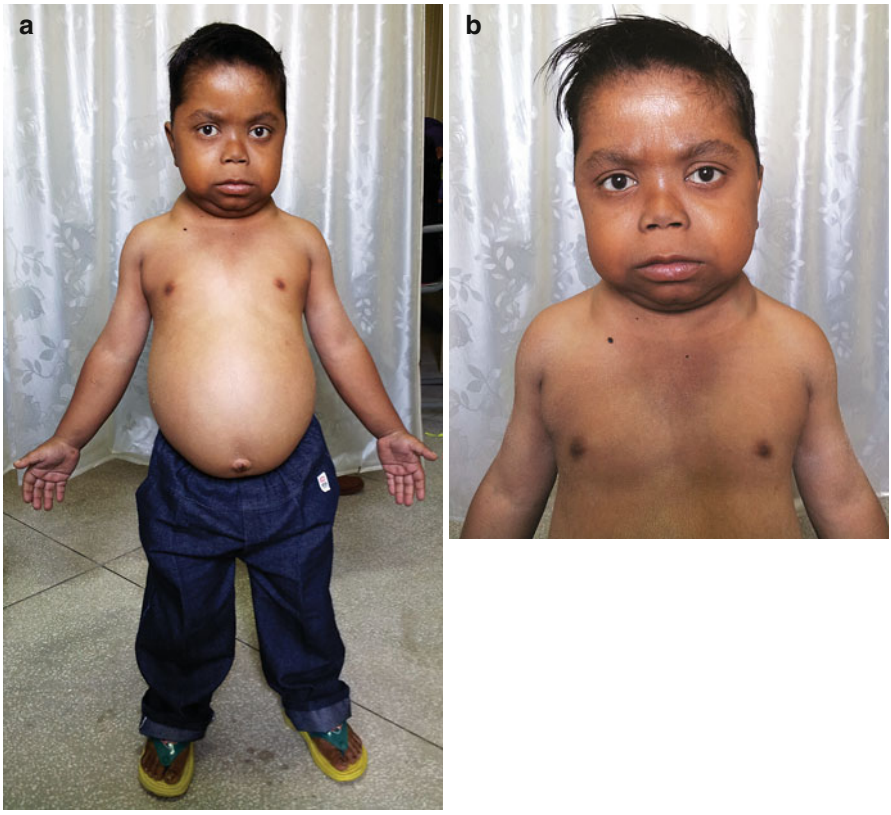

Fig. 3.9 (**a**) A 14-year-old boy with primary congenital hypothyroidism having short stature, distended abdomen, and umbilical hernia (**b**). Myxedematous features in the same child

23. *What is cretinism?*

Cretinism is a disorder characterized by irreversible mental disability and poor linear growth due to severe thyroid hormone deficiency during pre- and/or early postnatal period (<3 years of age). Cretinism can be endemic or sporadic. Endemic cretinism is due to severe maternal and fetal iodine deficiency during intrauterine period and manifests as either neurological or myxedematous cretinism. Sporadic cretinism is a result of long-standing untreated congenital hypothyroidism as a consequence of thyroid dysgenesis/dyshormonogenesis.

24. *What are the differences between endemic cretinism and sporadic congenital hypothyroidism?*

The differences between endemic cretinism and sporadic congenital hypothyroidism are summarized in the table given below.

Parameters	Endemic cretinism	Sporadic congenital hypothyroidism
Prevalence	1:25–1:100	1:2500
Geographical area	Iodine-deficient areas	Iodine-sufficient area
Etiology	Severe maternal–fetal iodine deficiency	Thyroid dysgenesis
		Dyshormonogenesis
Clinical presentation	Neurological/myxedematous	Myxedematous
Goiter	Present (neurological cretin)	Usually absent
	Absent (myxedematous cretin)	Present in dyshormonogenesis
Preventive strategies	Iodine supplementation	–
Therapy	L-thyroxine (myxedematous cretin)	L-Thyroxine
	Rehabilitation (neurological cretin)	

25. *Why myxedematous manifestations are severe in endemic myxedematous cretin as compared to sporadic congenital hypothyroidism in a neonate?*

Myxedematous manifestations are severe in newborns with endemic myxedematous cretinism as compared to those with sporadic congenital hypothyroidism at birth. This is because maternal T_4 (which is transferred to fetus during intrauterine period) is sufficient to prevent development of severe myxedematous manifestations in those with sporadic congenital hypothyroidism, whereas low maternal T_4 (due to severe iodine deficiency) accounts for severe myxedematous features in newborns with endemic myxedematous cretin.

26. *What is endemic cretinism?*

Endemic cretinism is characterized by irreversible mental disability in individuals born in endemic iodine-deficient regions and exhibit some or all of the following features including; neuromuscular dysfunction (spasticity, motor incoordination, and squint), deaf–mutism, impaired linear growth, and hypothyroidism with or without goiter. Endemic cretinism occurs in regions where intake of iodine is <25 μg/day.

27. *What are the differences between neurological and myxedematous cretin?*

Endemic cretinism can manifest as either neurological or myxedematous cretinism. The differences between the two are summarized in the table given below.

Parameters	Neurological cretin	Myxedematous cretin
Mental retardation	Severe	Less severe
Deaf-mutism	Present	May be present
Squint	Present	Absent
Cerebral diplegia	Often present	Absent
Linear growth	Usually normal	Severe retardation
Myxedematous features	Absent	Present
Goiter	Present	Absent
Thyroid function tests	Normal	Hypothyroid
X-ray knee	Normal	Epiphyseal dysgenesis
Therapy	Rehabilitation	L-Thyroxine

28. *Why do some individuals with severe iodine deficiency develop myxedematous cretinism, while others develop neurological cretinism?*

Severe maternal and fetal iodine deficiency results in endemic cretinism and may manifest as neurological or myxedematous cretin. The exact cause for variation in the presentation of endemic cretinism is not known; however, it is thought that clinical manifestation of endemic cretinism is the result of two pathophysiologic events. Severe thyroid hormone deficiency (as a consequence of severe maternal and fetal iodine deficiency) during early intrauterine period results in impaired brain development and, consequently, irreversible neuronal damage. This occurs in both variants of endemic cretinism. Subsequent manifestation as either neurological or myxedematous cretinism depends on the response of thyroid gland to severe iodine deficiency, i.e., either goiter or atrophy. Those who develop goiter (and consequently compensated euthyroxinemia) manifest as neurological cretins, whereas those with thyroid gland atrophy (and consequently severe and persistent hypothyroxinemia) manifest as myxedematous cretins. It is speculated that environmental factors like selenium deficiency and exposure to thiocyanate may modulate thyroid gland response to iodine deficiency. However, it should be noted that some individuals have features of both neurologic and myxedematous cretinism (mixed cretin).

29. *How does selenium deficiency and exposure to thiocyanate cause thyroid gland atrophy in endemic iodine-deficient areas?*

    Iodine deficiency results in hypothyroxinemia and consequent increased TSH drive leads to increased intrathyroidal hydrogen peroxide (H_2O_2) production. Glutathione peroxidase, a selenoprotein, protects thyroid gland from H_2O_2-mediated injury to thyroid follicular cells. In the presence of selenium deficiency, there is accumulation of intrathyroidal H_2O_2 which results in follicular cell damage and fibrosis. Thiocyanate not only competes with iodine for sodium–iodide symporter in the thyroid gland but has also been shown to induce follicular cell necrosis.

30. *What is the treatment for endemic cretinism?*

    Endemic cretinism is a preventable disorder and optimal iodine supplementation prior to conception prevents the development of cretinism. Therefore, effective strategies for iodine supplementation should be implemented in iodine-deficient areas. In myxedematous cretins, iodine therapy has been shown to improve myxedematous features when initiated prior to the age of 3–4 years; however L-thyroxine should be preferred in children with myxedematous cretin. Neurocognitive deficits do not improve either with iodine or L-thyroxine therapy in myxedematous cretins. Rehabilitation is the mainstay of therapy in neurological cretins.

31. *How to supplement iodine for daily requirement?*

    Iodine is present in alluvium soil and seawater. Therefore, vegetations grown in iodine-rich soil and food of marine origin are rich source of iodine. Because of recurrent floods and consequent soil erosion, iodine is leached away from the soil, and iodine deficiency is prevalent in many parts of the world. To prevent iodine deficiency disorders in the community, iodine needs to be supplemented through a vehicle which is widely consumed. This vehicle may be water, milk, salt, wheat flour, or bread. Common salt is universally and consistently consumed; hence it is the preferred medium to deliver recommended daily allowance for iodine. Potassium iodate (KIO_3), the most stable iodine compound, is preferred to iodize common salt.

32. *What are the precautions required for the optimal delivery of iodine from iodized salt?*

    The following precautions should be observed while using iodized salt. Salt should be purchased within 3 months from the date of manufacture and at the time of purchase it should be crystal clear and white. It must be stored in a dry,

airtight container along with plastic-pack and kept away from the furnace. Once the pack is opened, it has to be consumed within 4 weeks. Salt should preferably be added on table rather than during cooking, as iodine quickly sublimates on exposure to heat.

33. *What is the recommended daily allowance for iodine?*

Recommended daily allowance (RDA) for iodine as suggested by WHO is summarized in the table given below.

Age	RDA of iodine (μg/day)
0–5 years	90
6–12 years	120
>12 years	150
Pregnancy and lactation	250

34. *What are the causes of hypothyroidism in children?*

Iodine deficiency is the most common cause of primary hypothyroidism in children worldwide. However, in iodine-sufficient regions, the commonest cause is Hashimoto's thyroiditis. Other causes of hypothyroidism in children include neck irradiation, delayed-onset dyshormonogenesis, ectopic thyroid gland, consumptive hypothyroidism, and secondary hypothyroidism (pituitary transcription factor defects and sellar–suprasellar mass). The figure illustrated below shows primary hypothyroidism in a child with ectopic thyroid gland (Figs. 3.10 and 3.11).

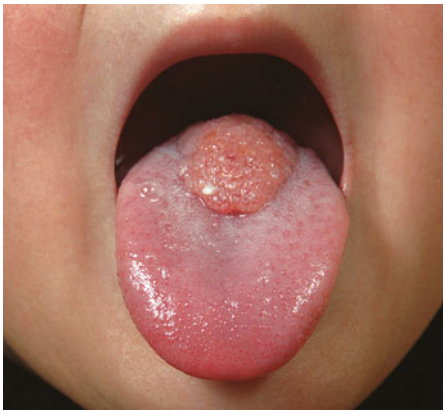

Fig. 3.10 Lingual thyroid in a child with juvenile hypothyroidism

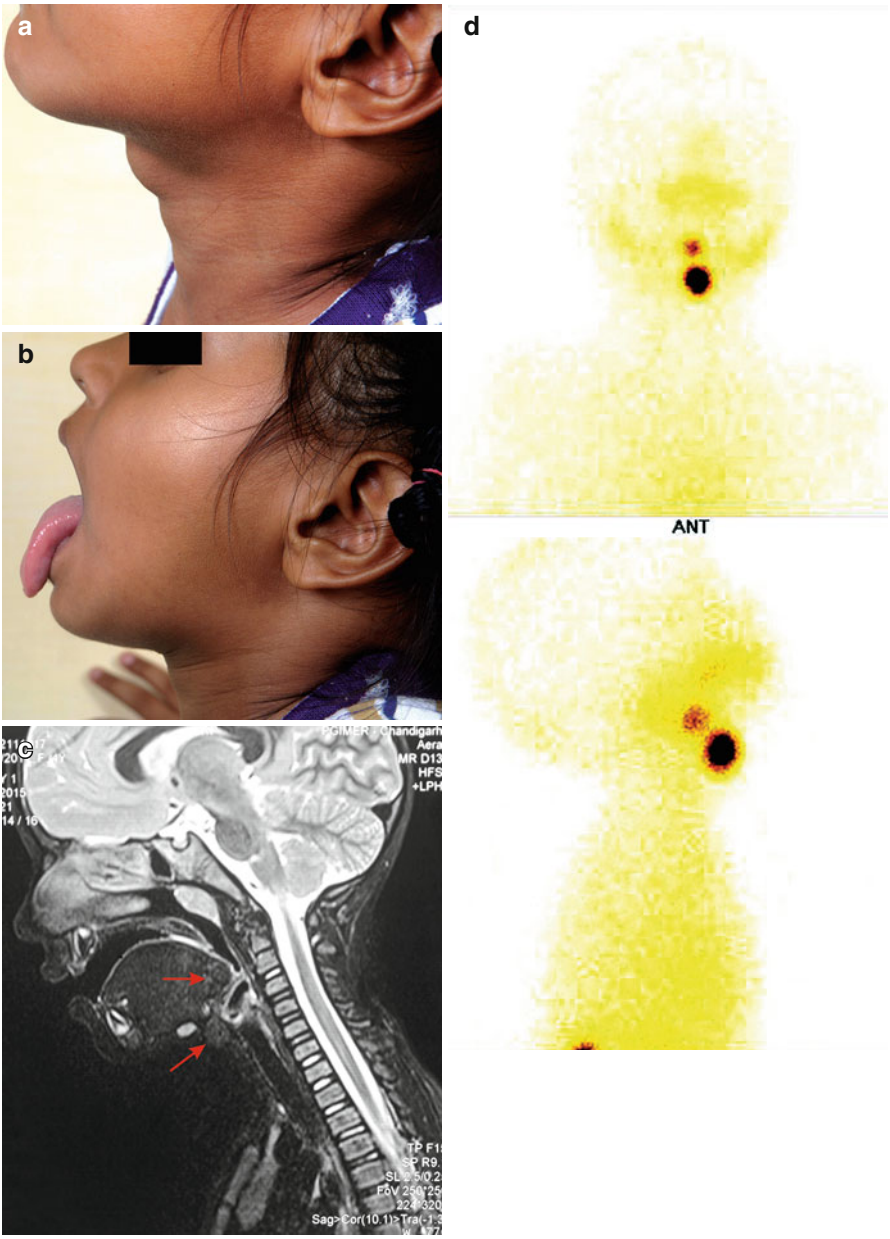

Fig. 3.11 (**a**) A 4-year-old child with midline swelling in upper part of the neck. (**b**) Upward movement of swelling with protrusion of the tongue. (**c**) MRI T2 sagittal image shows presence of lingual thyroid at posterior one-third of the tongue and ectopic thyroid tissue anterior to the glottis with absence of thyroid tissue at the eutopic site suggestive of "double ectopic" thyroid gland (*red arrows*). (**d**) ^{99m}Tc thyroid scan confirms the "double ectopic" thyroid gland

35. *What are the monosymptomatic presentations of juvenile hypothyroidism?*

The monosymptomatic presentations of juvenile hypothyroidism include short stature, delayed puberty, poor scholastic performance, precocious puberty (Van Wyk–Grumbach syndrome), myopathy (Kocher–Debre–Semelaigne syndrome), limping gait (stippled epiphysis and slipped femoral epiphysis), headache and visual field defects (thyro-lactotrope hyperplasia), diffuse goiter and, rarely, acute abdomen (huge multicystic ovaries and acute cholecystitis), and pericardial effusion (Figs. 3.12, 3.13, and 3.14).

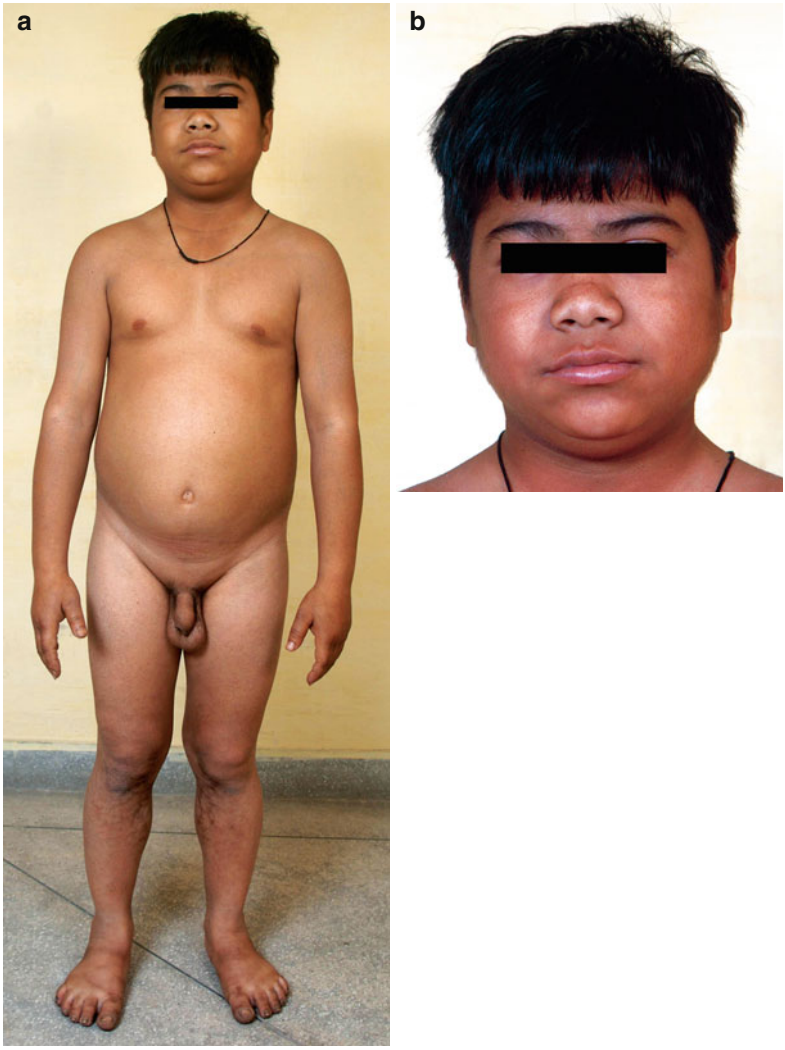

Fig. 3.12 (**a**) Short stature in a 14-year-old boy with juvenile primary hypothyroidism. (**b**) Myxedematous features in the same child

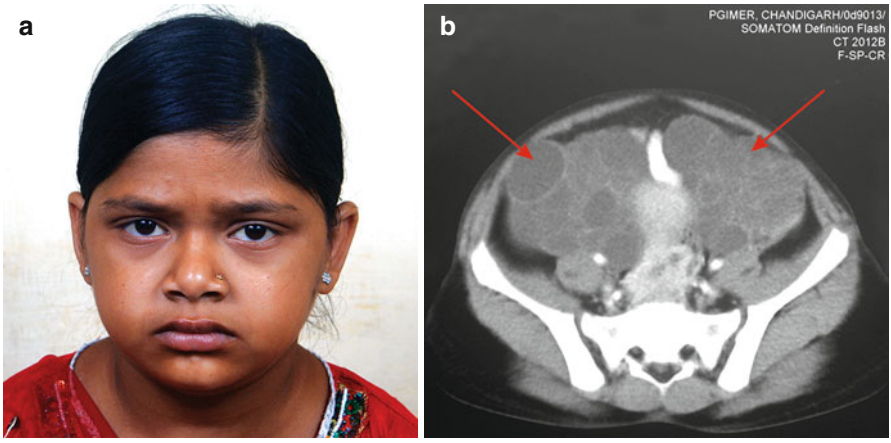

Fig. 3.13 (**a**) A 16-year-old girl with overt features of hypothyroidism (**b**). Axial CECT pelvis shows presence of enlarged bilateral multicystic ovaries (*red arrows*) in the same patient

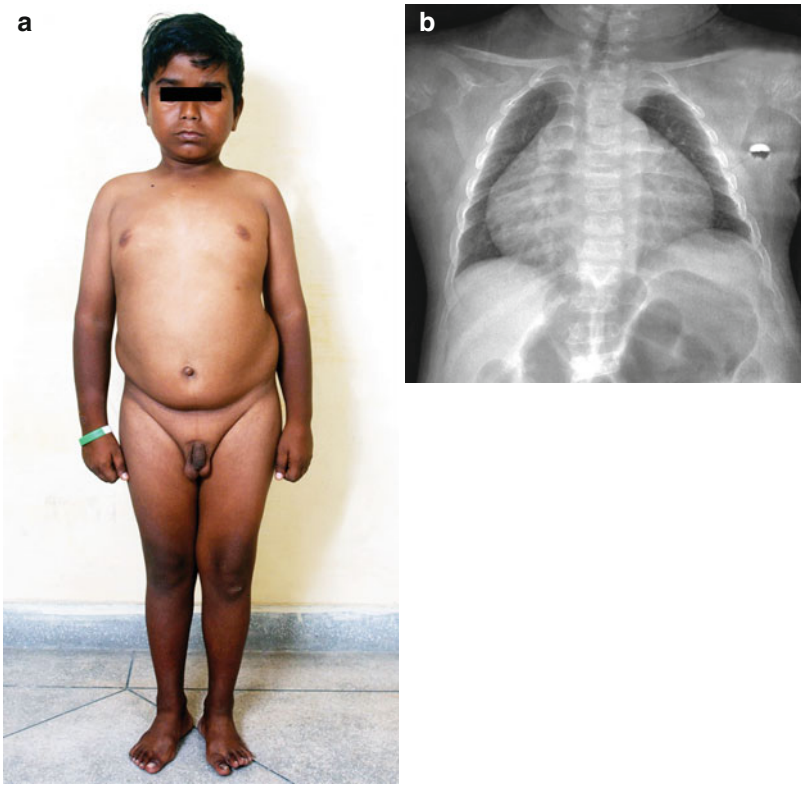

Fig. 3.14 A 14-year-old boy with juvenile primary hypothyroidism presented with (**a**) short stature, delayed puberty, and (**b**) pericardial effusion

36. *Why is short stature a common manifestation of juvenile hypothyroidism?*

    Short stature with retarded bone age is a common manifestation of juvenile hypothyroidism. This is because thyroid hormone is required for GH secretion and GH-mediated IGF1 generation. In addition, thyroid hormone has a direct effect on growth plate and promotes differentiation of chondrocytes to hypertrophic chondrocytes and enhances angiogenesis.

37. *What are the unique features of precocious puberty associated with primary hypothyroidism?*

    The characteristic features of precocious puberty associated with primary hypothyroidism are decreased growth velocity and retarded bone age, whereas other causes of precocious puberty are associated with increased growth velocity and advanced bone age. Girls with precocity due to primary hypothyroidism usually present with vaginal bleed and/or thelarche, whereas boys present with isolated testicular enlargement. Pubic/axillary hair is characteristically absent in both sexes despite precocity. This is because precocious puberty associated with hypothyroidism is predominantly mediated through FSH, and lack of LH drive results in decreased androgen production. In addition, thyroid hormone plays an important role in the growth and development of pilosebaceous unit.

38. *Why do patients with juvenile hypothyroidism develop precocious puberty?*

    Precocious puberty associated with primary hypothyroidism is gonadotropin-independent. Basal FSH is elevated; however, basal LH is low, and LH response to GnRH is prepubertal. Precocious puberty in patients with primary hypothyroidism is due to the action of TRH and TSH on GnRH and FSH receptors, respectively ("specificity-spillover"). The loss of feedback inhibition of T_4 on hypothalamus results in elevated levels of TRH, which acts on GnRH receptors and preferentially stimulates FSH secretion. LH secretion is suppressed as a result of hyperprolactinemia, which is a consequence of elevated TRH, whereas FSH secretion is driven by elevated TRH, even in presence of hyperprolactinemia. In addition, the clearance of FSH is also reduced as a consequence of hypothyroidism. As both TSH and FSH are glycoprotein hormones, elevated levels of TSH in patients with primary hypothyroidism act on FSH receptors in ovary and testes, to induce ovarian follicular growth and testicular enlargement, respectively.

39. *What are the causes of stippled epiphysis?*

    Stippled epiphysis is a feature of congenital/juvenile hypothyroidism, thyroid hormone resistance, and multiple epiphyseal dysplasias. Thyroid hormone is essential for the development of growth plate; therefore, thyroid hormone deficiency/resistance results in fragmentation of ossification centers leading to stippled epiphysis (Fig. 3.15).

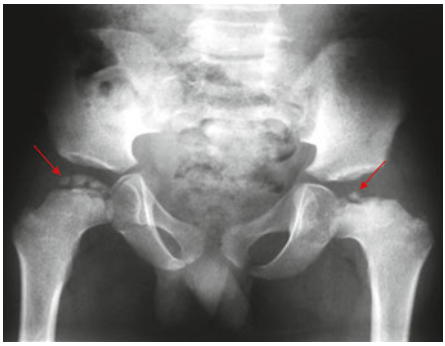

Fig. 3.15 Plain radiograph of pelvis depicting stippled femoral epiphysis (*red arrows*) in a patient with juvenile primary hypothyroidism

40. *What are the endocrine causes of slipped capital femoral epiphysis?*

    Primary hypothyroidism, growth hormone deficiency, rhGH therapy, Cushing's syndrome, obesity, and primary hyperparathyroidism are the important endocrine causes of slipped capital femoral epiphysis. It is commonly bilateral and occurs due to disproportionate growth of epiphyseal growth plate as compared to head of the femur.

41. *What are the disorders associated with increased prevalence of hypothyroidism?*

    Disorders associated with increased prevalence of hypothyroidism include Turner syndrome, Down syndrome, celiac disease, type 1 diabetes, William syndrome, and Klinefelter's syndrome.

42. *A 4-year-old girl presented with goiter. On evaluation, thyroid function test showed T4 3.5 µg/dl and TSH 29 µIU/ml. How to evaluate further?*

    The index child has primary hypothyroidism. The common causes of primary hypothyroidism in children include Hashimoto's disease and delayed-onset dyshormonogenesis. Therefore, further evaluation includes estimation of antithyroid peroxidase antibody and perchlorate discharge test. In addition, X-rays for bone age estimation should also be performed. In children without goiter, ^{99m}Tc pertechnetate scan should be performed to assess the presence/absence of ectopic thyroid tissue.

43. *What is perchlorate discharge test?*

In newborns/children with primary hypothyroidism who have goiter and/or normal or increased uptake on ^{99m}Tc pertechnetate/ ^{123}I scan, a diagnosis of dyshormonogenesis (other than NIS defect) should be considered. This can be confirmed by perchlorate discharge test. Perchlorate is an anion which competes with iodine for uptake by NIS. A baseline ^{123}I uptake study is performed after 2h of radioiodine ingestion. Thereafter, 200–1000 mg potassium perchlorate is administered orally and repeat ^{123}I uptake study is performed after 30min to 1h. Decrease in radioactivity by >10 % as compared to baseline uptake suggests dyshormonogenesis. This is because radioiodine is taken up by the gland in patients with dyshormonogenesis, but is not incorporated in tyrosine residues of thyroglobulin and hence discharged from the thyroid gland due to competetive inhibition by perchlorate at NIS.

44. *A 10-year-old boy presented with headache and visual impairment. Neuroimaging revealed a sellar–suprasellar mass and the child was referred to a neurosurgeon. Preoperative evaluation revealed T_4 2.4 µg/dl and TSH 150 µIU/ml; prolactin 104 ng/ml; cortisol 400 nmol/L with prepubertal LH, FSH, and testosterone; and low IGF1. Is surgery warranted?*

In all patients with sellar–suprasellar mass, anterior pituitary function tests including T_4, TSH, 0800h cortisol, prolactin, LH/FSH, testosterone/estradiol, and IGF1 should be done. In the index child, hormonal profile was suggestive of primary hypothyroidism with hyperprolactinemia. Low IGF1 in the index patient may be due to low T_4 or mass effect. In a patient with primary hypothyroidism, presence of a sellar–suprasellar mass should raise a suspicion of thyro-lactotrope hyperplasia. In this scenario, optimal replacement with L-thyroxine not only reverses features of hypothyroidism but also results in visual improvement in 1–4 months followed by regression of thyro-lactotrope hyperplasia. Hence, surgery should be avoided in these patients (Fig. 3.16).

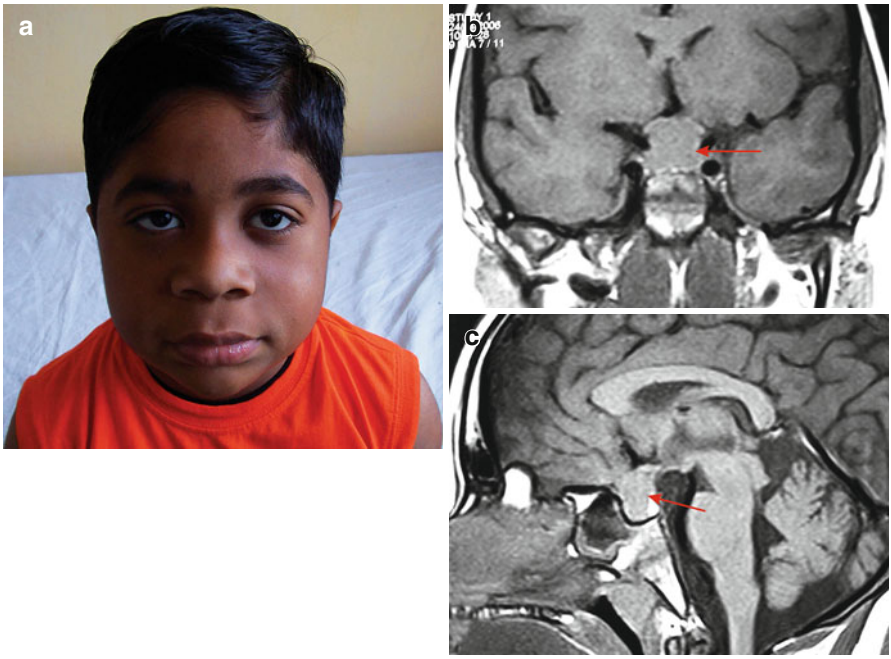

Fig. 3.16 (**a**) A 10-year-old boy with overt features of hypothyroidism. (**b**) Coronal and (**c**) sagittal T1W images showing presence of diffuse homogenous enlargement of the pituitary gland (*red arrow*) suggestive of pituitary hyperplasia (thyro-lactotrope) in the same patient

45. *What are the height prospects in children with juvenile hypothyroidism?*

Early diagnosis and optimal replacement therapy result in attainment of normal final adult height in children with juvenile hypothyroidism. Children with long-standing hypothyroidism will experience catch-up growth after initiation of L-thyroxine; however, catch-up growth is often incomplete in these patients due to diminished chondrocyte reserve. Children in the peripubertal age and those who are overzealously treated with L-thyroxine may experience rapid skeletal maturation and, hence, compromised final adult height. There are a few case studies where GnRH analogue has been used with or without rhGH in children with juvenile hypothyroidism during peripubertal period who had suboptimal catch-up growth. However, the results were variable (Fig. 3.17).

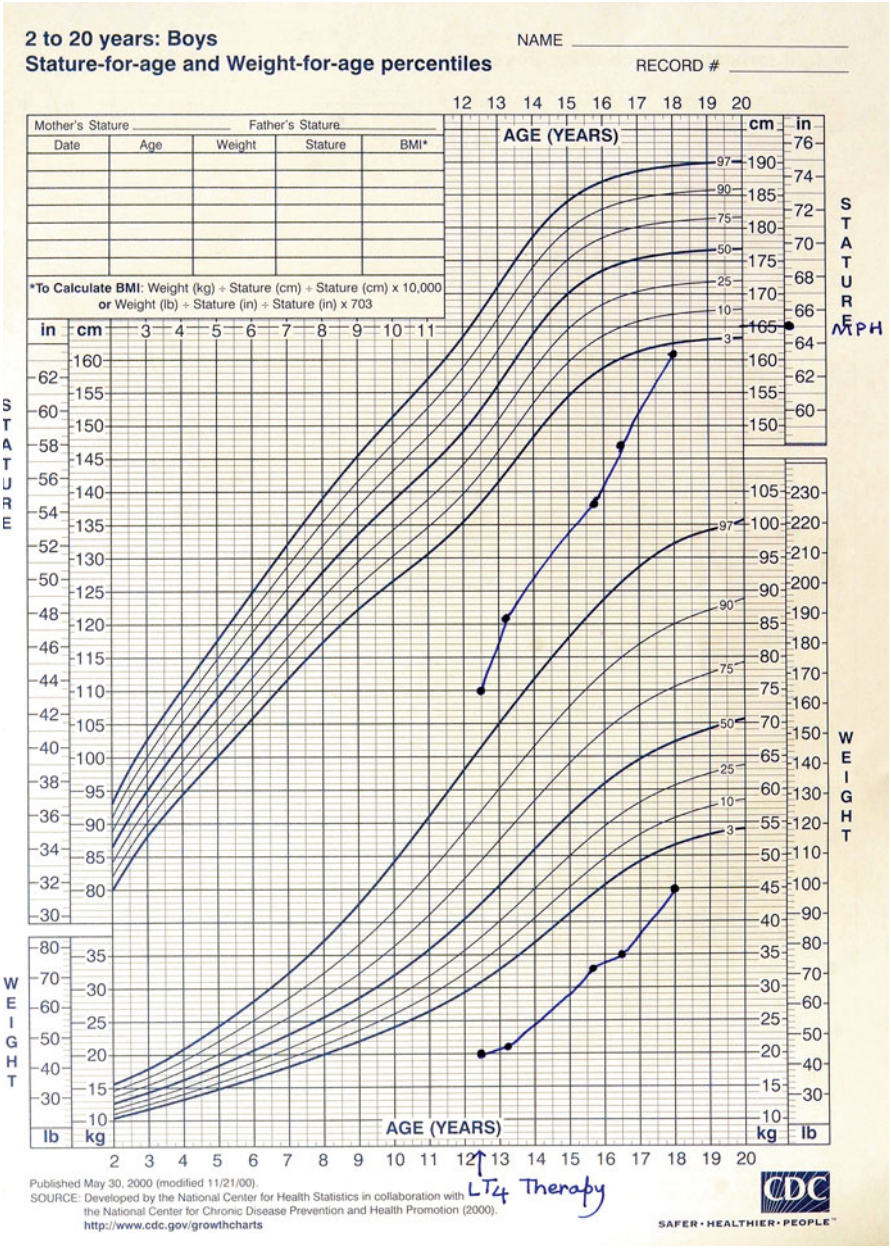

Fig. 3.17 Growth chart depicting improvement in height SDS in a boy with juvenile primary hypothyroidism after initiation of L-thyroxine

46. *What are the causes of lack of "catch-up growth" in a child with juvenile hypothyroidism who is on optimal L-thyroxine replacement?*

Failure to have a catch-up growth despite optimal L-thyroxine replacement in children with juvenile hypothyroidism should raise a suspicion of coexisting disorders like celiac disease, Turner syndrome, or growth hormone deficiency.

47. *How to treat and monitor juvenile hypothyroidism?*

The recommended dose of L-thyroxine is higher in children as compared to adults due to larger body surface area (in relation to body weight) and increased clearance of thyroid hormones in children. The recommended daily dose of L-thyroxine in children aged 1–5 years is 5 μg/Kg, while it is 4 μg/Kg in those between 6 and 12 years of age, and 3 μg/Kg in those aged >12 years, who have not completed the puberty. However, in postpubertal children L-thyroxine should be administered at dose of 1.6–1.8 μg/Kg/day. Both T_4 and TSH should be monitored till 3–4 years of age; TSH should be maintained in the lower half of normal reference range (0.5–2.5 μIU/ml) and T_4 in the upper half of normal reference range. Thereafter, TSH alone can be monitored every 3–6 monthly and maintained between 0.5 and 2.5 μIU/ml. Adverse effects are rare with L-thyroxine therapy and include pseudotumor cerebri, craniosynostosis, acute psychosis, and decline in scholastic performance. Bone age should be reassessed, if required.

48. *What is the outcome of complications related to long-standing juvenile hypothyroidism?*

Long-standing severe juvenile hypothyroidism is associated with short stature, delayed puberty, thyro-lactotrope hyperplasia, multicystic ovaries, precocious puberty, pseudo-myohypertrophy, epiphyseal dysgenesis, and slipped capital femoral epiphysis. Most of these manifestations usually reverse within 3–6 months of optimal L-thyroxine replacement. Although height improves after L-thyroxine therapy, final adult height is often subnormal in those with long-standing juvenile hypothyroidism. Stippled epiphysis normalizes with L-thyroxine therapy but slipped capital femoral epiphysis need surgical intervention.

49. *Why are clinical manifestations less severe in children with secondary hypothyroidism as compared to those with primary hypothyroidism?*

Secondary hypothyroidism is characterized by low FT_4 and low/normal to mildly elevated TSH. Clinical manifestations are less severe in children with secondary hypothyroidism as compared to those with primary hypothyroidism. This is because T_4 deficiency is usually mild in secondary hypothyroidism due to ongoing TSH-independent T_4 synthesis, which contributes to 10–15 % of circulating T_4. In addition, presence of concurrent multiple pituitary hormone deficiencies may mask the features of hypothyroidism. However, in primary hypothyroidism T_4 deficiency is usually severe, and markedly elevated TSH contributes to myxedematous manifestations.

50. *What are the causes of secondary hypothyroidism in children?*

Secondary hypothyroidism may be due to genetic or acquired causes. Genetic causes include pituitary transcription factor defects (PIT1, PROP1, HESX1, LHX3, LHX4,) or TSH-β/TRH receptor gene mutations. Sellar–suprasellar tumors (e.g., craniopharyngioma), infiltrative disorders of hypothalamo–pituitary region, and CNS insults like head injury, meningitis, and cranial irradiation are acquired causes of secondary hypothyroidism (Fig. 3.18).

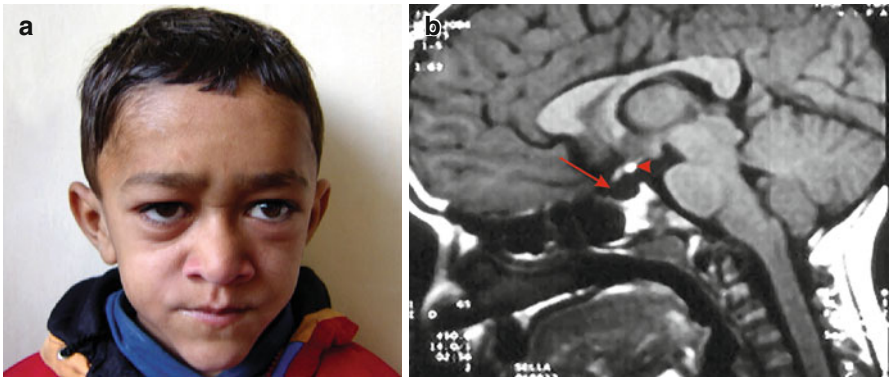

Fig. 3.18 (**a**) A 6-year-old child with multiple pituitary hormone deficiency. Note frontal bossing, midfacial hypoplasia, and cherubic face suggestive of GH deficiency. (**b**) Sagittal MRI T1-weighted image showing small sella, absent pituitary (*red arrow*) and stalk, and ectopic posterior pituitary bright spot (*arrow head*, MRI tetrad) suggestive of panhypopituitarism due to pituitary transcription factor defect

51. *How to approach a child with secondary hypothyroidism?*

In children with secondary hypothyroidism, assessment of other pituitary hormones including 0800h cortisol, prolactin, and gonadotropins (if the child is in peripubertal age) should be performed. GH–IGF1 axis should be assessed after normalization of serum T_4. In addition, imaging of sella is mandatory, which may reveal sellar–suprasellar mass lesions, structural defects of pituitary gland (small sella, hypoplastic/absent anterior pituitary, redundant/absent stalk, and ectopic posterior pituitary bright spot) or can be normal.

52. *How to treat a child with secondary hypothyroidism?*

Oral L-thyroxine is the therapy of choice in secondary hypothyroidism. In children with secondary hypothyroidism and coexisting glucocorticoid deficiency, glucocorticoid replacement should be initiated prior to L-thyroxine therapy, to prevent the risk of adrenal crisis. L-Thyroxine requirement is usually lower in secondary hypothyroidism, as TSH-independent thyroid hormone biosynthesis continues in these patients. However, requirement of L-thyroxine increases with concomitant growth hormone therapy (as GH therapy increases T_4 to T_3 conversion and reduces TSH as a result of increased

somatostatin tone) or estrogen replacement (due to estrogen-mediated increase in TBG). After optimal replacement with glucocorticoids and/or L-thyroxine, there may be unmasking of central diabetes insipidus, as both these hormones counteract the action of antidiuretic hormone. Serum FT_4 should be monitored in children with secondary hypothyroidism on L-thyroxine therapy and targeted in the upper half of normal reference range. Serum FT_4 is preferred over total T_4 as concurrent hormone deficiencies influence the circulating TBG levels. The underlying cause of secondary hypothyroidism should be appropriately treated.

53. *A 7-year-old boy was evaluated for poor scholastic performance and thyroid function test showed T4 4 µg/dl and TSH 2.3 µIU/ml. Patient was clinically euthyroid and serum-free T4 was done which was normal. What is the likely diagnosis?*

Low T_4 but normal FT_4 and TSH suggest the diagnosis of thyroxine-binding globulin (TBG) deficiency. TBG is a glycoprotein which binds approximately 70 % of circulating T_4 and 80 % of circulating T_3. TBG deficiency is inherited as X-linked recessive disorder, with a prevalence of 1 in 5000 newborns. The acquired causes of TBG deficiency include nephrotic syndrome, hepatic failure, and drugs like glucocorticoids and androgens. However, these patients do not require L-thyroxine therapy as FT_4 is normal.

54. *What are the causes of hyperthyroidism during infancy and childhood?*

During infancy, the causes of hyperthyroidism include transplacental transfer of TSH receptor-stimulating antibody (in neonates born to mother with Graves' disease), McCune–Albright syndrome and TSH receptor-activating mutations. In children, the most common cause of hyperthyroidism is Graves's disease and, rarely, toxic adenoma and toxic multinodular goiter may also result in hyperthyroidism.

55. *What are the alterations in thyroid function test in a neonate born to a mother with Graves' disease?*

Neonates born to a mother with Graves' disease may be euthyroid, thyrotoxic, or hypothyroid. Approximately 1–5 % of neonates born to mothers with Graves' disease have transient neonatal hyperthyroidism due to transplacental transfer of TSH receptor-stimulating antibody, which may manifest at birth or after few days (if mother is on antithyroid drugs). Neonatal transient primary hypothyroidism can occur as a result of transplacental passage of maternal antithyroid drugs or TSH receptor-blocking antibody. Rarely, central hypothyroidism can

also occur due to increased maternal transfer of T_4 before 32 weeks of pregnancy, which results in inhibition of fetal hypothalamo–pituitary–thyroid (HPT) axis; this suppression of HPT axis may persist up to 6 months.

56. *What is the natural history of TRAb-mediated hyperthyroidism in a neonate born to mother with Graves' disease?*

TRAb-mediated hyperthyroidism commonly resolves spontaneously within 3–12 weeks. Persistence of thyrotoxicosis beyond 6 months should raise a possibility of McCune–Albright syndrome or TSH receptor-activating mutations.

57. *What are the manifestations of neonatal hyperthyroidism?*

Neonatal hyperthyroidism is characterized by irritability, restlessness, poor feeding, failure to thrive, increased appetite, weight loss, diarrhea, sweating, tachycardia, and heart failure. Goiter is commonly present. Eye signs like periorbital edema, lid retraction, and proptosis may be present even if mother does not have thyroid-associated orbitopathy. Hepatosplenomegaly, lymphadenopathy, and thymic enlargement may also be present in newborns with hyperthyroidism. Skeletal manifestations of neonatal hyperthyroidism include microcephaly, advanced bone age, and craniosynostosis.

58. *How to evaluate a neonate born to mother with Graves' disease?*

Cord blood sample should be obtained for FT_4 and TSH in neonates born to mother with Graves' disease. If thyroid function is normal, repeat testing should be performed after 1–2 weeks, as transplacental passage of antithyroid drugs may delay the onset of thyrotoxicosis in these newborns. Estimation of TRAb is helpful in confirming the etiology of neonatal hyperthyroidism.

59. *How to treat neonatal hyperthyroidism?*

Neonatal hyperthyroidism should be managed as thyroid storm, because it is associated with high mortality (30%) in untreated neonates. Treatment includes antithyroid drugs (methimazole 0.25–1 mg/Kg/day in two to three divided doses), propranolol 2 mg/Kg/day, and iodides if required. Propylthiouracil is contraindicated as neonates are at a higher risk of hepatotoxicity. Glucocorticoids should be instituted to tide over the crisis. Antithyroid drugs are required only for short duration, as TRAbs-mediated neonatal thyrotoxicosis remits by itself within 2–3 months. However, infants with McCune–Albright syndrome and TSH receptor-activating mutations require definitive therapy later, after euthyroidism is attained with antithyroid drugs.

60. *What are the differences in clinical manifestation of hyperthyroidism in children as compared to adults?*

Most of the manifestations of childhood hyperthyroidism are similar to those seen in adults. However, there are some important differences between the two, which are summarized in the table given below.

Parameters	Hyperthyroidism in children	Hyperthyroidism in adults
Etiology	Graves' disease	Graves' disease
	McCune–Albright syndrome, TSH receptor-activating mutations	Toxic multinodular goiter
	Rarely, resistance to thyroid hormone	Toxic adenoma
Growth acceleration	Common	–
Weight	Weight loss uncommon, weight gain can occur	Weight loss common
Atrial fibrillation	Rare	Common
Congestive heart failure	Rare	Common
Nervousness, hyperactivity	Common	Less common
Thyroid-associated ophthalmopathy	Rare	Common
Infiltrative dermopathy/ acropachy	Rare	Less common

61. *What are the monosymptomatic presentations of childhood hyperthyroidism?*

The monosymptomatic presentations of childhood hyperthyroidism include accelerated growth velocity, poor scholastic performance and attention deficit hyperkinetic disorder, and diffuse goiter. In addition, children with thyrotoxicosis can rarely present with headache due to benign intracranial hypertension and polydypsia (Fig. 3.19).

Fig. 3.19 A 15-year-old boy with Graves' disease who has diffuse goiter and thyroid-associated orbitopathy

62. *How to evaluate children with hyperthyroidism?*

The most common cause of hyperthyroidism in children is Graves' disease. Other rare causes include McCune–Albright syndrome and TSH receptor-activating mutations. A careful examination for clinical features of McCune–Albright syndrome (cafe-au-lait macules, bony swellings, signs of sexual precocity, acro-gigantism) should be performed. Family history should be obtained, as TSH receptor-activating mutation is an autosomal dominant disorder. In the absence of these clinical clues, further investigation is not warranted and child should be managed as Graves' disease (Figs. 3.20 and 3.21).

Fig. 3.20 A 14-year-old girl with Graves' disease who had (**a**) diffuse goiter and asymmetrical proptosis (**b**) CT orbit showing thickening of extraocular muscles suggestive of thyroid-associated ophthalmopathy. Note the width of extraocular muscles (*arrow*) exceed the width of optic nerve (*arrow head*)

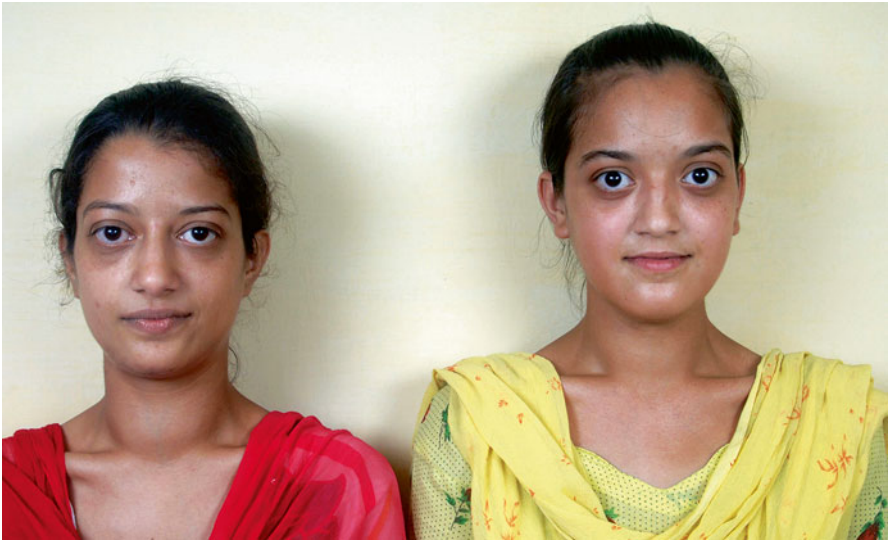

Fig. 3.21 Siblings with diffuse goiter and stare suggestive of familial Graves' disease. They showed positivity for TSH receptor antibody (TRAb)

63. *When to suspect thyroid-associated orbitopathy in children?*

Thyroid-associated orbitopathy (TAO) should be considered in a child who presents with proptosis, which is usually bilateral and asymmetrical, along with diffuse goiter and thyrotoxicosis. However, the unusual presentation of TAO includes unilateral proptosis and euthyroid TAO, and rarely 3–5 % of patients may have TAO associated with Hashimoto's thyroiditis. Elevated TSH receptor antibodies (TRAb) titer substantiates the diagnosis of TAO, which may be particularly useful in euthyroid TAO. Extraocular muscle belly thickening with sparing of tendon is characteristic radiological finding in TAO.

64. *How to treat a child with Graves' disease?*

The available treatment modalities for the management of Graves' disease in children include antithyroid drugs, radioactive iodine, and thyroid surgery. Among the antithyroid drugs, methimazole/carbimazole is preferred as there is a higher risk of hepatotoxicity with the use of propylthiouracil in children. Methimazole is initiated at a dose of 0.25–1 mg/Kg/day as a single daily dose. Addition of propranolol at a dose of 0.5–2 mg/Kg/day in three divided doses helps in alleviation of adrenergic manifestations. The response to antithyroid drugs is poor in children as compared to adults, and only 20–30 % of children achieve remission after 2 years of therapy. Further, predictors of remission of Graves' disease are not well defined in children. It is recommended that chil-

dren with Graves' disease who fail to achieve remission after 2 years of antithyroid drug therapy should be offered definitive treatment, either radioiodine ablation or surgery. Radioiodine ablation is an effective therapy for children with Graves' disease with a cure rate of 95 %. However, it is to be avoided in children <5 years of age, due to concern for possible risk of thyroid malignancy. In children with Graves' disease, high-dose radioiodine therapy should be employed for complete ablation of thyroid gland, as low-dose radioiodine therapy is associated with an increased risk of neoplasm in irradiated residual thyroid tissue. Total/near-total thyroidectomy is indicated in children with large goiter and in those with age <5 years who fail to achieve remission with antithyroid drugs.

65. *What are the peculiarities of hyperthyroidism associated with McCune–Albright syndrome?*

McCune–Albright syndrome (MAS) is due to post-zygotic activating mutations of Gsα subunit and is characterized by triad of fibrous dysplasia, café-au-lait macules, and endocrinopathy. Hyperthyroidism is the third most common endocrine abnormality (19 %) in MAS after precocious puberty (52 %) and acro-gigantism (27 %). The mean age of presentation of hyperthyroidism is 14 years, although it may present as early as in neonatal period. Children with hyperthyroidism associated with MAS may have diffuse goiter, multinodular goiter, or solitary nodule. Definitive therapy should be offered to these children as remission does not occur with the use of antithyroid drugs.

66. *What are the causes of coarse facial features?*

Various endocrine disorders are associated with coarse facial features including acromegaly, hypothyroidism, multiple endocrine neoplasia type 2B syndrome (MEN2B), pachydermoperiosteitis, and mucopolysaccharidosis. In addition, patients with morbid obesity, insulinoma, and prolactinomas may also have coarse facial features. The coarse facial features in acromegaly are due to soft tissue overgrowth as a result of GH–IGF1 excess. In patients with hypothyroidism, coarse facial features are accompanied with periorbital puffiness due to deposition of glycosaminoglycans (GAGs) as a result of high TSH as well as low T_4. Patients with mucopolysaccharidosis also manifest with coarse features due to increased extracellular accumulation of GAGs as a result of their impaired degradation by lysosomal enzymes. Patients with prolactinoma can also have coarse facial features due to "specificity-spillover" of prolactin at GH receptor as a result of structural similarity. Patients with MEN2B have blubbery lips as a result of mucosal neuromas which may contribute to coarse facial feature. Altered fibroblast activity and increased sensitivity to gonadal steroids and epidermal growth factor contribute to coarse facial features in patients with pachydermoperiostosis (Fig. 3.22).

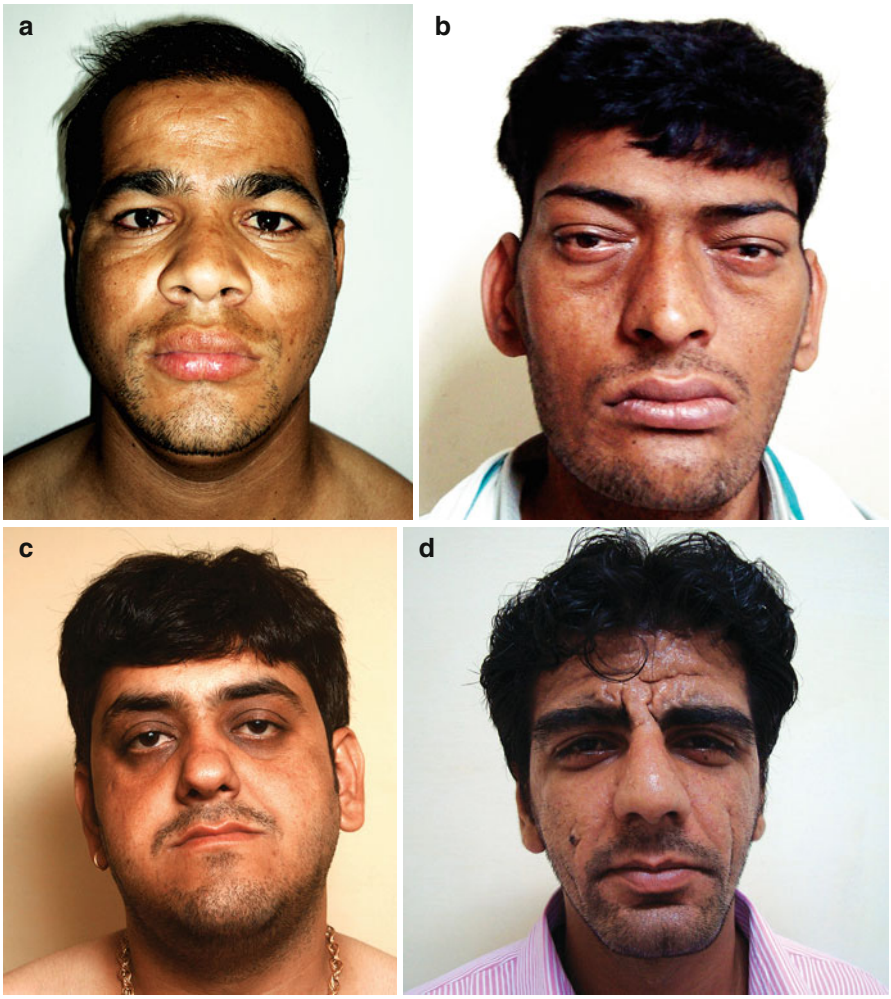

Fig. 3.22 Coarse facial features: varying etiology. Patients with (**a**) primary hypothyroidism, (**b**) acromegaly, (**c**) prolactinoma, (**d**) pachydermoperiostosis, (**e**) MEN2B, (**f**) and mucopolysaccharidosis

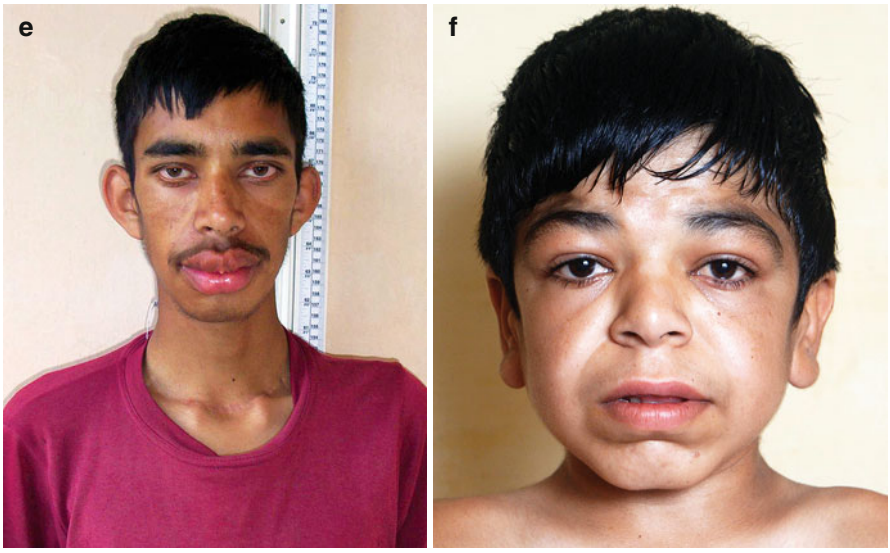

Fig. 3.22 (continued)

Further Readings

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Childhood Cushing's Syndrome

4

4.1 Case Vignette

A 16-year-old girl presented with weight gain, secondary amenorrhea, and increased facial hair for the past 2 years; however, her appetite was normal and there was no change in her lifestyle. She also complained of weakness and appearance of striae over the abdomen for the last 1 year. She had a history of poor gain in height during the last 4 years. Patient also had difficulty in climbing the stairs. However, there was no history of easy bruisibility, back pain, or pathological fracture. She had menarche at the age of 12 years and later oligomenorrhea, followed by secondary amenorrhea for the last 2 years. There was no history of any drug intake, or use of alternative medications, or steroid preparations, either inhalational or ointments. On examination, her height was 157 cm (<25th percentile, height age 13 years, target height 161 cm), weight 67 Kg (>75th percentile, weight age >20 years), BMI 24.1 Kg/m^2, and blood pressure 112/74 mmHg. She had florid features of Cushing's syndrome including moon facies, facial plethora, acne, dorso-cervical fat pad, and wide violaceous striae over lower abdomen and thighs. She also had cuticular atrophy, knuckle hyperpigmentation, and proximal muscle weakness. She had generalized obesity with waist circumference of 90 cm. However, she did not have bruise and pulp atrophy. Patient had hirsutism with Ferriman–Gallwey score of 15/36 and did not have features of virilization. There was no cafe-au-lait macule, lentigines, bony deformity, spine tenderness, or cutaneous fungal infection. Biochemical evaluation revealed serum potassium 4.2 mEq/L, liver, and renal function tests were normal. Fasting blood glucose was 92 mg/dl and 2h plasma glucose after glucose load was 112 mg/dl with HbA1c of 5.0 %. Lipid profile showed serum cholesterol 159 mg/dl, LDL-C 99.6 mg/dl, triglyceride 198 mg/dl, and HDL-C 32.2 mg/dl. Cortisol dynamics showed 0800h cortisol 567 nmol/L; awake 2300h cortisol 520 nmol/L; 0800h ACTH 16.8 pg/ml; awake 2300h ACTH 63.7 pg/ml; late-night salivary cortisol 12.5 nmol/L and 28.7 nmol/L on two consecutive days, respectively; and 24h urinary free cortisol on three consecutive days was 448 μg,

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469 μg, and 532.8 μg, respectively. Serum cortisol after overnight dexamethasone suppression test (ONDST) was 464 nmol/L, low-dose dexamethasone suppression test (LDDST) 408 nmol/L, and after high-dose dexamethasone suppression test (HDDST) was 157 nmol/L. Serum prolactin was 8.63 ng/ml (N 4.7–23.3), T_4 7.02 μg/dl (N 4.8–12.7), TSH 0.894 μIU/ml (N 0.27–4.2), testosterone 0.32 nmol/L (N 0.2–2.9), LH 0.24 mIU/ml (N 2.4–12.6), and FSH 0.32 mIU/ml (N 3.5–12.5). Ultrasound pelvis showed normal ovaries and uterus. Dynamic contrast-enhanced MRI sella revealed differentially enhancing 4.8 × 4.0 mm lesion which was hypointense on T1W and hyperintense on T2W images in the left half of the pituitary gland. She underwent bilateral inferior petrosal sinus sampling (IPSS) with 100 μg (IV bolus) human CRH. IPSS localized the source of ACTH excess to the pituitary and lateralized to right side of the pituitary gland. Serum ACTH and cortisol profile during IPSS are summarized in the table given below. She underwent transsphenoidal surgery; intraoperatively tumor was localized to the left side of the pituitary, and excision of the tumor was accomplished. Patient developed left lateral rectus palsy postoperatively, which recovered within 2 weeks. She was documented to have hypocortisolemia (83.5 nmol/L) on day 1 postoperatively and was started on hydrocortisone supplementation. Histopathology of the tumor tissue showed pituitary adenoma, while immunostaining for ACTH was negative. At 6 weeks, she had weight loss of 5 Kg, resolution of plethora, disappearance of acne, and violaceous striae started fading. An 0800h serum cortisol after the omission of hydrocortisone for 24h was 4.6 nmol/L, and serum T_4 was 10.5 μg/dl. She is continued with hydrocortisone supplementation and advised to follow up every three monthly (Fig. 4.1).

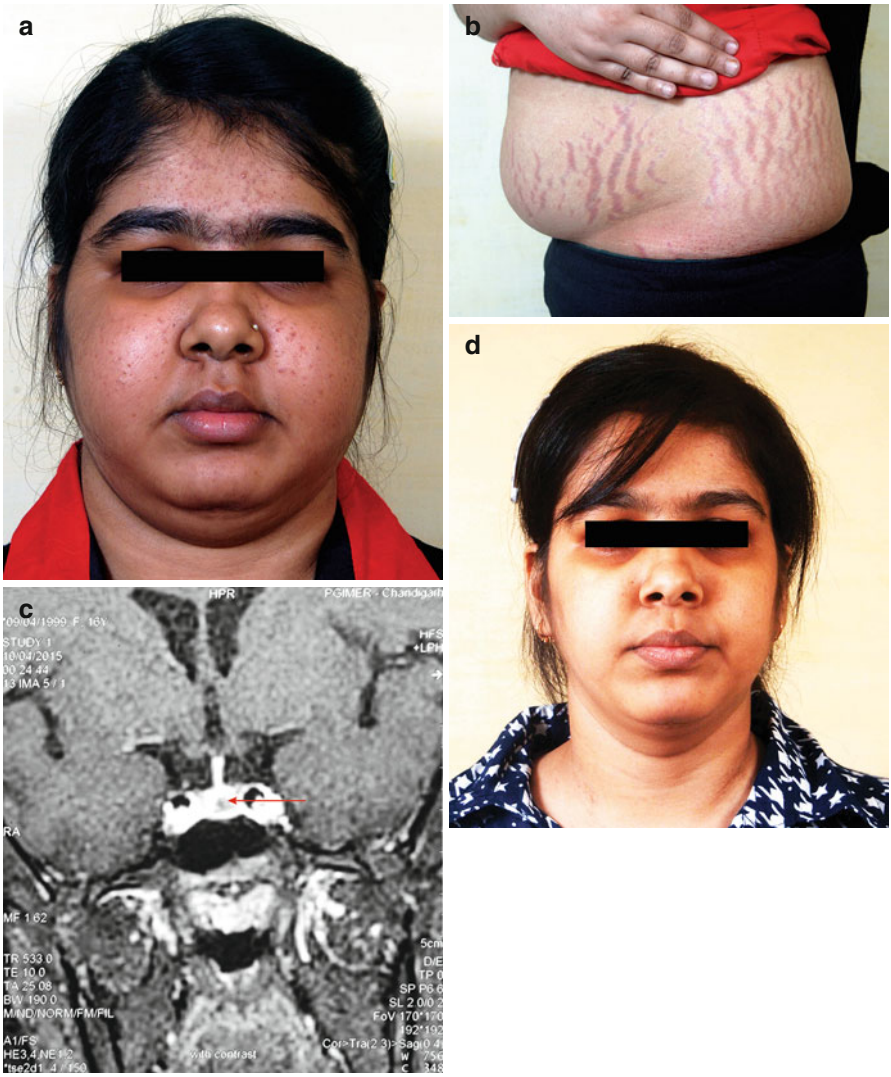

Fig. 4.1 (**a**) A 16-year-old girl with moon facies, acne, lanugo hair, and facial plethora (**b**) Typical wide violaceous striae. (**c**) CEMRI sella showing hypointense lesion measuring 4.8×4 mm (*red arrow*). (**d**) Regression of Cushingoid features after 3 months of transsphenoidal surgery in the same patient

Results of inferior petrosal sinus sampling after human CRH (100 μg) stimulation

Time (min)	Right central (RC) ACTH (pg/ml)	Left central (LC) ACTH (pg/ml)	Peripheral (P) ACTH (pg/ml)	RC/P ACTH ratio	RC/LC ACTH ratio	RC/LC-PRL-adjusted ACTH ratio
0	75.65	17.29	19.81	3.81	4.37	1.38
2	1516.2	25.55	27.92	54.30	59.34	4.27
3	770.2	41.41	30.55	25.21	18.59	0.94
5	614.0	41.90	51.69	11.87	14.65	0.92
15	701.4	33.54	36.33	19.30	20.91	1.54

4.2 Stepwise Analysis

This 16-year-old girl presented with weight gain, menstrual abnormalities, and hirsutism. On evaluation, though she was overweight for age, her height age was less than the chronological age which suggests underlying endocrine disorder for her growth failure, as opposed to children with exogenous obesity, who usually grow taller due to hyperinsulinemia-mediated increased IGF1 generation. However, the attained height in the index patient was within 1.5 SD of her target height, which indicates relatively recent onset of the disease. She had wide violaceous striae, proximal myopathy, and cuticular atrophy, which are the specific features of Cushing's syndrome and represent the manifestations of protein catabolism. Though the children of younger age with Cushing's syndrome usually present with growth failure and delayed puberty and do not have features of protein catabolism, the index patient had manifestations of protein catabolism. The presence of features of protein catabolism in the index patient distinguishes it from polycystic ovarian syndrome which usually presents in the peripubertal age with weight gain and menstrual irregularities. Though the patient denied history of use of steroid preparations or alternative medications, 0800h serum cortisol was estimated to exclude the exogenous Cushing's syndrome as inadvertent use of steroids is not uncommon in children and adolescents. In addition, 0800h serum cortisol is also useful to establish the loss of diurnal rhythm and for the interpretation of HDDST. The presence of hypercortisolemia in the index patient was confirmed by elevated urinary free cortisol, midnight salivary cortisol, and non-suppressible ONDST. However, at least two tests are required to establish the presence of hypercortisolemia in a patient with suspected Cushing's syndrome to increase sensitivity of the screening tests. Additional screening test like midnight serum cortisol may be useful in a situation where the results of the screening tests are discordant or negative, and the clinical suspicion of Cushing's syndrome is strong. A 2300h serum cortisol >207 nmol/L in awake state has a sensitivity and specificity of 94 % and 100 %, respectively, or in sleeping state >50 nmol/L has a sensitivity and specificity of 100 % and 20 %, respectively. Further, 2300h serum cortisol <207 nmol/L in awaken state or <50 nmol/L in sleeping state virtually excludes the diagnosis of Cushing's syndrome with a negative predictive value of 96 % and 100 %, respectively. The LDDST should be preferred over other screening tests in those who have subtle features of Cushing's syndrome (pseudo-Cushing's syndrome). Nevertheless, it should be remembered that the doses of dexamethasone used for performing the dynamic tests are different in children and adolescents as compared to adults (ONDST, LDDST, and HDDST – 15 μg/Kg, 30 μg/Kg/day for 2 days, and 120 μg/Kg/day for 2 days, respectively, in children weighing <40 Kg). After confirmation of hypercortisolemia, measurement of plasma ACTH is recommended to establish the etiological diagnosis of Cushing's syndrome. A 2300h plasma ACTH >22 pg/ml has highest discriminatory value to establish the diagnosis of ACTH-dependent Cushing's syndrome. An 0800h plasma ACTH <5 pg/ml supports the diagnosis of ACTH-independent

Cushing's syndrome. An 0800h plasma ACTH >90 pg/ml is suggestive of ectopic source of ACTH secretion. After confirmation of ACTH dependency, dynamic contrast-enhanced MRI sella should be performed to localize the source of ACTH excess, as pituitary Cushing's is the most common (98 %) cause of Cushing's syndrome during peripubertal period. The index patient had 2300h ACTH value of 63.7 pg/ml and dynamic CEMRI sella localized the 4.8 × 4.0 mm tumor in the left half of the pituitary gland. In adults, the pituitary tumor size ≥6 mm has a specificity of 98 %, whereas such data is not available for patients with childhood Cushing's syndrome. Therefore, additional investigations like HDDST and IV CRH stimulation tests should be performed to substantiate the diagnosis of pituitary ACTH-dependent Cushing's syndrome. However, the data for ACTH and cortisol cutoffs in response to IV CRH are limited in children. Therefore, this patient was subjected to IPSS and it localized the source of ACTH to the pituitary gland but lateralized, contrary to the MRI findings, to the right half of the pituitary gland. Basal central to peripheral (C/P) ACTH ratio >2 and stimulated ratio >3 localizes the source of ACTH to the pituitary, whereas a ratio of >1.4 between the right and left inferior petrosal sinus lateralizes the source of ACTH excess. The index patient had basal C/P ratio of 3.8 and stimulated peak of 54.3, which localized the source of ACTH excess to the pituitary. The ratio of right to left inferior petrosal sinus ACTH after CRH stimulation was 59.3, lateralizing the source of ACTH excess to the right side. The discordance in lateralization of pituitary tumor between MRI and IPSS can be explained by intercavernous venous mixing, right-sided dominance in pituitary venous drainage pattern (in 40 % of healthy adults), or presence of the epicenter of the tumor on one side with extension of the tumor to the contralateral side. The pretest probability of finding a tumor during surgical exploration is approximately 92 % as lateralized by MRI, compared to 69 % as lateralized by IPSS. Therefore, a decision was taken first to explore the left half of the pituitary in the index patient, and the tumor was found on the same side and was resected. Histopathology of the tumor tissue was consistent with pituitary adenoma, but immunohistochemistry on tumor tissue for ACTH was negative, which may be a poor predictor in achieving long-term remission. Postoperatively, serum cortisol should be estimated at 0800h from day 1 to day 5 and a serum cortisol value <100 nmol/L merits for immediate replacement with hydrocortisone, whereas patients with serum cortisol level >450 nmol/L do not require supplementation with hydrocortisone. However, 0800h serum cortisol between 100 and 450 nmol/L requires a close watch and, if symptomatic, should be supplemented. Postoperative 0800h serum cortisol <50 nmol/L predicts long-term remission; however, some studies have shown that 0800h serum cortisol <140 nmol/L up to 6 weeks also predicts the similar long-term outcome. The immediate postoperative hypocortisolemia (<50 nmol/L) is a result of peritumoral corticotropes suppression due to long-standing hypercortisolemia. The index patient had day 1, 0800h serum cortisol <100 nmol/L and she was initiated with hydrocortisone replacement. Postoperatively, she had desquamation which is the earliest clue of curative surgery and occurs as a result of increased cuticular

turnover as suppressive effect of hypercortisolemia on stratum corneum is eliminated. She had rapid disappearance of plethora within a week after surgery. At 6 weeks, she had weight loss of 5 Kg, diminution of acne, and fading away of striae suggestive of likelyhood to achieve remission. The probability of cure in the index case is more likely as she had microadenoma, no parasellar extension, immediate postoperative 0800h cortisol <140 nmol/L, and histopathological documentation of adenoma, though prolonged follow-up is required to recognize recurrence at the earliest as the disease can resurge at any time and a prolonged remission phase (>10 years) predicts long-term cure.

4.3 Clinical Rounds

1. *What is the most common cause of Cushing's syndrome in children*?

   As in adults, the most common cause of Cushing's syndrome (CS) in children is the use of exogenous glucocorticoids. A detailed history of glucocorticoid exposure (including topical and inhalational use) should be elicited in all children presenting with Cushing's syndrome. Eczema, bronchial asthma, and nephrotic syndrome are common disorders during childhood for which glucocorticoids are often prescribed.

2. *What are the causes of endogenous Cushing's syndrome in children*?

   Both Cushing's disease and adrenal disorders contribute almost equally to the etiology of endogenous Cushing's syndrome in children. However, adrenal disorders are the most common cause of endogenous Cushing's syndrome in children <5 years of age, whereas Cushing's disease is the commonest cause after 5 years of age. Ectopic Cushing's syndrome is rare in children (Figs. 4.2 and 4.3). The common causes of childhood Cushing's syndrome at various ages are listed in the table given below.

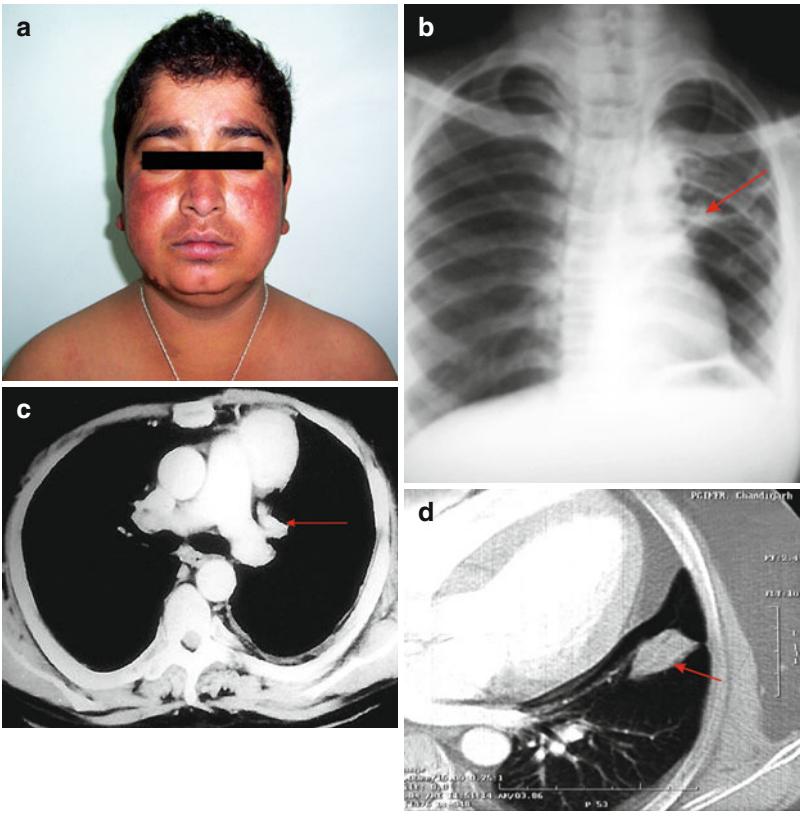

Fig. 4.2 (**a**) Moon facies and plethora in a 14-year-old boy with ectopic Cushing's syndrome. (**b**) Chest X-ray shows left para-hilar soft tissue mass (*red arrow*). (**c**, **d**) Axial CT chest confirms the left para-hilar mass suggestive of bronchial carcinoid (*red arrows*)

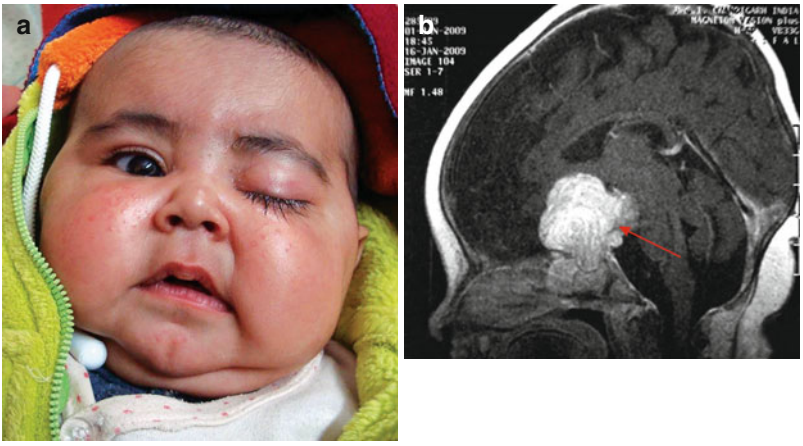

Fig. 4.3 (**a**) A 2-month-old girl with Cushing's syndrome having round facies, facial plethora, and ptosis of the left eye. (**b**) Sagittal CEMR demonstrates a large sellar–suprasellar enhancing mass (*red arrow*) causing sellar expansion in the same child. The histopathology of the lesion revealed ACTH-secreting congenital immature teratoma

Age	Common etiology
Infancy	McCune–Albright syndrome
	Adrenocortical neoplasm
1–5 years of age	Adrenocortical neoplasm
>5 years	Cushing's disease
	Primary pigmented nodular adrenocortical disease (PPNAD)

3. *Is there a gender difference in the etiology of Cushing's syndrome in children as compared to adults*?

   In children, Cushing's disease (CD) is more prevalent in boys during prepubertal years. During adolescence, the male to female ratio is equal and in adults, Cushing's disease predominates in women. Both adrenocortical adenoma and carcinoma have a female preponderance in children as well as in adults. Ectopic Cushing's syndrome (although rare in children) is more common in girls during childhood; however, during adulthood it is more frequent in men.

4. *What are the manifestations of Cushing's syndrome in children*?

   The common presenting manifestations of childhood Cushing's syndrome include moon facies (100 %), weight gain (90 %), growth failure (84 %), and delayed puberty (60 %). In addition, fatigue, hypertension, features of protein catabolism (striae, bruise, proximal myopathy, and plethora), and hyperandrogenism (in girls) may also be present. The unusual features of childhood Cushing's syndrome include precocious puberty, gait abnormalities (slipped capital femoral epiphyses, osteonecrosis of head of femur), and abdominal mass (Figs. 4.4 and 4.5).

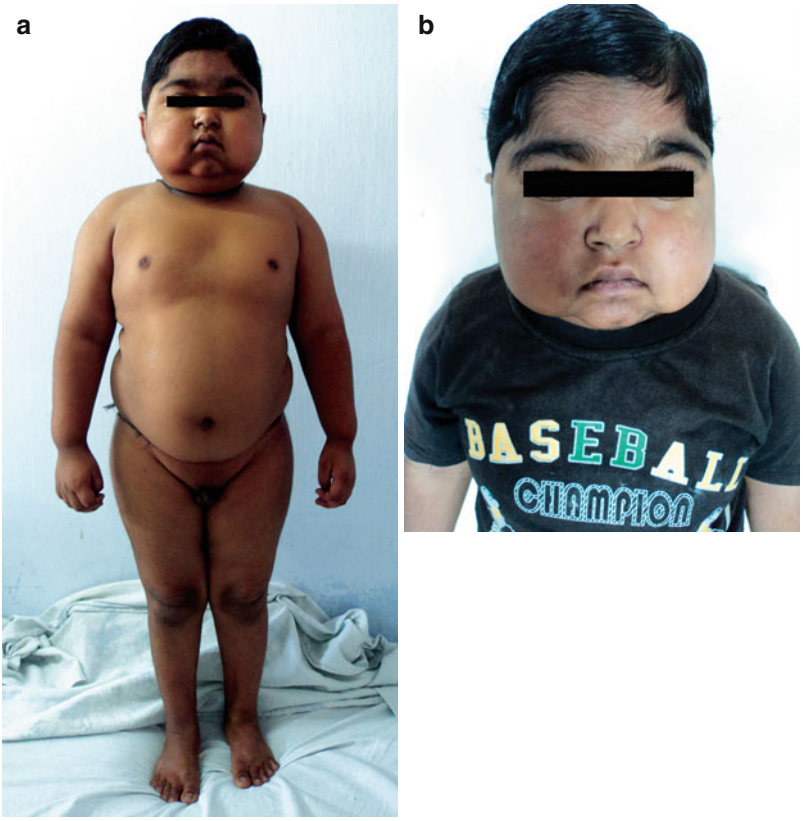

Fig. 4.4 (**a**, **b**) A 3-year-old child with Cushing's syndrome. Note the characteristic moon facies and no features of protein catabolism

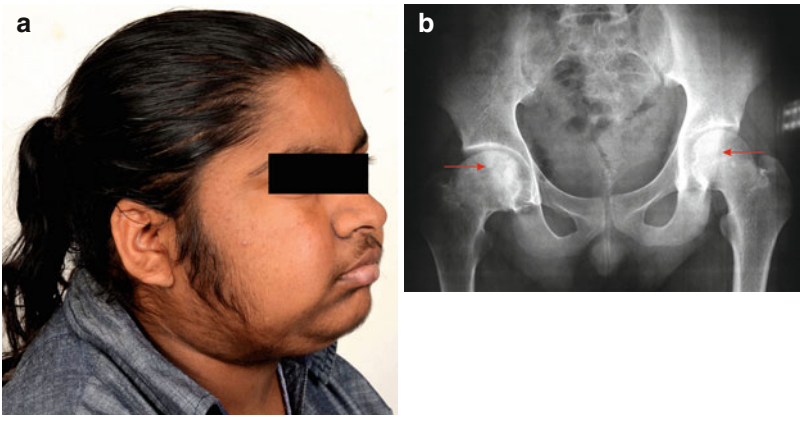

Fig. 4.5 (**a**) A 17-year-old girl with Cushing's syndrome having hirsutism and acne (**b**) X-ray of pelvis shows avascular necrosis of head of right femur with loss of contour and areas of radiolucency and early changes in head of left femur (*red arrows*)

5. *What are the differences in clinical manifestations of Cushing's syndrome in children as compared to adults*?

   The differences in clinical manifestations of Cushing's syndrome in children as compared to adults are summarized in the table given below.

Parameters	Cushing's syndrome in children (%)	Cushing's syndrome in adults (%)
Moon facies	100	81
Weight gain	90–92	90–95
Growth failure	84	–
Hypertension	51–63	71–75
Glucose intolerance	–[a]	75
Hirsutism	46	81
Striae	36	56
Bruise	28	62
Proximal myopathy	13	56
Plethora	46	94
Osteopenia	74	50

[a]Data not available

   It is to be noted that the features of protein catabolism are less common in children as compared to adults, due to relatively higher IGF1 levels in children.

6. *How to differentiate between Cushing's syndrome and exogenous obesity in children*?

   Children with exogenous obesity present with rapid weight gain and sometimes with violaceous striae, mimicking CS. The presence of growth failure in an obese child is highly suggestive of Cushing's syndrome. On the contrary, children with exogenous obesity commonly have normal to increased height (or growth velocity) because of increased IGF1 generation due to hyperinsulinemia. Height SDS and BMI SDS are increased in children with exogenous obesity, whereas in children with CS, BMI SDS is increased and height SDS is reduced. In addition, striae are usually thin (<1 cm) in children with exogenous obesity, whereas wide (>1 cm) purplish striae are characteristic of CS. Other features of protein catabolism, if present, can also help in the diagnosis of childhood CS.

7. *Why is there growth failure in children with Cushing's syndrome*?

   The impaired growth in children with CS is attributed to cortisol-mediated inhibition of GHRH–GH axis, decreased pre-chondrocyte to chondrocyte differentiation, chondrocyte apoptosis, impaired local IGF1 generation and action, and

increased bone collagen breakdown. In addition, cortisol-mediated suppression of hypothalamo–pituitary–gonadal axis and alterations in calcium–vitamin D homeostasis (decreased calcium absorption and hypercalciuria) also contributes to poor linear growth.

8. *Why is growth failure more common in children with Cushing's disease as compared to Cushing's syndrome due to adrenocortical tumors*?

   Growth retardation is more common in children with Cushing's disease as compared to those with adrenocortical tumors. This is because of long lag time prior to diagnosis of Cushing's disease in children. Majority of adrenocortical tumors in children are malignant and have short lag time prior to diagnosis. In addition, concurrent androgen excess in children with adrenocortical carcinoma may result in normal/accelerated growth velocity.

9. *How does hypercortisolemia affect pubertal development*?

   Puberty is commonly delayed in children with CS due to the inhibitory effect of cortisol on hypothalamo–pituitary–gonadal axis. However, patients with concurrent androgen excess due to androgen and cortisol co-secreting adrenal tumors may present with heterosexual precocity in girls and isosexual precocity in boys (gonadotropin-independent precocious puberty). Children with Cushing's disease may also have increased adrenal androgen secretion and may have premature pubarche; however, gonadarche is delayed.

10. *What are the characteristics of adrenocortical tumors in children*?

    Adrenocortical tumors are rare in children and account for only 0.3–0.4 % of all neoplasms in childhood, except in southern Brazil where the incidence is high. Majority of adrenocortical tumors in children are functional (90 %) and malignant (88 %). Among the functional tumors, 61 % have features of androgen excess, 33 % have both androgen and cortisol excess, and 6 % of patients have isolated cortisol excess. Cortisol-secreting adrenal tumors are common during infancy and early childhood with a peak incidence at 4.5 years. Androgen-secreting adrenocortical tumors may present with heterosexual precocity in girls and isosexual precocity in boys.

11. *What are the disorders associated with adrenocortical tumors in children*?

    Li–Fraumeni, Beckwith–Wiedemann, isolated hemihypertrophy syndromes, and, rarely, MEN 1 are associated with adrenocortical tumors in children.

12. *What are the peculiarities of Cushing's syndrome associated with McCune–Albright syndrome*?

Cushing's syndrome is rare in McCune–Albright syndrome (MAS) and occurs only in 5–7 % of patients. CS associated with MAS may even manifest at birth (due to intrauterine hypercortisolemia), but majority of children present before 5 years of age. CS associated with MAS is due to ACTH-independent diffuse/nodular adrenal hyperplasia. Cushingoid facies, low birth weight, and failure to thrive are the common presenting features. Hypercortisolemia associated with MAS may resolve spontaneously as fetal zone of adrenal cortex (which over expresses Gs-α) regresses by 1 year of age and is replaced by the definitive adult zone. Children who have spontaneous resolution of CS may develop adrenal insufficiency subsequently. The presence of café-au-lait macules, fibrous dysplasia, and other endocrinopathies helps in the diagnosis of MAS. The definitive therapy of CS associated with MAS is bilateral adrenalectomy. Medical therapy with ketoconazole is not preferred as many children with MAS have coexisting liver disease; however, metyrapone may be used in critically ill infants awaiting surgery.

13. *What is primary pigmented nodular adrenocortical disease*?

Primary pigmented nodular adrenocortical disease (PPNAD) is an ACTH-independent cause of Cushing's syndrome. Approximately 70 % patients with PPNAD have overt Cushing's syndrome and the rest have subclinical CS. PPNAD commonly presents in second/third decade of life and has a female preponderance. Multiple pigmented nodules (<5 mm) with internodular atrophy is the characteristic histological feature of PPNAD. Pigmentation is due to accumulation of lipofuscin pigment, whereas internodular atrophy due to suppression of ACTH by hypercortisolemia as a result of autonomously functioning nodules. PPNAD is commonly (>90 %) associated with Carney's complex. The genes implicated in pathogenesis of PPNAD are PRKAR1A, PDE11A, PDE8B, and MYH8 and are involved in increased cyclic adenosine monophosphate (cAMP) generation, thereby facilitating tumorigenesis.

14. *What are the distinctive features of childhood Cushing's syndrome associated with PPNAD*?

The distinctive features of childhood Cushing's syndrome associated with PPNAD include young age of onset (usually <15 years), subtle features of protein catabolism, normal growth velocity (due to insidious onset and mild hypercortisolemia), and osteoporosis. Biochemical characteristics include mild hypercortisolemia and paradoxical increase in serum cortisol/UFC following high-dose dexamethasone suppression test. Adrenal imaging is normal; occasionally, CT adrenal may demonstrate characteristic "beads on a string appearance." Bilateral adrenalectomy is curative (Fig. 4.6).

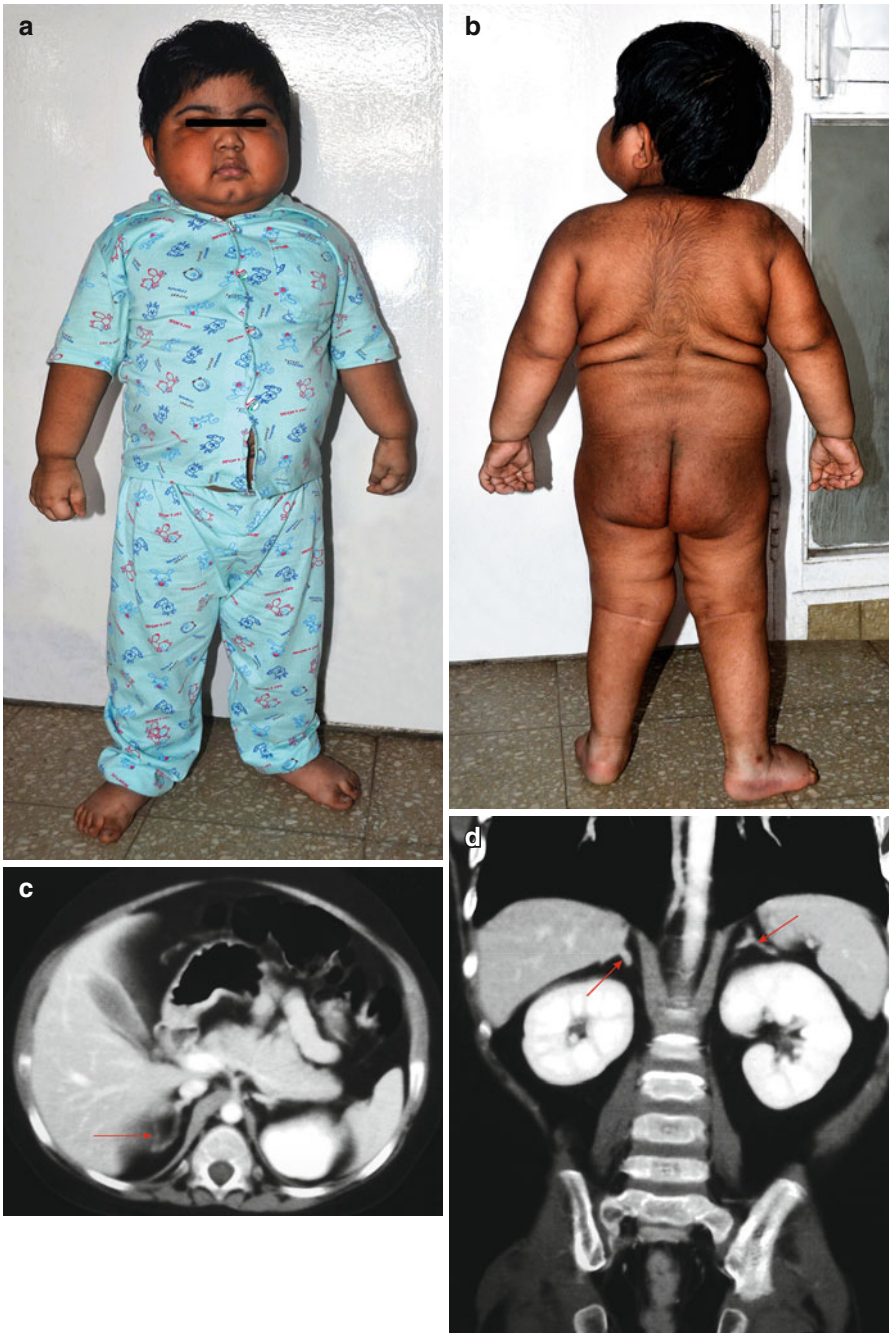

Fig. 4.6 (**a**) A 3-year-old child with obesity, plethora, and moon facies. (**b**) Excessive vellus hair in the same child. (**c**) Axial and (**d**) coronal CECT abdomen of the same patient showing bilateral adrenal hyperplasia with "beads on a string" appearance on the right side (*red arrows*) suggestive of PPNAD

15. *What is the atypical presentation of PPNAD*?

PPNAD may present as classical, subclinical, or cyclical Cushing's syndrome. In addition, some children with PPNAD may present with "atypical Cushing's syndrome" which manifests as asthenia, lean habitus, severe muscle wasting, short stature, and osteoporosis. Biochemically, 24h urine free cortisol is usually normal. However, loss of normal diurnal cortisol rhythm and paradoxical increase in cortisol/UFC following HDDST points towards the diagnosis of PPNAD.

16. *What is the paradoxical cortisol response to high-dose dexamethasone suppression test*?

In response to high-dose dexamethasone, >50 % decline in serum cortisol from baseline (0800h) cortisol is considered as suppressible HDDST, while failure to suppress serum cortisol by >50 % from baseline indicates non-suppressible HDDST. The paradoxical response to dexamethasone is defined as >50 % rise in serum cortisol from baseline cortisol after HDDST during Liddle's protocol. PPNAD is the most common cause of paradoxical cortisol response to HDDST. Rarely, paradoxical response to HDDST may also be seen in patients with adrenocortical carcinoma. This phenomenon is due to overexpression of glucocorticoid receptors on adrenal nodules with consequent activation of protein kinase A signaling pathway, which is involved in cortisol synthesis.

17. *What is Carney's complex*?

Carney's complex is an autosomal dominant disorder and is characterized by cardio-cutaneous manifestations and endocrine hyperactivity. The cutaneous manifestations are seen in 80 % and include lentigines, blue nevus, and myxomas. Cardiac myxomas are present in 30–60 % of patients; usually multiple and can develop in any cardiac chamber. The most common endocrinopathy associated with Carney's complex is Cushing's syndrome due to PPNAD and is present in 25–60 %, followed by acromegaly (10 %). Other features of Carney's complex are testicular tumors, breast fibroadenoma, ovarian cysts, thyroid tumors, and schwannomas. Inactivating germline mutations of protein kinase A regulatory subunit 1α-gene (PRKAR 1A) has been found in 45–65 % of patients with Carney's complex.

18. *How to monitor a patient with Carney's complex*?

In children with Carney's complex, echocardiography and testicular ultrasonography should be performed annually. Growth rate and pubertal development must be monitored closely in these children for the early detection of endocrinopathies. Adolescents and adults with Carney's complex should be monitored annually with echocardiography, testicular and thyroid ultrasonography, serum IGF1, and late-night salivary/midnight serum cortisol.

19. *How to approach a child with suspected Cushing's syndrome*?

An approach to a child with suspected Cushing's syndrome is depicted in the figure given below (Fig. 4.7).

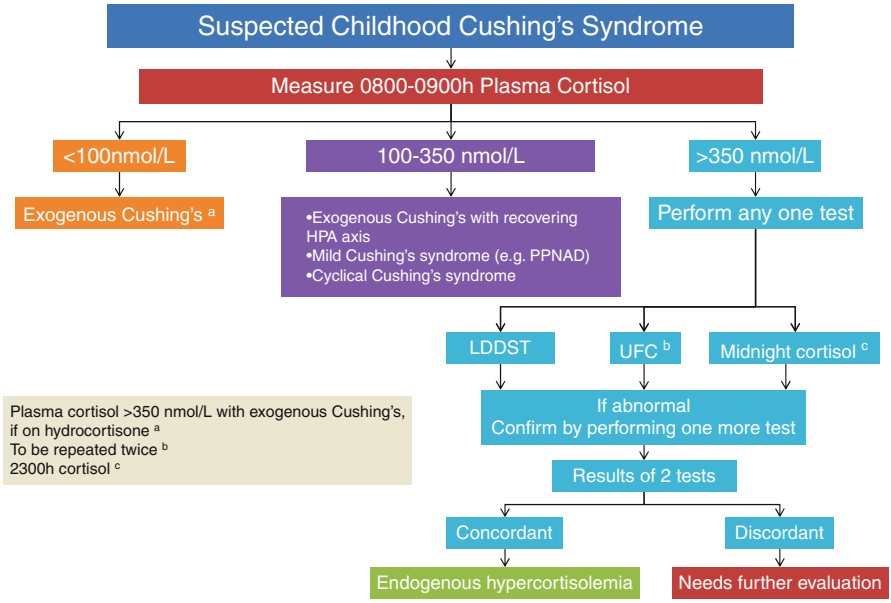

Fig. 4.7 Approach to a child with suspected Cushing's syndrome

20. *What is the role of estimation of 0800–0900h cortisol in the diagnosis of Cushing's syndrome*?

Estimation of 0800–0900h cortisol is helpful in the differentiation of exogenous from endogenous Cushing's syndrome, as it is suppressed in patients with exogenous Cushing's syndrome. This is important because rampant use of alternative medications is not uncommon in clinical practice. Baseline morning cortisol is also required for the interpretation of HDDST, as well as for the assessment of circadian rhythm.

21. *How to perform dexamethasone suppression tests in children*?

The dose of dexamethasone used for various suppression tests in the diagnosis of childhood Cushing's syndrome are summarized in the table given below. The diagnostic cutoffs of serum cortisol after dexamethasone suppression tests are same as in adults. However, it should be noted that ONDST is less preferred in children.

Test	Weight ≥40 Kg	Weight ≤40 Kg
ONDST	1 mg at 2300h	15 μg/Kg at 2300h
LDDST	0.5 mg, 6 hourly × 2 days	30 μg/Kg/day in four divided doses × 2 days
HDDST	2 mg, 6 hourly × 2 days	120 μg/Kg/day in four divided doses × 2 days

22. *What is the importance of diurnal variation of cortisol secretion in the diagnosis of Cushing's syndrome*?

Cortisol secretion peaks at 0400–0800h and troughs at 2300–2400h and this diurnal rhythm is established by 2–3 years of age. Diurnal variation of cortisol secretion prevents sustained hypercortisolemia, which may be detrimental to neuronal function and sleep. Loss of diurnal rhythm of cortisol secretion is defined as a 1600h serum cortisol level >50 % of 0800h serum cortisol or 2300h serum cortisol ≥207 nmol/L. This is the earliest abnormality of hypothalamo–pituitary–adrenal axis in patients with Cushing's syndrome. Other causes of altered diurnal rhythm include pseudo-Cushing syndrome, seizure disorder, depression, use of anticonvulsants, and shift-workers. However, pregnancy and glucocorticoid resistance syndrome are associated with preserved diurnal rhythm of cortisol secretion despite high serum cortisol.

23. *A 13-year-old female with specific features of Cushing's syndrome has 0800h cortisol 540 nmol/L (20 μg/dl), normal urine free cortisol, and suppressible LDDST. Is the diagnosis of Cushing's syndrome excluded*?

No. In the index patient with a high clinical suspicion of Cushing's syndrome, a normal UFC and a suppressible LDDST does not rule out the diagnosis of Cushing's syndrome. A midnight serum cortisol should be performed in this scenario, as the earliest biochemical abnormality in Cushing's syndrome is loss of diurnal rhythm. If sleeping midnight serum cortisol is ≥50 nmol/L (1.8 μg/dl), a possibility of mild Cushing's syndrome should be considered. In such circumstances, patients should be kept under close surveillance with periodic revaluation of cortisol dynamics. Further, even if the midnight serum cortisol is normal, a possibility of cyclical Cushing's syndrome should be kept and serial monitoring of UFC should be performed.

24. *What is the role of late-night salivary cortisol for the diagnosis of Cushing's syndrome in children*?

Late-night salivary cortisol (LNSC) measures free cortisol and is devoid of interference from alterations in cortisol-binding globulin. It is a simple noninvasive test and sampling for LNSC can be done even at home. Therefore, it is one of the first-line screening tests for the diagnosis of Cushing's syndrome in adults. However, it is not preferred as a screening test in childhood Cushing's syndrome as limited data is available regarding the use of LNSC in children.

25. *How to establish the etiological diagnosis of a patient with Cushing's syndrome*?

The approach to establish the etiological diagnosis of a patient with Cushing's syndrome is summarized in the figure given below (Fig. 4.8).

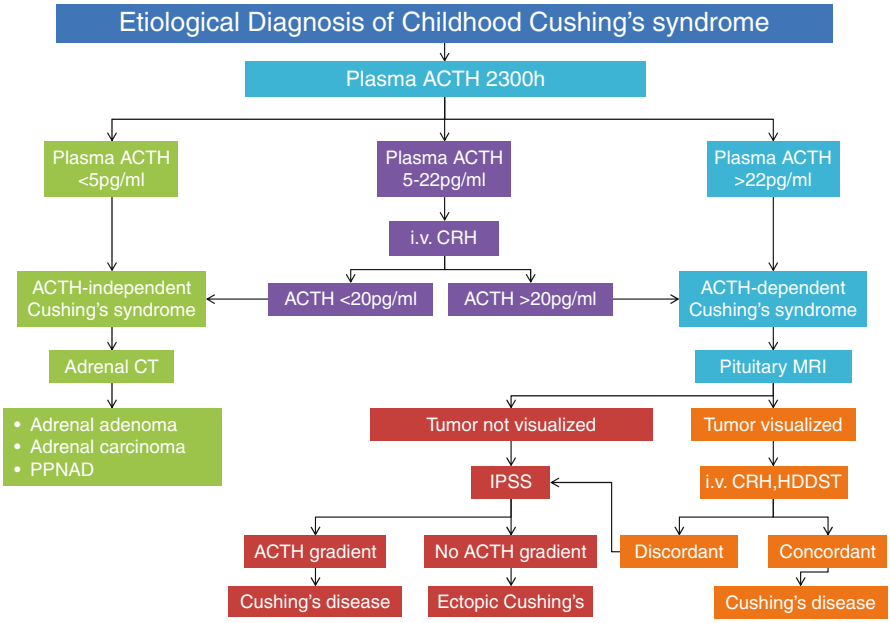

Fig. 4.8 Approach to a child for differential diagnosis of Cushing's syndrome

26. *What is the role of MRI in localization of pituitary tumor in children with Cushing's disease*?

Cushing's disease is the commonest cause of CS in children above 5 years of age. Majority of the ACTH-secreting pituitary tumors are microadenomas and the tumor is commonly <5 mm. Contrast-enhanced MRI sella is the preferred imaging modality to localize pituitary adenoma in children with Cushing's disease. On MR sellar imaging, microadenomas are visualized as hypointense lesions as compared to normally enhancing pituitary tissue after contrast administration (differential enhancement). The sensitivity of conventional CEMRI to localize a pituitary microadenoma in children is approximately 50 %; however, despite localization on MRI, concordance rate with surgery is only 50 %. Recently, it has been shown that use of spoiled gradient recalled acquisition MRI (SPGR-MRI) is associated with improved sensitivity (75 %) and accuracy (88 %) as compared to conventional MRI (Figs. 4.9 and 4.10).

Fig. 4.9 (**a**) A 15-year-old boy with Cushing's syndrome. (**b**) Coronal CEMR showing a hypointense pituitary lesion on the right side causing focal glandular expansion suggestive of microadenoma (*red arrow*)

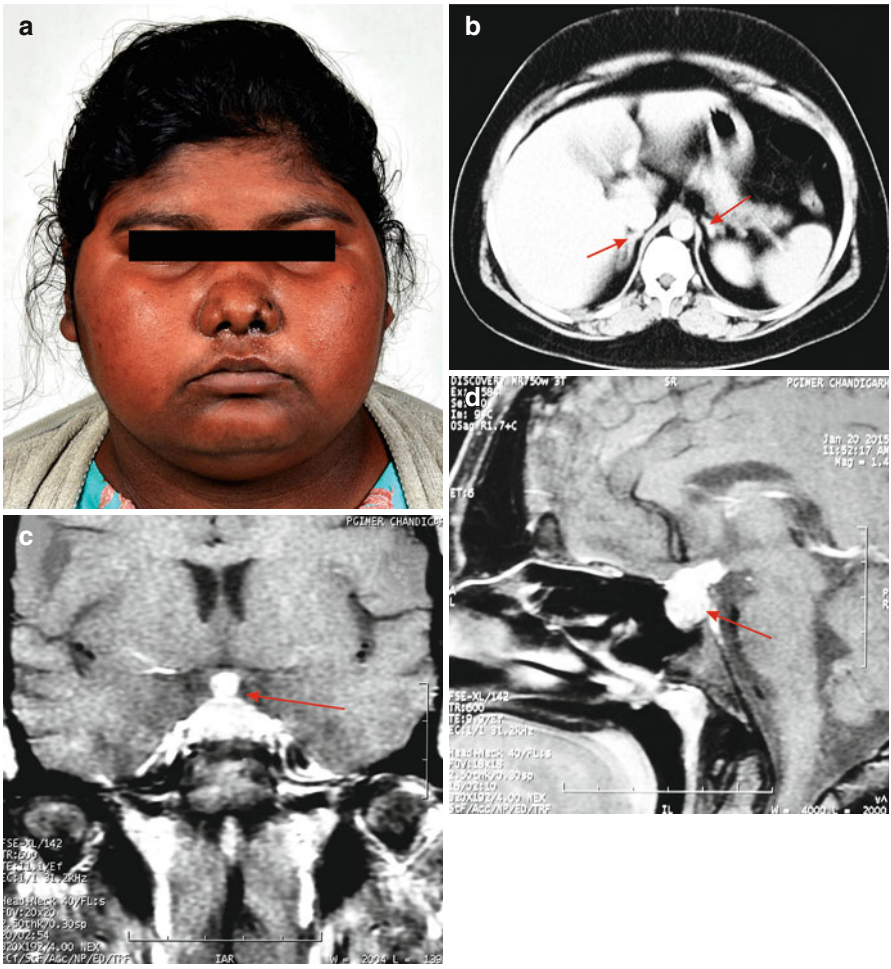

Fig. 4.10 (**a**) An 18-year-old girl with Cushing's syndrome. (**b**) CECT abdomen showing bilateral adrenal hyperplasia (*red arrows*). (**c**) Coronal and (**d**) sagittal CEMRI showing pituitary macroadenoma with extension into stalk (*red arrows*)

27. *How to evaluate a child for comorbidities associated with Cushing's syndrome*?

A detailed clinical examination including height, weight, and blood pressure should be performed in all children with Cushing's syndrome. These children should also be examined for cutaneous/systemic signs of infection. Biochemical investigations include fasting and postprandial plasma glucose, lipid profile, serum potassium, and assessment of anterior pituitary hormones. X-ray of dorsolumbar spine and DXA scan should also be performed.

28. *What are the difficulties in transsphenoidal surgery in children with Cushing's syndrome*?

Transsphenoidal surgery (TSS) is the preferred treatment modality in children with Cushing's disease; however, TSS in children is technically challenging and requires greater expertise as compared to adults. The small size of adenoma (commonly <5 mm) and non-pneumatization of sphenoid sinus also make TSS a difficult surgery in children.

29. *What is the role of radiotherapy in children with Cushing's disease*?

Pituitary radiotherapy is an effective second-line modality for residual/recurrent disease after transsphenoidal surgery (TSS). The cure rate after external beam radiotherapy (EBRT) is better in children (92 %) as compared to adults (60–70 %). In addition, the mean time to response is also faster in children (0.8–1 year) than in adults (1.5–4 years). Growth hormone deficiency is the most common anterior pituitary hormone deficiency after pituitary irradiation in children, while other pituitary hormones are usually preserved. The data regarding the use of stereotactic radiotherapy in children is limited; however, it seems to be equally effective.

30. *How to optimize linear growth in a child with Cushing's disease after curative surgery*?

Children with Cushing's disease may not have optimal catch-up growth even after curative therapy. This is due to suppressive effect of long-standing hypercortisolemia on GH–IGF1 axis and growth plate or as a result of insult to somatotropes after TSS. Children with suboptimal catch-up growth after curative TSS should be subjected to GH dynamic tests after 3 months of surgery and if found to have GH deficiency, they should be supplemented with rhGH. Addition of GnRH analogues may be considered in children who are in peripubertal age and have compromised predicted adult height. In addition, children who had undergone radiotherapy should be treated with ketoconazole/metyrapone during the interim period. GH dynamics should be performed once eucortisolemia is attained and treated with rhGH, if found to be GH deficient. Other hormone deficiencies, if present, should be adequately replaced. If the child is on glucocorticoid supplementation, overdosage should be avoided.

31. *How to evaluate a child with ACTH-independent Cushing's syndrome*?

Adrenal CT is the first-line investigation in children with ACTH-independent Cushing's syndrome. Presence of a heterogeneous unilateral mass >4 cm with irregular margin, necrosis, hemorrhage or calcification, attenuation value >10 HU, and delayed contrast washout (<50 % at 10 min) favors a diagnosis of adrenocortical carcinoma (ACC, Fig. 4.11). In contrast, adrenal adenomas are small (<4 cm), homogenous, and have regular margins, with attenuation value <10 HU and have early contrast washout (>50 % at 10 min). Children with PPNAD usually have normal adrenals on imaging; however, occasionally "beads on a string appearance" may be seen on adrenal CT.

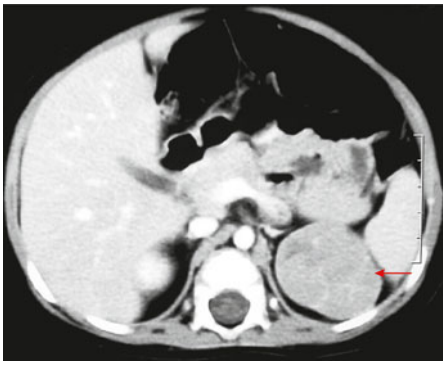

Fig. 4.11 Axial CT upper abdomen showing heterogeneous mass in the left adrenal region abutting the pancreatic tail suggestive of adrenocortical carcinoma (*red arrow*)

Further Readings

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Rickets–Osteomalacia

5

5.1 Case Vignette

A 25-year-old female presented with fracture neck of the left femur following trivial trauma. She had history of diffuse aches and pains and proximal muscle weakness for the last 6 months. After sustaining fracture, she became bedbound and was referred to our institute. There was no history of recurrent pain abdomen, diarrhea, steatorrhoea, polyuria, graveluria, or periodic paralysis. She had history of poor exposure to sunlight and deficient intake of dairy products. There was no past history of fracture, renal stone disease, gallstone disease, or pancreatitis. She had no history of use of glucocorticoids in any form, alternative medications, bisphosphonates, or calcium and vitamin D preparations. However, she complained of difficulty in swallowing and foreign-body sensations in her eyes. She had two live children and the last child birth was 3 years earlier, and she continues to menstruate regularly. Family history was noncontributory. On examination, she was lean, thin, emaciated, diffusely hyperpigmented, and had pallor, cheilosis, and glossitis. Her blood pressure was 100/60 mmHg. She had genu varum, kyphoscoliosis, diffuse bony tenderness, proximal muscle weakness, and severe attrition of her teeth with pigmentation. She had no goiter and deep tendon reflexes were delayed. Her eyes were suffused and tongue was dry. Movements at left hip joint were restricted and painful. Systemic examination was unremarkable. On investigations, hemoglobin was 10.2 g/dl, serum creatinine 1.2 mg/dl (eGFR 40 ml/min), Na^+ 145 mEq/L, K^+ 3.4 mEq/L, corrected calcium 9 mg/dl, phosphorus 3.0 mg/dl, alkaline phosphatase 919 IU/L (N < 128), iPTH 220 pg/ml (N 15–65), and 25(OH)D 30 ng/ml (N 30–70). Twenty-four-hour urinary calcium was 211 mg, phosphate 500 mg, and protein 1.2 g. Arterial blood gas analysis revealed pH 7.28, HCO_3 9.5 mEq/L, and calculated anion gap 11 mEq/L, and corresponding urine pH was 6.5. Serum T_4 was 4.6 μg/dl (N 4.8–12.7), TSH 78 μIU/ml (N 0.27–4.2), and anti-TPO antibody 480 IU/ml

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(N < 34). Antinuclear antibody was present (speckled pattern, 3+) and celiac serology (IgA tTG) was negative. Ultrasonography of the neck and sestamibi parathyroid scan was noncontributory. Ultrasound abdomen showed bilateral small kidneys. X-ray of spine and pelvis showed osteopenia and fracture neck of the left femur, respectively. X-ray of chest revealed pseudo-fracture at the outer border of scapula. ^{99m}Tc MDP bone scan revealed increased uptake in sternum, mandible, ribs, and long bones suggestive of metabolic bone disease. Schirmer's test was positive and lip biopsy was consistent with Sjogren's syndrome. With this profile, Sjogren's syndrome with distal renal tubular acidosis (RTA) and primary hypothyroidism due to Hashimoto's thyroiditis were considered, and the patient was initiated on sodium bicarbonate, potassium chloride, and calcium carbonate tablets. In addition, she was also started with L-thyroxine. With this treatment, her bone pain resolved, proximal myopathy improved, and she was able to walk with support. Repeat blood gas analysis showed pH 7.4, HCO_3 20 mEq/L, and serum K^+ 4.2 mEq/L. Later, calcitriol was added due to declining eGFR. She is planned for renal biopsy for the initiation of immunosuppressive therapy as she had proteinuria and declining eGFR (Fig. 5.1).

Fig. 5.1 (**a**) A 25-year-old female with distal renal tubular acidosis due to Sjogren syndrome. (**b**) Note genu varum, (**c**) attrition of teeth with loss of enamel. (**d**) X-ray of pelvis showing fracture neck of the left femur, right coxa vara, deformed pelvis, and cortical thinning. (**e**) X-ray of chest showing bilateral pseudo-fractures at the lateral border of scapula (*red arrows*)

5.2 Stepwise Analysis

The index patient presented with fragility fracture at a young age along with proximal muscle weakness. The clinical possibilities with this symptomatology include primary hyperparathyroidism (PHPT), severe osteomalacia with secondary hyperparathyroidism, hypophosphatemic osteomalacia, and renal tubular acidosis. Secondary osteoporosis due to Cushing's syndrome and hyperthyroidism can also result in fragility fracture along with proximal muscle weakness. PHPT is less likely in the index patient as she did not have symptoms related to hypercalcemia (painful bones, abdominal groans, renal stones, psychic moans, and fatigue overtones) and had normal calcium profile, which excludes this diagnosis. However, the patient had elevated serum iPTH level in the presence of 25(OH)D sufficiency, which can be explained by concurrent stage 3 chronic kidney disease. Severe osteomalacia due to vitamin D deficiency usually presents with bone pains and myopathy. Presence of Looser's zone, raised alkaline phosphatase, and high iPTH further supports the diagnosis of vitamin D deficiency. However, osteomalacia to manifest as fracture is very rare and presence of normal calcium and phosphate levels, with normal 25(OH)D at presentation in the index patient, make this diagnosis unlikely. Further, hypokalemia is not a feature of vitamin D deficiency-related osteomalacia. Celiac disease is an important consideration in this scenario, as it may manifest with osteomalacia/osteoporosis as a monosymptomatic presentation. Absence of gastrointestinal symptoms and negative celiac serology make this diagnosis unlikely. Hypophosphatemic osteomalacia manifests with bone pains, proximal myopathy, and severe hypophosphatemia, normal or mildly elevated alkaline phosphatase and iPTH. The index patient had bone pains and proximal myopathy, but normal serum phosphate and moderately elevated alkaline phosphatase and iPTH do not support the diagnosis of hypophosphatemic osteomalacia. Secondary osteoporosis due to Cushing's syndrome and hyperthyroidism, as both these disorders are associated with proximal myopathy and fragility fractures, were ruled out. Proximal myopathy, fragility fracture, hypokalemia, normal anion gap metabolic acidosis with failure to acidify urine (urine pH >5.5), hypercalciuria, and radiological evidence of osteomalacia establishes the diagnosis of distal RTA. Diagnosis of RTA should be suspected in a patient who presents with periodic paralysis, proximal myopathy, bony pains and deformities, and nephrolithiasis/nephrocalcinosis. Biochemically, it can be confirmed by the presence of normal anion gap metabolic acidosis. Further, the estimation of urine pH helps in differentiating between proximal and distal RTA, as proximal RTA is associated with urine pH <5.5, whereas distal RTA >5.5. As the index patient had urine pH of 6.5 in presence of normal anion gap metabolic acidosis, it suggests the diagnosis of distal RTA. However, in milder/incomplete forms of distal RTA, blood pH may be in a normal range due to compensation; therefore, ammonium chloride loading test is recommended to establish the diagnosis of distal RTA in such scenario. Hypokalemia, hypercalciuria, and hypocitraturia are the additional evidences of the distal renal tubular dysfunction, whereas, aminoaciduria,

glucosuria, and phosphaturia support the diagnosis of proximal RTA. Further, proximal RTA can be confirmed by bicarbonate loading test. Nephrocalcinosis/nephrolithiasis is usually a feature of distal RTA due to hypercalciuria and hypocitraturia. High urinary calcium in the presence of low urinary citrate allows the calcium to precipitate in the renal interstitium, thereby resulting in nephrocalcinosis. The index patient had concurrent stage 3 CKD (eGFR 40 ml/min and bilateral shrunken kidneys) which itself may be contributing to the metabolic acidosis. However, normal anion gap metabolic acidosis even in presence of mild to moderate CKD suggests the possibility of associated RTA. Metabolic acidosis leads to increased bone resorption and impaired bone mineralization. Activation of RANK ligand by systemic acidosis results in increased bone resorption. Further, the acidosis also inhibits bone mineralization due to its effect on osteoblast, thereby resulting in decreased bone collagen production and reduced alkaline phosphatase activity. The common cause of distal RTA in majority of patients is idiopathic; however, some patients may have concurrent autoimmune disorders or use of drugs associated with distal RTA. These include Sjogren's syndrome, systemic lupus erythematosus, PHPT, Wilson's disease, sarcoidosis, and use of certain drugs like amphotericin B, ifosfamide, and lithium carbonate. As the patient had history suggestive of dry mouth and dry eyes, a possibility of Sjogren's syndrome as the etiology of distal RTA was considered. Antinuclear antibody and lip biopsy confirmed the same in the index patient. The presence of primary hypothyroidism in the index patient is another component of autoimmunity. The primary aim of treatment is effective correction of metabolic acidosis. The other concurrent biochemical abnormalities like hypokalemia and hypercalciuria get spontaneously normalized with correction of metabolic acidosis. However, potassium and calcium supplementation may be required initially. Short-term use of calcitriol may be required for rapid healing of osteomalacia; however, it is associated with increased risk of nephrocalcinosis. Therefore, periodic monitoring of urinary calcium excretion and renal ultrasonography should be performed.

5.3 Clinical Rounds

1. *What are the constituents of bone*?
   Bone is made up of cells and matrix. The cellular component contributes to only 2 % of the dry weight of bone, while the rest is by matrix. The cellular component includes osteoblasts, osteoclasts, bone-lining cells, and osteocytes; the latter accounts for approximately 95 % of cellular compartment. The bone matrix comprises of inorganic (60–70 %) and organic constituents (30–40 %). The inorganic components include calcium hydroxyapatite and magnesium. Approximately 90 % of the organic component is constituted by type 1 collagen and the rest by non-collagenous proteins like bone sialoprotein, osteopontin, osteonectin, and osteocalcin. The constituents of bone are shown in the figure given below (Fig. 5.2).

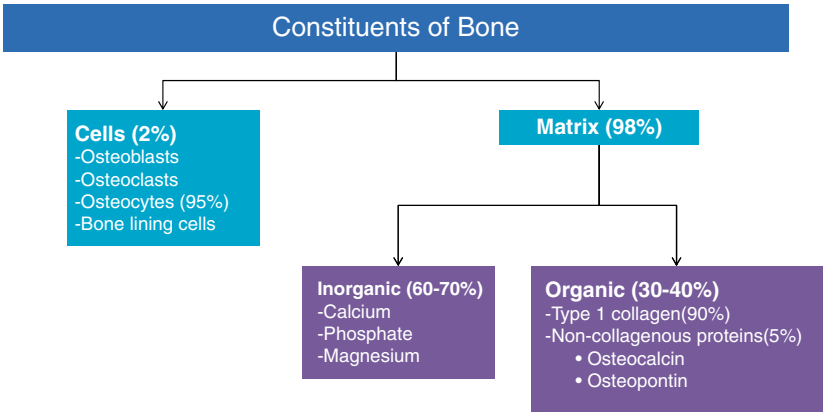

Fig. 5.2 Constituents of bone

2. *What are the functions of different types of cells present in bone*?

   Osteocytes are the most abundant cells present in bone. They are mature osteoblasts and are embedded in the bone matrix. They act as mechanosensor and initiate bone remodeling. These cells also secrete various phosphatonins including FGF-23 which play a crucial role in the phosphate homeostasis. In addition, osteocytes also secrete dickkopf-1 and sclerostin which inhibit bone formation. Osteoblasts lay down the matrix (osteoid) and promote mineralization of osteoid by secreting osteocalcin, osteopontin, and alkaline phosphatase. Once the process of mineralization is complete, mature osteoblasts may undergo apoptosis or can differentiate into osteocyte or bone-lining cells. Osteoblasts also promote osteoclastogenesis through RANK-RANKL pathway. Osteoclasts secrete various proteolytic enzymes like cathepsin K which results in bone resorption, a key step in bone remodeling. Bone-lining cells serve as a blood–bone barrier and regulate the influx and efflux of calcium and phosphorus from extracellular fluid. Further, these cells can redifferentiate into osteoblasts, when they are exposed to PTH.

3. *What are the types of bone*?

   Human skeleton can be classified on the basis of anatomy or structure. Anatomically, bone can be classified into long bone (e.g., femur), short bone (e.g., carpal bones), and flat bone (e.g., skull bones). Structurally, bone can be classified either as cortical and trabecular or as woven and lamellar bone.

4. *What are the differences between cortical and trabecular bone*?

   The differences between cortical and trabecular bone are summarized in the table given below.

Parameters	Cortical	Trabecular
Synonyms	Compact bone	Cancellous, spongy
Contribution to total bone mass	80 %	20 %
Predominant sites	Shaft of long bones	End of long bones, vertebra
Porosity	5–15 %	30–90 %
Haversian system	Present	Absent
Metabolic activity	Low	High
Remodeling rate	Low	High
Predominant hormonal control	PTH, thyroxine	Gonadal steroids, glucocorticoids

5. *What are the differences between woven and lamellar bone*?
   The differences between woven and lamellar bone are summarized in the table given below.

Parameters	Woven bone	Lamellar bone
Architecture	Disorganized collagen	Organized collagen (parallel or concentric)
Cell-to-matrix ratio	High	Low
Bone turnover	High	Low
Strength	Weak	Strong
Formation	Formed by rapid osteoid production by osteoblast	Formed by maturation of woven bone
Sites	Fetal bone Site of fracture in adults Paget's disease	All bones in the adult

6. *How is bone formed*?

   New bone is formed by either endochondral or intramembranous ossification. Endochondral ossification is a stepwise integrated process of bone formation involving differentiation of mesenchymal stem cells into cartilage that forms a scaffolding for deposition of bone matrix by osteoblasts. Intramembranous ossification involves direct formation of bone from mesenchymal stem cells without intermediate stage of cartilage formation. The flat bones (craniofacial) are formed by intramembranous ossification, while vertebrae, ribs, and long bones are developed by endochondral ossification (Fig. 5.3).

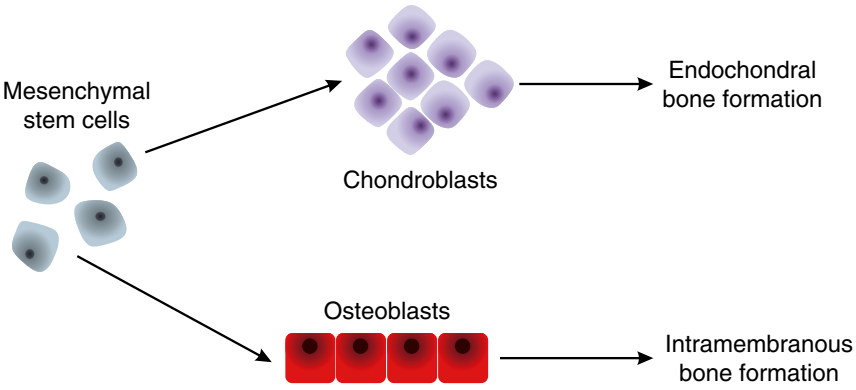

Fig. 5.3 Types of bone formation

7. *What are the regulators of calcium homeostasis*?

Intracellular calcium is 10,000 times lower than serum ionized calcium, and normal levels of both serum and intracellular calcium are required for neuromuscular excitability and cardiac contractility. Therefore, minute-to-minute regulation of serum calcium is essential for maintenance of these vital functions. Normal levels of serum calcium are maintained by coordinated action of parathyroid hormone (PTH), $1,25(OH)_2D$, and possibly, FGF-23 and calcitonin. PTH is the prime regulator of serum calcium, and the circulating level of calcium in turn regulates PTH secretion by acting through calcium-sensing receptors (CaSR) present in the parathyroid gland. PTH directly promotes bone resorption, increases renal reabsorption of calcium in distal convoluted tubule (DCT), and facilitates intestinal calcium absorption by stimulation of renal 1α-hydroxylase activity in proximal convoluted tubule (PCT). Serum calcium also regulates its own excretion independent of PTH by acting through calcium-sensing receptor present in thick ascending limb of loop of Henle (TALH) in the kidney. FGF-23 inhibits 1α-hydroxylase activity and possibly suppresses PTH secretion. The role of calcitonin in calcium homeostasis is uncertain in humans; however, it inhibits bone resorption and increases calcium excretion. Further, these hormones also maintain the steep gradient between extracellular and intracellular calcium levels by regulation of the activity of calcium exchange pumps present on cell membranes (Fig. 5.4).

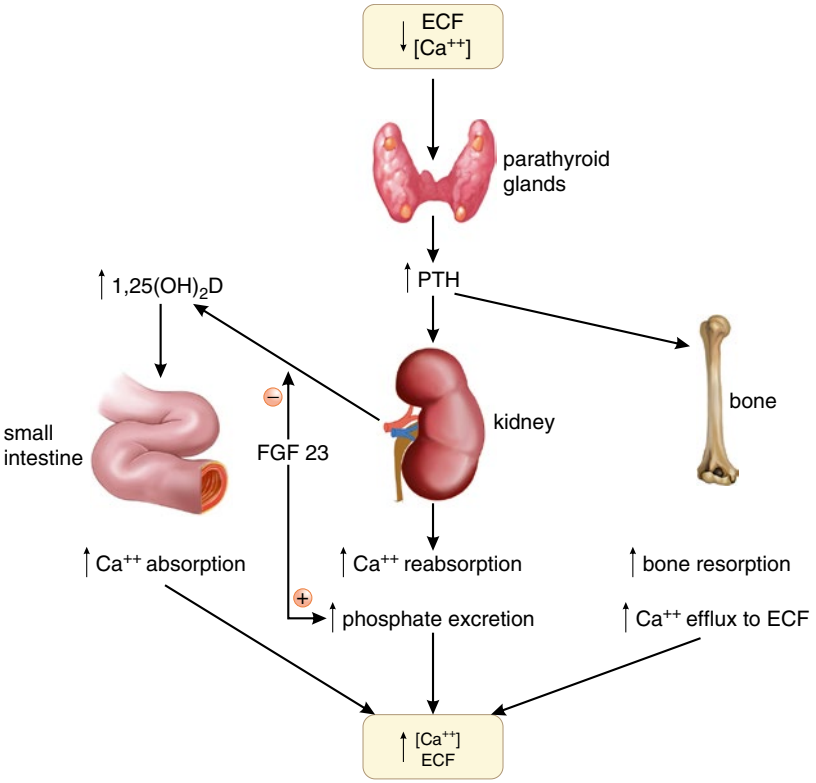

Fig. 5.4 Regulation of calcium homeostasis

8. *How is serum calcium maintained in the normal range*?

Calcium absorption from the intestine, resorption from the bone, and reabsorption from the kidney are tightly regulated to maintain serum calcium within the normal range. Only 20–30 % of the ingested calcium is reabsorbed from the upper intestine (duodenum and upper jejunum). Of the total calcium absorbed, passive absorption accounts for only 8–23 %, while $1,25(OH)_2D$-mediated active absorption contributes to the rest. PTH-mediated bone remodeling results in exchange of 200–500 mg of calcium between extracellular fluid and bone per day. In the kidney, 98 % of the filtered calcium is reabsorbed and the rest is excreted in urine. Majority of the filtered calcium is passively reabsorbed in proximal convoluted tubules (60–70 %) and thick ascending limb of loop of Henle (TALH, 20 %), whereas PTH-mediated active reabsorption in distal convoluted tubule and collecting ducts contribute to the rest (8–10 %). Calcium reabsorption in TALH is regulated by CaSR, independent of PTH and $1,25(OH)_2D$. FGF-23 also contributes to calcium homeostasis through the regulation of 1α-hydroxylase activity in the proximal convoluted tubule (Fig. 5.5).

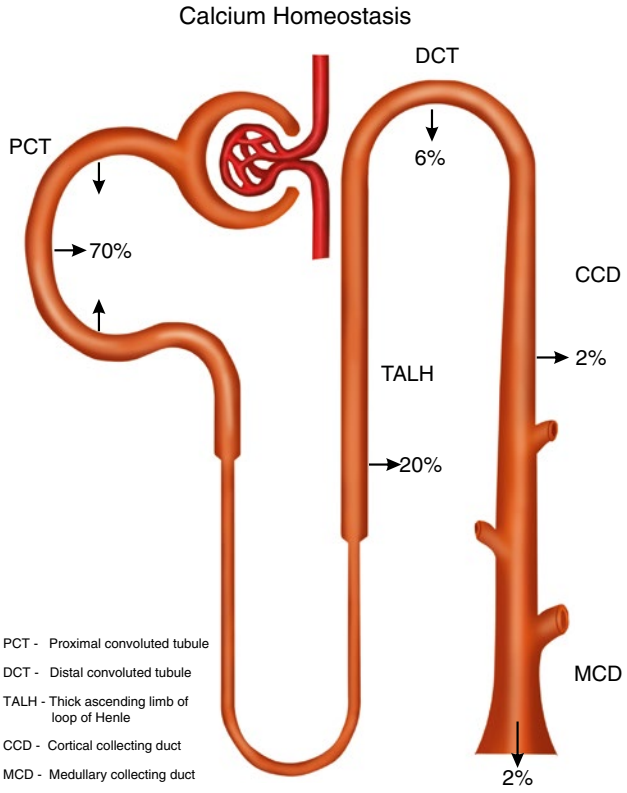

Fig. 5.5 Sites of calcium reabsorption from the kidney

9. *What are the regulators of phosphorus homeostasis*?

   The level of phosphate in the intracellular compartment is one to two times higher than in the extracellular fluid. The regulators of phosphate homeostasis are $1,25(OH)_2D$, PTH, and FGF-23. $1,25(OH)_2D$ increases the reabsorption of phosphate from the gut. PTH and FGF-23 promote the excretion of phosphate by decreasing the renal tubular reabsorption of phosphate. The effect of PTH is immediate, whereas that of FGF-23 takes several hours. However, PTH and FGF-23 have opposing effects on renal 1α-hydroxylase activity; PTH stimulates it, whereas FGF-23 inhibits it. PTH promotes bone remodeling and hence maintains the phosphate exchange between bone and extracellular fluid. The role of other phosphatonins apart from FGF-23 in phosphate homeostasis is not precisely defined.

10. *How is serum phosphorus maintained in the normal range*?

    Approximately 90 % of ingested phosphorus is absorbed from the upper intestine (duodenum and jejunum). Out of this, 60–70 % is passively absorbed and

$1,25(OH)_2D$-mediated active absorption contributes to the rest. The kidney is the prime organ involved in phosphate homeostasis, and it is accomplished by modifying renal phosphate excretion. Eighty-five to 90 % of the filtered phosphate is reabsorbed and the rest is excreted in urine. Out of this, approximately 85 % is reabsorbed in PCT, while the rest in distal tubules. Phosphate reabsorption is mediated through active transport via sodium–phosphorus co-transporters present at these sites (Na-Pi 2a and 2c). PTH inhibits the expression of Na-Pi 2a in PCT, while FGF-23 inhibits the expression of Na-Pi 2a and 2c in the PCT. PTH also inhibits the reabsorption of phosphate in distal convoluted tubule (Fig. 5.6).

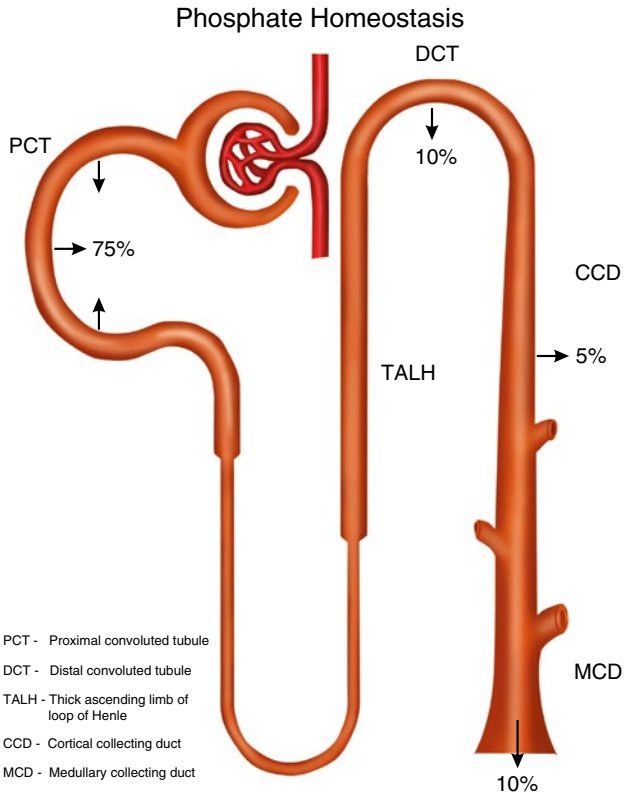

Fig. 5.6 Sites of phosphate reabsorption from the kidney

11. *What are the differences between calcium and phosphate homeostasis*?

The differences between calcium and phosphate homeostasis are enlisted in the table given below.

Parameters	Calcium	Phosphorous
Distribution		
Bone	99 %	85 %
Non-osseous tissue	<1 %	15 %
Extracellular: intracellular	10,000:1	1:1–2
Protein binding	50 %	12 %
Diurnal variation	Almost nil	Nadir between 0800 and 1100h
Influence of meal on serum level	No change	Decrease
Intestinal absorption	Duodenum and jejunum	Duodenum and jejunum
Active	70–90 %	20–30 %
Passive	8–20 %	60–70 %
Renal reabsorption		
PCT	60–70 %	85 %
TALH	20 %	Nil
DCT and CT	8–10 %	15 %
Key organs in homeostasis	Bone and intestine	Kidney
Prime hormonal regulator	PTH and 1,25 $(OH)_2D$	PTH and FGF-23
Bone mineralization	Important	Essential

12. *What is the most important mineral for bone mineralization*?

Bone mineralization is a coordinated process which involves deposition of calcium, phosphate, and magnesium on matrix, laid down by osteoblasts. Optimal levels of serum calcium, phosphate, PTH, 1,25$(OH)_2D$, bone-specific alkaline phosphatase, pH, and FGF-23 are required for bone mineralization. The solubility product of calcium and phosphate is the major determinant of bone mineralization rather than the serum level of individual minerals, calcium, or phosphate. Minor alterations in serum phosphate concentration lead to marked variation in the solubility product, whereas minor alterations in serum calcium do not significantly influence the same. Hence, the most important metabolite for mineralization is phosphate not calcium. This is best evidenced in patients with hypophosphatemic osteomalacia, who have impaired mineralization despite normal serum calcium level.

13. *What is FGF-23*?

FGF-23 is a 251 amino acid peptide predominantly secreted by osteocytes. It is a major phosphatonin (possibly a misnomer as it is a phosphaturic hormone)

involved in phosphate homeostasis. It acts in association with its co-receptor klotho and inhibits the translocation of intracellular sodium phosphorus co-transporter (NaPi 2a and 2c) to the cell membrane in proximal convoluted tubule, resulting in phosphaturia. In addition, it also inhibits renal 1 α-hydroxylase activity, thereby decreasing intestinal phosphate reabsorption. Further, FGF-23 possibly suppresses PTH secretion. Recently, it has been shown that FGF-23 has a role in calcium homeostasis by promoting calcium reabsorption in the distal tubule through upregulation of transient receptor potential vanilloid-5 (TRPV-5).

14. *What is klotho*?

"Klotho" is named after a Greek Goddess, who spins the thread of human life. Klotho is a gene that encodes a protein which is present in three forms; transmembrane, secreted, and soluble form. The transmembrane klotho is a membrane-bound form, while soluble and secreted klotho are present in circulation. Soluble klotho is a truncated form of the extracellular domain of transmembrane klotho, whereas secreted klotho represents the entire molecule. The transmembrane form is expressed in multiple tissues, especially in the kidney and acts as a co-receptor for FGF-23 and results in phosphaturia and suppression of renal 1α-hydroxylase and PTH. In addition, it has antiaging and anti-IGF1 effects. Secreted as well as soluble klotho may act alone or in concert with FGF-23 and has antioxidant, antiapoptotic, and anti-*wnt* signaling effects. FGF-23-klotho complex is possibly regulated by phosphate, PTH, and 1,25 $(OH)_2D$.

15. *How is vitamin D formed in the body*?

Endogenous vitamin D synthesis occurs in the Malpighian layer of epidermis on exposure to ultraviolet B rays (wave length 290–315 nm). The cutaneous synthesis contributes to 80 % of circulating vitamin D and the rest is provided by diet. On exposure to sunlight, 7-dehydrocholesterol is converted to pre-vitamin D_3 which rapidly photoisomerizes to vitamin D_3 (cholecalciferol). This form of vitamin D is biologically inactive and requires hepatic and renal hydroxylations to form 25-hydroxy vitamin D_3 [25(OH)D] and 1,25-dihydroxy vitamin D_3 [1,25$(OH)_2D$], respectively. The enzyme 1α-hydroxylase involved in renal hydroxylation is tightly regulated. 1,25$(OH)_2D$ is considered as the active form of vitamin D as the affinity of 1,25$(OH)_2D$ to vitamin D receptor (VDR) is 500- to 1000-fold higher as compared to 25(OH)D. However, the serum concentration of 25(OH)D is about 500 times higher than that of 1,25$(OH)_2D$, hence biological activity of 25(OH)D cannot be excluded. The synthesis of vitamin D is summarized in the figure given below (Fig. 5.7).

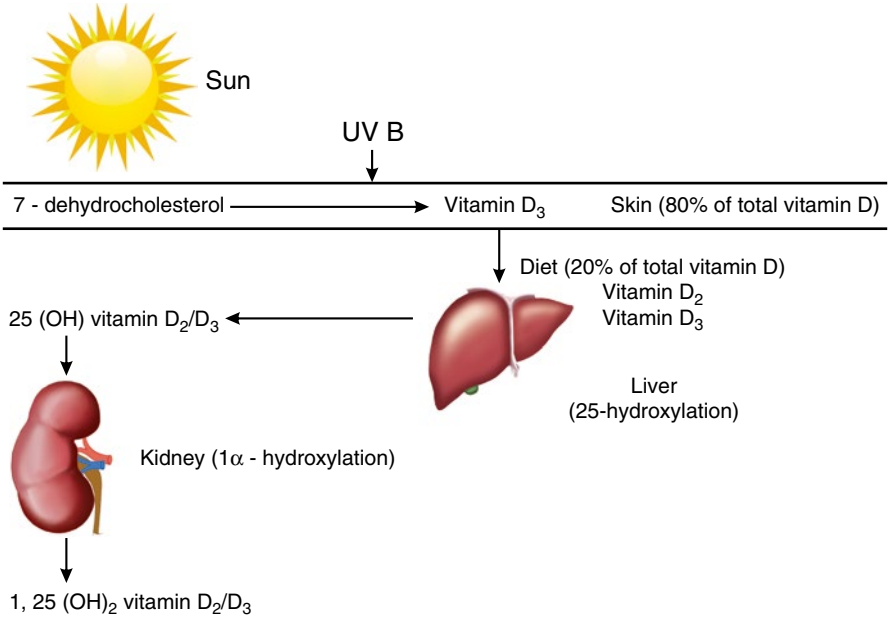

Fig. 5.7 Vitamin D biosynthesis

16. *Why is vitamin D considered as a hormone*?

Vitamin D is not truly a vitamin, but it is a steroid and qualifies the criteria for a hormone. These include endogenous production (Malpighian layer of epidermis), direct release into circulation [1,25$(OH)_2$D release from kidney], presence of binding protein (vitamin D-binding protein), action through a receptor, existence of a feedback system [between 25(OH) vitamin D and 1,25$(OH)_2$D], and site of action distal to organ of production (kidney, intestine, and bone).

17. *How is vitamin D important in bone physiology*?

1,25$(OH)_2$D, the active form of vitamin D, plays an important role in bone health. It is the prime regulator of calcium and phosphorus absorption from small intestine. 1,25$(OH)_2$D by increasing the synthesis of osteocalcin promotes maturation of mineralized matrix. In addition, it activates the differentiation of osteoclast precursors and thereby promotes bone remodeling. Although vitamin D is important for bone mineralization, it is not essential as evidenced by healing of rachitic lesions in patients with inactivating mutations of vitamin D receptor (vitamin D-resistant rickets type 2) after treatment with intravenous calcium.

18. *What are the non-osseous effects of vitamin D*?

Vitamin D plays an important role in the maintenance of bone health. In addition, it also has been demonstrated to have various non-osseous effects, which include improvement of muscle strength and cardiovascular health, modulation of immune system and glucose–insulin homeostasis, and antiproliferative effects on various tissues including breast, prostate, and colon. Although the role of vitamin D in muscle function is well established, data regarding other non-osseous benefits are not so robust.

19. *How does vitamin D deficiency cause myopathy*?

Proximal myopathy is one of the common manifestations of vitamin D deficiency, although it is more common in children than in adults. Vitamin D is required for ATP-dependent calcium uptake into sarcoplasmic reticulum, synthesis of muscle proteins (e.g., actin, troponin), and differentiation of myogenic stem cells into myoblasts. Further, vitamin D deficiency has been shown to be associated with atrophy of type 2 muscle fibers. Concurrent hypophosphatemia associated with vitamin D deficiency also contributes to myopathy. Secondary hyperparathyroidism consequent to vitamin D deficiency also adds to proteolysis and atrophy of type 2 muscle fibers. Therefore, patients with vitamin D deficiency manifest with reduced muscle strength and increased tendency to fall. The latter is due to atrophy of type 2 muscle fibers as these fast-twitching fibers need to be recruited on sudden change of posture to prevent a fall. Treatment with vitamin D (cholecalciferol) has been shown to improve muscle strength and reduce the incidence of falls.

20. *What is metabolic bone disease*?

Metabolic bone disease refers to heterogeneous group of disorders characterized by abnormalities of bone mineral metabolism, bone cells, or matrix.

21. *What is growth plate*?

The growth plate, also known as physis, is present between the epiphysis and metaphysis at the ends of long bones. It comprises of five zones: resting zone, proliferative zone, hypertrophic zone, calcification zone, and ossification zone, from epiphysis to metaphysis. The process of linear growth initiates at the epiphyseal end of growth plate and new bone is laid down at the metaphysis, resulting in new bone formation at the metaphyseal end of the long bone (Fig. 5.8).

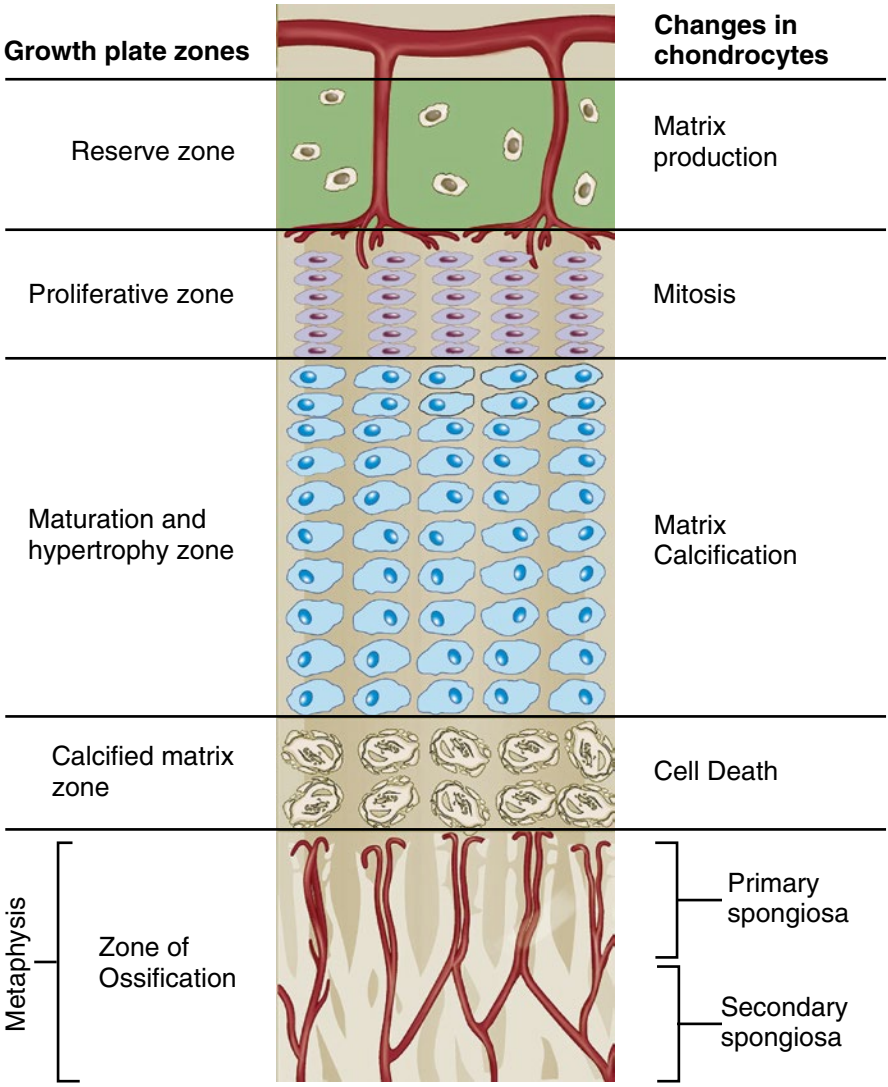

Fig. 5.8 Different zones of growth plate

22. *What is osteomalacia*?

Osteomalacia is a disorder characterized by defective mineralization of osteoid. In normal physiology, mineralization of osteoid requires optimal calcium-phosphate solubility product, alkaline pH (7.6) at mineralization site, and presence of alkaline phosphatase (which suppresses the inhibitors of mineralization). Therefore, abnormalities in mineral homeostasis, presence of acidic pH (e.g., chronic kidney disease), and hypophosphatasia result in osteomalacia.

23. *What is rickets*?

Rickets is a disorder of epiphyseal growth plate characterized by defective development and impaired mineralization of growth plate as a result of abnormal mineral homeostasis. The defective development of epiphyseal growth plate is due to lack of apoptosis of hypertrophic chondrocytes, which is essential for invasion by the bone cells (osteoblasts and osteoclasts) for the new bone formation. Impaired apoptosis is the result of hypophosphatemia, as optimal levels of serum phosphate are required for caspase-9-mediated apoptosis of hypertrophic chondrocytes. Failure of removal of hypertrophic chondrocytes results in secondary defect in osteoid synthesis and hence impaired mineralization. Besides the involvement of epiphyseal growth plate, there is a generalized defective mineralization (osteomalacia) of bone matrix in rickets.

24. *What is the difference between rickets and osteomalacia*?

The difference between rickets and osteomalacia are summarized in the table given below.

Parameters	**Rickets**	**Osteomalacia**
Site of involvement	Growth plate and bone matrix	Bone matrix
Deformities	Common (genu valgum, genu varum)	Uncommon (bowing)
Presentation	Bone pain, deformities, and poor linear growth	Bone pain, fractures
Fractures	Uncommon	Common
Radiology	*Epiphysis*: indistinct and irregular margins	Cortical thinning
	Physis: widening of growth plate	Looser's zone
	Metaphysis: cupping, fraying, and splaying	Osteopenia
	Diaphysis: cortical thinning, Looser's zone	Triradiate pelvis

25. *How to classify rickets*?

Rickets can be classified as vitamin D-deficient, vitamin D-dependent, or vitamin D-resistant. In addition, rickets can also be classified on the basis of abnormalities of mineral homeostasis as calcipenic or phosphopenic rickets. Since hypophosphatemia is considered as the common denominator for all types of rickets, a new classification based on the mechanism of hypophosphatemia, i.e., PTH-dependent and FGF 23-dependent has been recently proposed.

26. *How does vitamin D deficiency cause rickets*?

Although the most common cause of rickets is vitamin D deficiency, neither vitamin D nor its receptor (VDR) is directly involved in the development of rickets. This is evidenced in *VDR* knockout mice where administration of intravenous calcium and phosphate is associated with healing of rachitic lesions. Vitamin D deficiency is associated with hypophosphatemia, low normal calcium, and secondary hyperparathyroidism. Decreased calcium

phosphate solubility product results in impaired mineralization and consequently rickets–osteomalacia.

27. *How to classify vitamin D-related rickets*?

Rickets can be classified as vitamin D-deficient or vitamin D-dependent on the basis of serum 25(OH)D levels and therapeutic response to calciferol (ergocalciferol or cholecalciferol) and/or calcitriol. Vitamin D deficiency is characterized by low levels of 25(OH)D and excellent therapeutic response to calciferol. Vitamin D-dependent rickets (VDDR) can be due to inherited deficiency of 1α-hydroxylase enzyme (VDDR type 1) or inactivating mutations in vitamin D receptor (VDDR type 2). The differences among various forms of vitamin D-related rickets are summarized in the table given below.

Parameters	**Vitamin D deficiency**	**VDDR type 1**	**VDDR type 2**
Synonyms	–	Pseudo-vitamin D-deficiency rickets	Hereditary vitamin D-resistant rickets
Age of presentation	3–18 months	Infancy	Infancy
Mode of inheritance	Sporadic	Autosomal recessive	Autosomal recessive
Pathophysiology	Deficiency of vitamin D	Deficiency of renal 1α-hydroxylase	Defective vitamin D receptor
Associated features	Nil	Nil	Alopecia
Serum calcium	Low/low normal	Low	Low
Serum phosphorus	Low	Low	Low
ALP	Elevated	Elevated	Elevated
iPTH	Elevated	Elevated	Markedly elevated
25 (OH)D level	Low	High	Normal
1,25 $(OH)_2D$ level	Normal/high	Very low	Very high
Response to calciferol	Excellent	No response	No response
Response to calcitriol	Good	Excellent	Most children are resistant to therapy even with high dose

28. *What is vitamin D-resistant rickets*?

The term vitamin D-resistant rickets encompasses disorders associated with rickets that are nonresponsive to therapy with optimal doses of calciferol. The causes of vitamin D-resistant rickets include hypophosphatemic rickets/osteomalacia, chronic renal failure, and renal tubular acidosis. VDDR type 1 and VDDR type 2 are also considered as vitamin D-resistant rickets. Presence of vitamin D-resistant rickets should be suspected if there is failure of appearance of line of provisional calcification after 3 months of optimal therapy (600,000 IU) with vitamin D (Fig. 5.9).

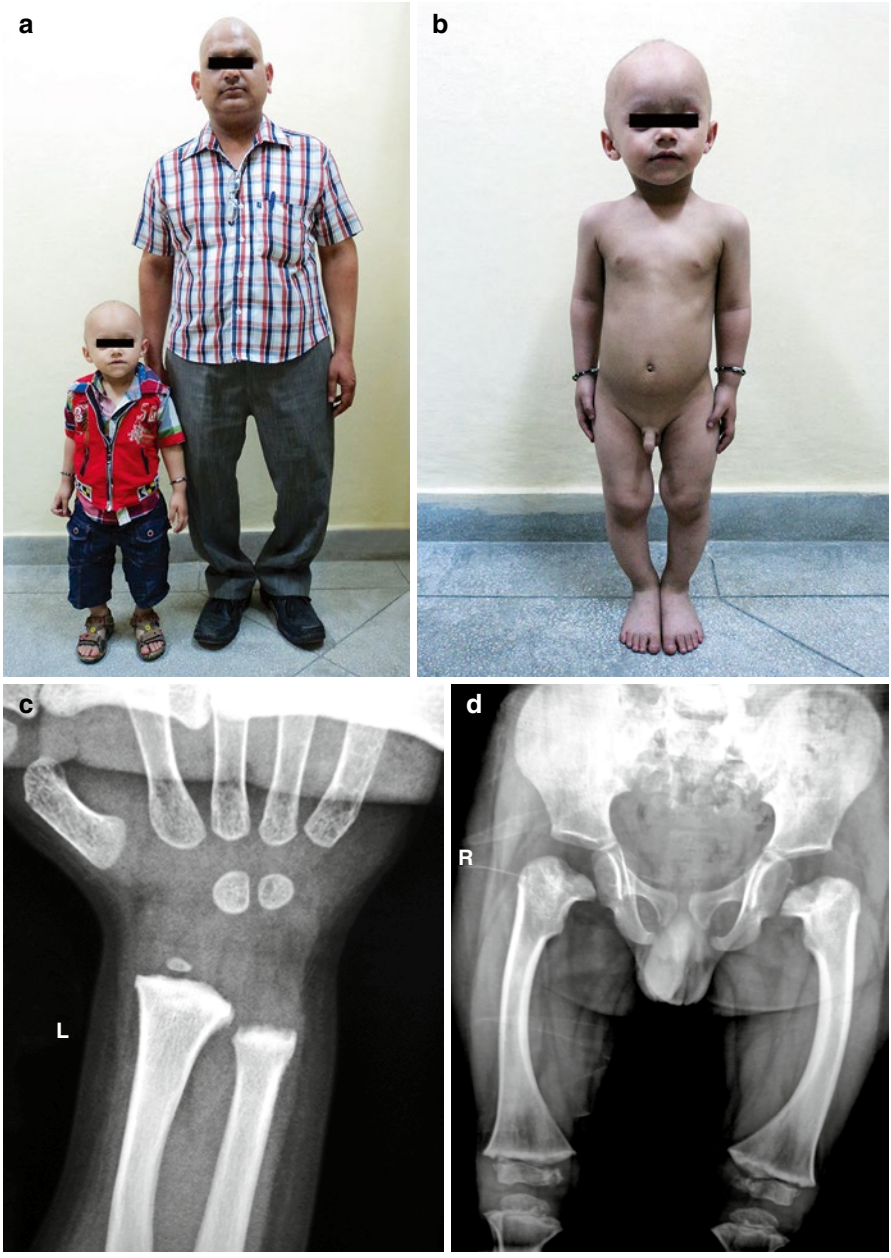

Fig. 5.9 (**a**) Familial vitamin D-dependent rickets type 2. (**b**) Genu varum and alopecia in the same child. (**c**, **d**) Plain radiographs of the wrist and lower limbs showing fraying and splaying of metaphysis, indistinct and irregular epiphysis, femoral bowing, and cortical thinning

29. *How to differentiate between calcipenic and phosphopenic rickets*?

Rickets can be classified on the basis of primary abnormality in mineral homeostasis as calcipenic or phosphopenic. Calcipenic rickets is due to calcium/vitamin D deficiency or impaired vitamin D action, whereas phosphopenic rickets is primarily due to hypophosphatemia as a consequence of renal phosphate wasting. The differences between calcipenic and phosphopenic rickets are summarized in the table given below.

Parameters	Calcipenic rickets	Phosphopenic rickets
History	Inadequate sun exposure	Family history of rickets/ osteomalacia
	Poor dietary calcium intake	
	Malabsorption	
	Anticonvulsant therapy	
Growth plate abnormalities	Less severe	More severe
Deformities	Less severe	More severe
Enthesopathy	Absent	Present
Myopathy	Present	Absent[a]
Bone pain	Present	Absent[a]
Dental abscess	Absent	Present
Enamel hypoplasia	Present	Absent
Tetany	May be present	Absent
Seizures	May be present	Absent
Osteopenia	May be present	Absent
Osteitis fibrosa cystica	May be present	Absent
Calcium	Low/low to normal	Normal
Phosphorous	Low/normal	Very low
Alkaline phosphatase	Markedly elevated	Elevated
Serum PTH	Markedly elevated	Normal to mildly elevated

[a]Except in tumor-induced osteomalacia (TIO)

30. *What are the clinical features of rickets*?

The clinical features of rickets are enlisted in the table given below.

Site	Clinical features
Skull	Craniotabes
	Frontal bossing
Dentition	Delayed tooth eruption
	Enamel hypoplasia
Extremities	Widening of wrist
	Genu valgum, genu varum
	Windswept deformity
	Double malleolus
	Saber shin (anterior convexity of tibia)

Site	Clinical features
Thorax	Rachitic rosary
	Harrison's sulcus
	Pectus carinatum
Spine and pelvis	Kyphosis
	Scoliosis
	Lumbar lordosis
Neuromuscular	Hypotonia, pot belly
	Delayed motor development
	Tetany, seizures

31. *How does the age of onset of rickets help in the differential diagnosis of rickets/osteomalacia*?

The age of onset of rickets/osteomalacia is an important clue for differential diagnosis as different disorders typically manifest at specific ages. These are summarized in the table given below.

Age	Etiology	Remarks
Infancy	Vitamin D deficiency	Exclusively breast-fed infants
	Vitamin D-dependent rickets type 1	Hypocalcemic tetany
	Vitamin D-dependent rickets type 2	Alopecia
	Hypophosphatasia	Craniosynostosis
		Low alkaline phosphatase
		Hypercalciuria
Childhood	Vitamin D deficiency	Nutritional
		Malabsorption
		Celiac disease
	Renal rickets	Renal tubular acidosis
		Proximal RTA
		Fanconi's syndrome
		Isolated
		Distal RTA
		Chronic kidney disease
	Familial hypophosphatemic rickets	X-linked variant
		Enthesopathy
		Fractures Dental caries/dental abscess
	Hypophosphatasia	Low alkaline phosphatase
Adult onset	Vitamin D deficiency	Bone pain, pseudo-fracture
	Tumor-induced osteomalacia	Severe hypophosphatemia
	Familial hypophosphatemic rickets	Autosomal dominant variant
	Hypophosphatasia	Stress fractures
		Osteoarthritis
		Low alkaline phosphatase

32. *What are the manifestations of vitamin D deficiency in a newborn*?

A neonate born to vitamin D-sufficient mother has vitamin D stores for 8–12 weeks as a result of transplacental passage of 25(OH)D. Therefore, manifestations of vitamin D deficiency are seen only in neonates born to severely vitamin D-deficient mother. These include craniotabes, wide and open fontanelle, rachitic rosary, widening of growth plate, and osteopenia. Craniotabes is a manifestation of intrauterine vitamin D deficiency as the skull bones grow rapidly during intrauterine period and early infancy.

33. *What are the manifestations of vitamin D deficiency during infancy*?

Human breast milk is a poor source of vitamin D and contains approximately 25 IU/L. Hence, infants who are exclusively breast fed for a prolonged duration are at risk for the development of severe vitamin D deficiency, unless the mother is supplemented with supraphysiological doses of vitamin D (4000–6500 IU/day). Peak age of presentation of vitamin D deficiency is 3–18 months, and the common manifestations are growth failure, irritability, lethargy, delayed dentition, recurrent respiratory tract infections, delayed motor milestones, hypotonia (floppy infant), and hypocalcemic seizures. Onset of rickets during infancy manifests as wrist deformities and rachitic rosary due to rapid growth of upper limb and rib cage during this period, respectively. Further, the wrist deformities become more evident as the child starts crawling and commonly involve the ulnar side of wrist, due to the rapid growth of distal ulna as compared to radius.

34. *What are the manifestations of vitamin D deficiency during preschool period and adolescence*?

In a growing child, vitamin D deficiency manifests with deformities in lower limbs due to rapid growth and weight bearing. These include genu valgum, genu varum, windswept deformity, and anterior bowing of legs. In addition, proximal myopathy is a common manifestation of vitamin D deficiency in children and adolescence and manifests with waddling gait, even without any deformity. Chest deformities associated with vitamin D deficiency include pectus carinatum, pectus excavatum, rachitic rosary, and Harrison's sulcus. Looser's zone and vertebral involvement are usually a feature of adult osteomalacia, but presence of these features in a child suggests severe disease.

35. *What are the nonskeletal manifestations of vitamin D deficiency*?

The nonskeletal manifestations of vitamin D deficiency include carpopedal spasm, hypotonia, delayed motor milestones, delayed dentition, enamel hypoplasia, proximal myopathy, seizures, and hypocalcemic cardiac failure. Hypocalcemia due to vitamin D deficiency commonly manifests during infancy and adolescence because of increased demand of calcium for rapidly growing skeleton during this period.

36. *Why all patients with vitamin D deficiency do not manifest rickets/osteomalacia*?

Although vitamin D deficiency is rampant, clinical manifestations do not occur in all patients. There is a poor correlation between serum vitamin D levels and clinical manifestations. The exact cause for this dichotomy is not clear; the possible reasons include vitamin D receptor polymorphisms, normal $1,25(OH)_2D$ level despite vitamin D deficiency, and inappropriate PTH response to hypocalcemia. In addition, diagnosis of vitamin D deficiency based on total 25(OH)D levels, rather than "free" 25(OH)D levels, may also account for this disparity between clinical symptoms and vitamin D levels. Further, some patients with vitamin D deficiency may only have abnormal bone histomorphometry (subclinical osteomalacia).

37. *What is genu varum*?

In Latin, the words *genu* means knee and *varus* denotes deformity involving oblique displacement of part of a limb toward the midline. Genu varum is also called as "bowed legs." Genu varum may be physiological till 2 years of age and resolves spontaneously by next 1–2 years. The onset of genu varum after the age of 3 years or presence of asymmetrical deformity, rapid progression (>1.5 cm within 6 months), bone pain, or difference in leg length should raise a suspicion for pathologic causes like rickets (vitamin D deficiency, hypophosphatemic rickets/osteomalacia, renal tubular acidosis), skeletal dysplasias (achondroplasia, metaphyseal chondrodysplasia), and osteogenesis imperfecta.

38. *What is genu valgum*?

In Latin, the words *genu* means knee and *valgus* denotes deformity involving oblique displacement of part of a limb away from the midline. Genu valgum is also called as "knock knees." Genu valgum may be physiological between 3 and 7 years of age and resolves spontaneously thereafter. However, onset of genu valgum <3 years or >7 years of age or presence of asymmetrical deformity, rapid progression (>1.5 cm within 6 months), bone pain, or difference in leg length should raise a suspicion for pathologic causes like rickets (vitamin D deficiency, hypophosphatemic rickets/osteomalacia, renal tubular acidosis, renal osteodystrophy), osteochondrodysplasias, osteogenesis imperfecta, or poliomyelitis. However, the most common cause of pathologic genu valgum is post-traumatic.

39. *What is windswept deformity*?

Presence of genu valgum in one lower limb and genu varum in the contralateral limb is called as windswept deformity. The common causes of windswept deformity include severe vitamin D deficiency, hypophosphatemic rickets/osteomalacia, renal tubular acidosis, renal osteodystrophy, and hypophosphatasia.

40. *How to examine for genu valgum and genu varum*?

Genu varum is determined by the measurement of intercondylar distance. Examination can be performed in sitting or supine posture with both the lower limbs kept in straight position. Patient is asked to approximate the malleoli, and intercondylar distance is measured thereafter with a metallic tape. A distance of >5–6 cm is suggestive of genu varum. Genu valgum is determined by the measurement of intermalleolar distance. Examination can be performed in the same position as for genu varum. Patient is asked to approximate the femoral condyles and intermalleolar distance is measured with a metallic tape. A distance of >8–10 cm is suggestive of genu valgum.

41. *What are the radiological manifestations of rickets*?

Rickets is a metabolic bone disease that involves epiphysis, physis (growth plate), metaphysis, and diaphysis.

Site	Features
Epiphysis	Irregular margins
Physis	Widening along longitudinal axis
Metaphysis	Fraying, cupping, splaying
	Coarse trabecular pattern
Diaphysis	Osteopenia, cortical thinning, Looser's zone

The other radiological findings include deformities in the extremities (e.g., bowing), rachitic rosary, slipped capital femoral epiphysis, triradiate pelvis, and wormian bones (Fig. 5.10).

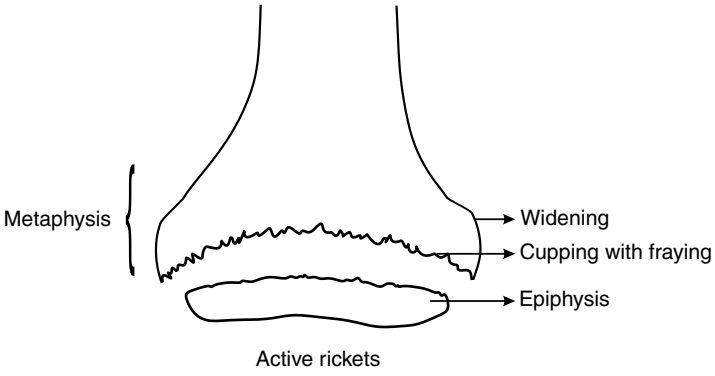

Fig. 5.10 Diagrammatic illustration of metaphyseal changes in rickets

42. *How to explain the radiological abnormalities in rickets*?

The earliest radiologic feature in rickets is appearance of indistinct margins and fraying of metaphysis. This is due to impingement of metaphysis along the longitudinal axis by proliferating hypertrophic chondrocytes. The unrestricted proliferation of hypertrophic chondrocytes results in expansion of growth plate which is visualized as increased distance between epiphysis and metaphysis. Later, weight bearing on unmineralized osteoid at metaphysis leads to splaying and cupping of metaphysis. These alterations in growth plate (physis) and metaphysis clinically manifest as widening of end of long bones, typically at wrist. The changes in the diaphysis and epiphysis reflect poor mineralization due to low calcium-phosphorus solubility product.

43. *What is pseudo-fracture*?

Pseudo-fractures or Looser's zone is visualized as a thin transverse band of rarefaction oriented perpendicular to the long-axis of bone. They are characteristic of rickets/osteomalacia; however, they can also be seen in Paget's disease and osteogenesis imperfecta. Looser's zone occurs due to mechanical stress of arterial pulsations on poorly mineralized bone and represents cortical stress fractures, which are filled with poorly mineralized callus, osteoid, and fibrous tissue. These lesions heal with optimal therapy of underlying disease.

44. *What are the differences between true fracture and pseudo-fracture*?

The differences between true fracture and pseudo-fractures are summarized in the table given below.

Parameters	**True fracture**	**Pseudo-fracture**
History of trauma	Usually present	Absent
Symmetry	Usually unilateral	Bilateral and symmetrical
Sites	Any	Inner margin of femoral neck Axillary margin of scapula
		Pubic and ischial rami
		Ribs
Involvement of bone	Through and through	Incomplete
Direction	Can be oblique/ perpendicular	Perpendicular to the long axis of bone
Visible callus (on X-ray)	Present	Absent

45. *What is bone histomorphometry*?

Bone histomorphometry is the measurement and analysis of bone structure and remodeling. This requires transiliac bone biopsy and histological examination of undecalcified bone. The parameters which are examined for bone structure include trabecular width, cortical width, and trabecular volume. The bone remodeling parameters may be static or dynamic; static parameters include osteoid volume and osteoid thickness, while the dynamic parameters include mineralization lag time and mineral apposition rate. Double tetracycline labeling is required for the assessment of dynamic parameters of bone remodeling. Normal values for some of the commonly used histomorphometric parameters are summarized in the table given below.

Parameters	**Male**	**Female**
Cortical thickness	915 μm	823 μm
Cancellous bone volume	19.7 %	21.8 %
Osteoid thickness	11.1 μm	12.3 μm
Osteoid volume	3.19 %	1.58 %
Mineral apposition rate	0.89 μm/day	0.88 μm/day
Mineralization lag time	27.6 days	21.1 days

46. *What are the indications for bone histomorphometry*?

Bone histomorphometry is indicated in patients with unexplained low bone mineral density or unexplained fractures. In addition, patients with renal osteodystrophy also require bone histomorphometry for evaluation of bone pain, unexplained fractures, or before initiation of anti-osteoporotic therapy.

47. *What are the characteristic findings of rickets/osteomalacia on bone histomorphometry*?

The histomorphometric characteristics of osteomalacia include osteoid volume >15 %, osteoid thickness >20 μm, and mineralization lag time>100 days.

48. *How to approach a patient with rickets–osteomalacia*?

A detailed history and clinical examination usually provide clues to the diagnosis in patients with rickets/osteomalacia. Biochemical investigations include serum calcium, albumin, phosphorous, alkaline phosphatase, parathyroid hormone, 25(OH)D and renal function tests. The results of these investigations help to guide further evaluation and management. An approach to a patient with rickets–osteomalacia is given below (Fig. 5.11).

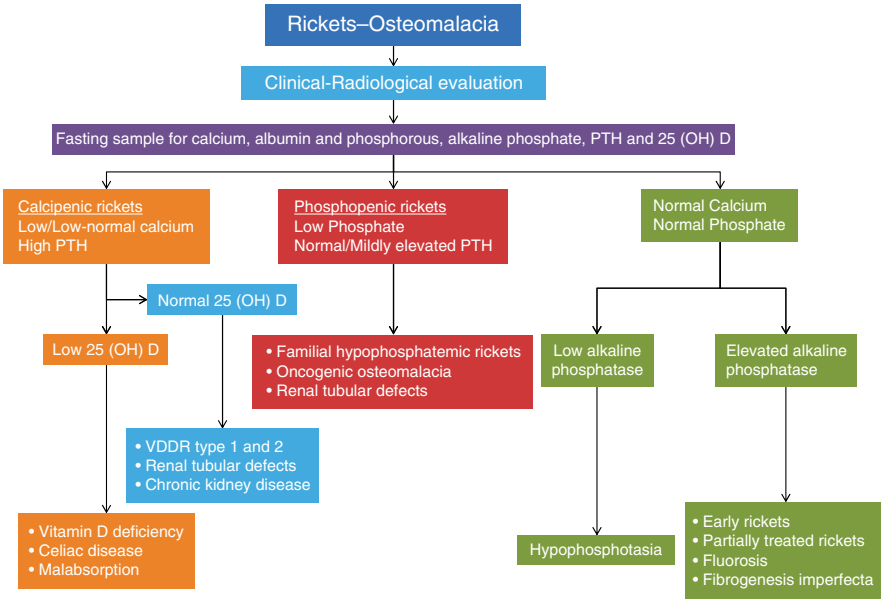

Fig. 5.11 Approach to a patient with rickets–osteomalacia

49. *How to diagnose vitamin D deficiency*?

Vitamin D deficiency/insufficiency is defined on the basis of serum 25(OH)D levels. This is because 25(OH)D levels are stable (half-life of 2–3 weeks) and precisely reflect the vitamin D status of an individual. Serum $1,25(OH)_2D$ is not used to define the vitamin D status of an individual because vitamin D deficiency is associated with normal/high levels of $1,25\ (OH)_2D$ as a consequence of secondary hyperparathyroidism. There is also high variability in the levels of $1,25(OH)_2D$, as it has a short half-life of 4hours. $1,25(OH)_2D$ is highly lipophilic, is labile, and circulates at a very low concentration. Further, assays for $1,25(OH)_2D$ are technically challenging because of difficulties in extraction of $1,25\ (OH)_2D$ from its binding proteins.

50. *How was the cutoff for defining vitamin D deficiency derived*?

Various cutoffs have been proposed to define vitamin D deficiency by different organizations based on clinical and biochemical parameters, including fracture, serum alkaline phosphatase, PTH, intestinal calcium absorption, and bone mineral density. Currently, the level of 25(OH)D at which iPTH starts rising is used to define vitamin D deficiency. Various studies have shown that 25(OH)D level <30 ng/ml is associated with rise in serum iPTH, and a level <20 ng/ml is associated with impaired intestinal calcium absorption. Hence, 25(OH)D level <30 ng/ml constitutes vitamin D insufficiency, and a level <20 ng/ml constitutes vitamin D deficiency.

51. *What are the stages of vitamin D deficiency*?

Depending on the alterations in mineral homeostasis, vitamin D deficiency may be classified into three stages, stage 1–3 as depicted in the table given below.

Stages	Serum calcium	Phosphorus	ALP	25(OH)D	iPTH	$1,25(OH)_2D$	Radiography
Stage 1	↓	Normal	↑	↓	↑	Normal	Osteopenia
Stage 2	Normal	↓	↑↑	↓↓	↑↑	↑	Mild rachitic changes
Stage 3	↓↓	↓↓	↑↑↑	↓↓↓	↑↑↑	Normal/↑/↓	Marked rachitic changes

52. *Who should be screened for vitamin D deficiency*?

Routine screening for vitamin D deficiency is not cost-effective, hence not recommended. However, individuals who are at risk for vitamin D deficiency should be screened by estimation of serum 25(OH)D; these include patients with chronic kidney disease, hepatic failure, malabsorption syndromes, patients on anticonvulsant or glucocorticoid therapy, morbidly obese individuals, and those undergoing bariatric surgery. In addition, estimation of 25(OH)D should also be performed in patients with rickets–osteomalacia, osteoporosis, hyperparathyroidism, granulomatous disorders, and unexplained hypercalcemia for the differential diagnosis and further management.

53. *What are the foods rich in vitamin D*?

The dietary sources of vitamin D are limited. Plant products are poor source of vitamin D and there are only limited sources of vitamin D of animal origin. Vitamin D obtained from plant sources contains ergocalciferol (vitamin D_2), while that of animal origin is cholecalciferol (vitamin D_3); both vitamin D_2 and D_3 are equipotent precursors for the synthesis of 25(OH)D. The vitamin D content of different foods is shown in the table given below.

Sources	Quantity (IU)
Cod liver oil (1 teaspoon)	400–1000
Salmon fish (100 g)	600–1000
Sardine fish (100 g)	300
Mackerel fish (100 g)	250
Egg yolk (per egg)	20
Human breast milk (1 l)	25
Cow milk (1 l)	4–40
Mushroom, sun-dried (100 g)	1600

54. *What is adequate sun exposure for vitamin D synthesis*?

Sunlight is the richest source for UV-B rays, which is required for endogenous vitamin D synthesis. Exposure to sunlight between 1000 and 1500h is recommended as the ratio of ultraviolet-B rays (UV-B) to UV-A rays is highest during this period. Exposure of whole body in a bathing suit to one minimal erythemal dose (MED) results in synthesis of approximately 10,000–20,000 IU of vitamin D_3, whereas exposure of 6–10 % of body (face and hands) to 0.5 MED provides 600–1000 IU of vitamin D_3. Minimal erythemal dose is defined as the amount of exposure to UV-B rays which results in persistent perceptible redness of skin, 24h after sun exposure. Redness which occurs immediately after sun exposure and disappears within 3–5h is mainly caused by heat and does not reflect adequate UV-B exposure. The approximate time for 1 MED is 4–10 min for white-skinned individuals and 60–90 min for dark-skinned individuals.

55. *How to treat vitamin D deficiency rickets–osteomalacia*?

Various regimens have been advocated to treat vitamin D deficiency rickets–osteomalacia. The recommended dose of vitamin D_3 (cholecalciferol) is depicted in the table given below. Vitamin D_2 (ergocalciferol) is as effective as vitamin D_3 in the treatment of vitamin D deficiency. These dosing schedules are aimed to achieve and maintain 25(OH)D levels >30 ng/ml.

Age group	Intensive phase therapy	Maintenance therapy
0–1 year	60,000 IU once weekly for 6 weeks	400–1000 IU/day
	or	
	2000 IU/day for 6 weeks	
1–18 years	60,000 IU once weekly for 6 weeks	600–1000 IU/day
	or	
	2000 IU/day for 6 weeks	
Adults	60,000 IU once weekly for 8 weeks	1500–2000 IU/day
	or	
	6000 IU/day for 8 weeks	

In addition to vitamin D supplementation, adequate intake of calcium must be ensured (30–75 mg/Kg/day of elemental calcium in three divided doses) to prevent hungry bone syndrome in children with rickets–osteomalacia.

56. *What is "stoss therapy"*?

"Stoss therapy" (*stossen* in German means *to push*) involves administration of massive doses of vitamin D to treat vitamin D deficiency rickets–osteomalacia, particularly in those patients whose compliance to therapy

cannot be ensured. Various dose schedules have been used and are shown in the table given below.

Intensive phase therapy	Maintenance therapy
1,00,000 IU of vitamin D_2 orally every 2hours for 12h (total dose 6,00,000)	400 IU/day after 3 months
or	
1,50,000–300,000 IU orally as a single dose	400 IU/day after 3 months
or	
6,00,000 IU intramuscularly as a single dose	400 IU/day after 3 months

57. *How to monitor a child with rickets on vitamin D therapy*?

After initiation of therapy, children with rickets should be monitored for efficacy and adverse effects of therapy. After 1 month of therapy, calcium profile (serum calcium, albumin, phosphorus, and alkaline phosphatase) should be monitored. The earliest response is improvement in serum phosphorus, which can be seen as early as 1–2 weeks, accompanied with rise in alkaline phosphatase. At 3 months of therapy, calcium profile, serum 25(OH)D, iPTH, urine calcium/creatinine ratio, and radiology should be performed. Optimal therapy usually results in resolution of biochemical and radiological abnormalities within 3 months. These patients should be followed at periodic intervals of 6 months, and 25(OH)D level must be estimated at 1 year, and annually thereafter.

58. *What are the radiological features of healing rickets*?

After initiation of therapy, a thin radiolucent line (a line of provisional calcification) appears adjacent to the metaphyseal end of long bone by 2–3 weeks, which represents calcification of chondroid matrix in the calcification zone of epiphyseal growth plate. This is followed by progressive mineralization of "cupping defect," which represents ossification of osteoid matrix present between line of provisional calcification and metaphyseal end of long bone. Slight cupping may remain as stigma of old rickets. The resolution of radiological abnormalities occurs within 3 months of optimal therapy (Fig. 5.12).

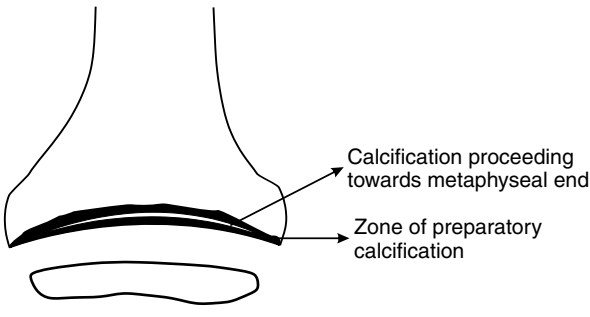

Fig. 5.12 Healing rickets

59. *Why is cholecalciferol/ergocalciferol and not calcitriol used for the treatment of vitamin D deficiency*?

Supplementation with cholecalciferol/ergocalciferol results in normalization of 25(OH)D levels, which is the precursor for 1,25 $(OH)_2D$. Therapy with calciferol results in regulated synthesis of 1,25$(OH)_2D$ (calcitriol) due to exquisite regulation of 1α-hydroxylase activity in PCT. In addition, 25(OH)D may also have direct effects on vitamin D receptor. Therapy with calcitriol does not normalize 25(OH) D levels, is expensive, requires frequent administration (as it has a half-life of 4h), and has the risk of iatrogenic hypercalcemia. Hence, cholecalciferol/ ergocalciferol is used for the treatment of vitamin D deficiency, rather than calcitriol.

60. *What are the indications for therapy with calcitriol*?

1,25$(OH)_2D$ is synthesized from 25(OH)D by the enzyme 1α-hydroxylase, which is present in the proximal convoluted tubule of the kidney. The activity of 1α-hydroxylase is stimulated by PTH and inhibited by FGF-23. Therefore, disorders associated with impaired 1α- hydroxylase activity or deficient secretion/ action of PTH or increased FGF-23 require calcitriol therapy. These include hypoparathyroidism, pseudohypoparathyroidism, advanced stage chronic kidney disease, and hypophosphatemic osteomalacia. In addition, vitamin D-dependent rickets type 1 (inactivating mutations of 1α-hydroxylase) and type 2 (vitamin D receptor defects) are indications for calcitriol therapy (Fig. 5.13).

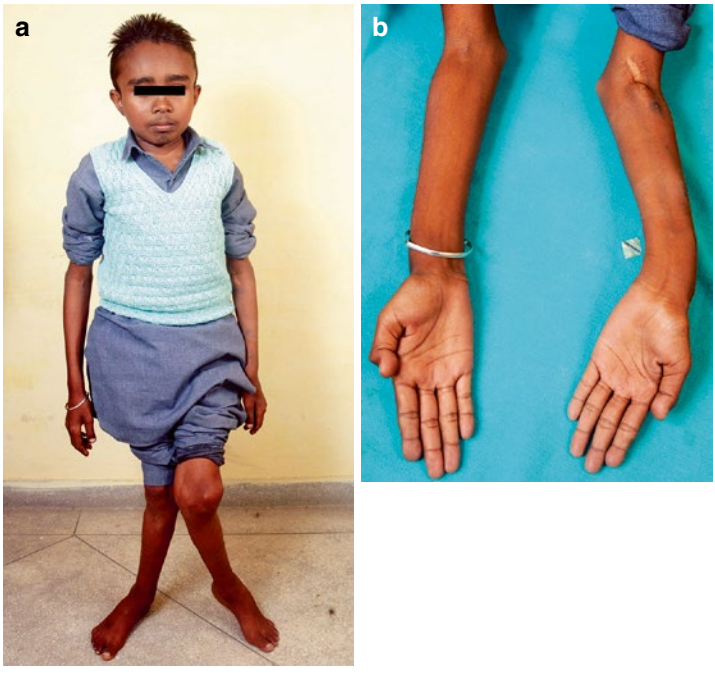

Fig. 5.13 (**a**) A 15-year-old boy with genu valgum and left knee joint deformity due to stage V chronic kidney disease (renal osteodystrophy). (**b**) Bowing of both forearms (L>R) in the same patient. Note the presence of A–V fistula

61. *An 8-year-old child was diagnosed to have chronic kidney disease* (*stage 3*). *His calcium profile was normal*, *25*(*OH*)*D 5 ng/ml and iPTH 600 pg/ml. Whether to treat this patient with calcitriol or cholecalciferol*?

Patients with chronic kidney disease beyond stage three have impaired 1α-hydroxylase activity; hence, calcium profile, 25(OH)D, and iPTH should be monitored in these individuals. It is recommended that calcitriol therapy should be initiated in these patients if iPTH is elevated more than CKD stage-specific cutoffs despite 25 (OH)D >30 ng/ml or there is hypocalcemia. Hence, therapy in the index patient should be cholecalciferol and not calcitriol. After adequate supplementation with cholecalciferol and normalization of 25 (OH)D, serum iPTH should be reassessed. If iPTH is still elevated above CKD stage specific cutoffs, additional therapy with calcitriol is indicated. The CKD stage-specific cutoffs for iPTH are summarized in the table given below.

Stage of CKD	**Target iPTH (pg/ml)**
Stage 3 (eGFR 30–59 ml/min)	35–70
Stage 4 (eGFR 15–29 ml/min)	70–110
Stage 5 (eGFR <15 ml/min)	150–300

62. *A patient with chronic kidney disease on maintenance hemodialysis was found to be vitamin D deficient* [*25*(*OH*)*D 10 ng/ml*]. *He was already receiving calcitriol therapy. Does this patient require calciferol supplementation*?

The index patient with stage 5 CKD is on therapy with calcitriol, the active form of vitamin D. He also has concurrent vitamin D deficiency. Despite calcitriol therapy, the index patient should be treated with calciferol and 25(OH)D levels should be normalized. Supplementation with calciferol normalizes 25(OH)D levels and provides a substrate for extrarenal 1α-hydroxylase enzyme, thereby reducing the requirement of calcitriol. Further, 25(OH)D is not only a precursor for $1,25(OH)_2D$ but also has effects independent of $1,25(OH)_2D$, which include suppression of PTH and improvement in skeletal muscle function. In addition, it has been shown that normalization of 25(OH)D levels in patients with CKD on maintenance hemodialysis, who were on calcitriol therapy, results in improvement in hemoglobin, reduction in doses of erythropoietin and sevelamer, and has beneficial effects on left ventricular muscle index.

63. *How to treat vitamin D-dependent rickets type 1 and 2*?

Children with VDDR type 1 and 2 present in infancy with classical features of rickets including growth retardation, bony deformities, dental abnormalities, delayed milestones, and hypocalcemic seizures. Presence of alopecia and high levels of $1,25(OH)_2D$ suggest VDDR type 2. Calcitriol is the treatment of choice for VDDR type 1, and the recommended dose is 1–3 μg/day in divided doses, with good therapeutic response. The therapy in patients with VDDR type

2 is challenging; high doses of cholecalciferol (5000–40,000 U/day), calcitriol (17–20 μg /day), and oral calcium therapy have been tried with varying success. In case of failure with these regimens, therapy with intravenous calcium is indicated. This not only results in normalization of serum calcium, but also in healing of rickets. Therapy with intravenous calcium leads to correction of hypocalcemia and secondary hyperparathyroidism, thereby resulting in improvement in serum phosphorus and healing of rachitic lesions in patients with VDDR type 2, even without vitamin D or phosphorus therapy. Intravenous calcium therapy can be given on a continuous or intermittent basis and can be stopped once the growth of child is complete.

64. *What are the familial disorders of phosphate homeostasis*?

The familial disorders of phosphate homeostasis are due to mutation of genes involved in phosphatonin secretion and degradation, or renal phosphate transporters. They are enlisted in the table given below.

Disease	Gene defect	Remarks
X-linked hypophosphatemic rickets	Loss-of-function mutation of PHEX gene	Decreased cleavage of FGF-23
Autosomal dominant hypophosphatemic rickets	Gain-of-function mutation of FGF-23 gene	Proteolysis-resistant FGF-23
Autosomal recessive hypophosphatemic rickets	Loss-of-function mutation of DMP1, EMPP1	Increased FGF-23
Hereditary hypophosphatemic rickets with hypercalciuria	Loss-of-function mutation of NaPi 2c	Phosphaturia
Tumoral calcinosis	Gain-of-function mutation of GALNT3	Associated with hyperphosphatemia
	Loss-of-function mutation of FGF-23 or Klotho	

PHEX phosphate-regulating endopeptidase homolog, X linked, *DMP1* dentin matrix protein-1, *EMPP1* ectonucleotide pyrophosphatase/phosphodiesterase 1, *GALNT3* polypeptide *N*-acetylgalactosaminyltransferase 3

65. *What are the characteristic manifestations of X-linked hypophosphatemic rickets–osteomalacia*?

Children with X-linked hypophosphatemic rickets/osteomalacia (XLH) usually present between 2 and 3 years of age with bowing of legs and short stature. Upper limb deformities are uncommon as the disease does not manifest during crawling stage of life. Dental abscess and enthesopathy are other characteristic abnormalities present in these patients. Poorly mineralized dentine provides an easy access to microbes to the pulp of teeth, thereby resulting in dental abscess. The cause of enthesopathy in patients with XLH is not clear; however, FGF-23 has been implicated. The biochemical characteristics of XLH include hypophosphatemia, normocalcemia, normal to mildly elevated PTH and alkaline

phosphatase, low or inappropriately normal 1,25-$(OH)_2D$, and elevated FGF-23 (Fig. 5.14).

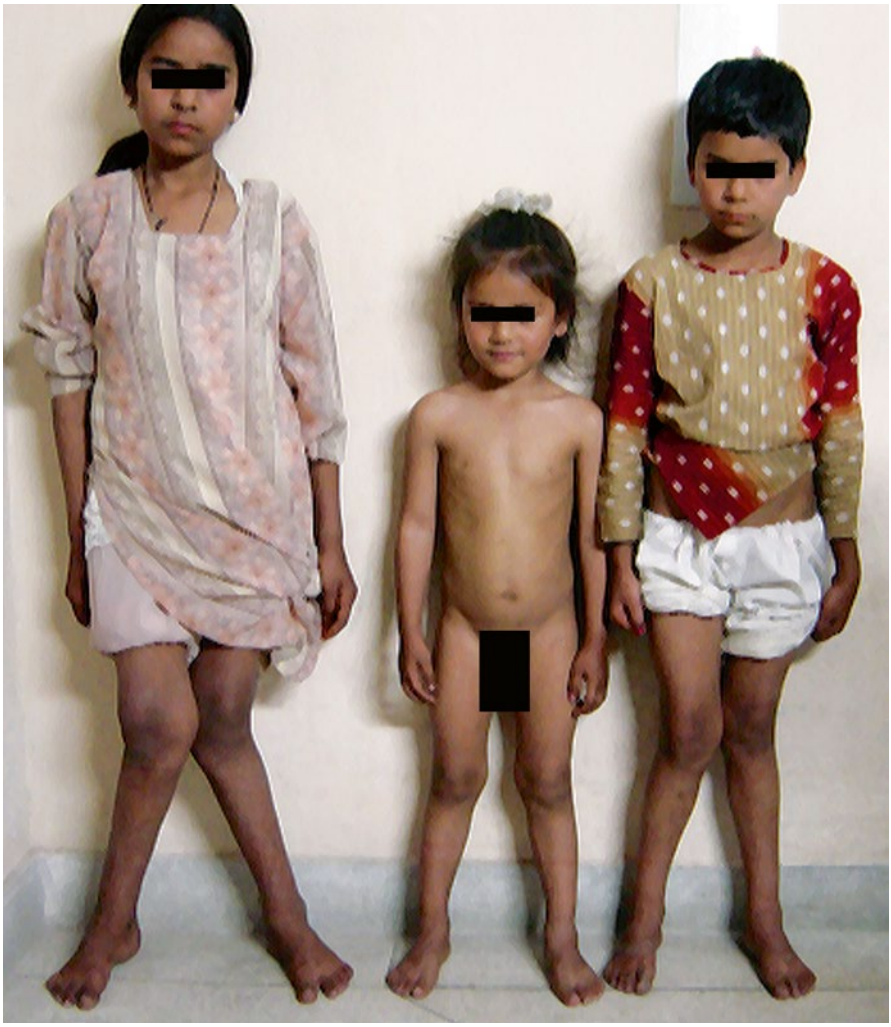

Fig. 5.14 Familial hypophosphatemic rickets–osteomalacia

66. *What are the causes of hypophosphatemic rickets/osteomalacia with normal FGF-23*?

The causes of hypophosphatemic rickets–osteomalacia with normal FGF-23 levels are hereditary hypophosphatemic rickets with hypercalciuria, Fanconi's syndrome, and tumor-induced osteomalacia associated with secreted frizzled related protein 4, FGF7, and matrix extracellular phosphoglycoprotein (MEPE).

67. *How to treat a child with X-linked hypophosphatemic rickets–osteomalacia*?

The cornerstone in the management of XLH is phosphate supplementation. The recommended dose of oral phosphate is 15–60 mg/Kg/day in three to five divided doses. In addition, calcitriol should also be given at doses of 15–60 ng/Kg/day in three to four divided doses and adequate calcium intake should be ensured. The goal of therapy is to maintain serum phosphate in low normal range (to avoid metastatic calcification), normalization of alkaline phosphatase, restoration of growth velocity, and healing of rickets. In addition, serum iPTH should be maintained in normal range. The management of autosomal dominant and recessive forms of hypophosphatemic rickets–osteomalacia is similar to XLH.

68. *What is the rationale of calcitriol supplementation in addition to phosphate therapy in patients with XLH*?

Patients with XLH have decreased 1α-hydroxylase activity due to elevated FGF-23. Further, therapy with phosphate may result in hypocalcemia and secondary hyperparathyroidism. The concurrent administration of calcitriol not only increases intestinal calcium and phosphate absorption but also prevents development of secondary/tertiary hyperparathyroidism.

69. *How to monitor a child with XLH on therapy*?

A child with X-linked hypophosphatemic rickets–osteomalacia should be clinically monitored by improvement in bone pain, growth velocity, and deformities. Biochemical monitoring includes serum phosphate, serum ALP, iPTH, and urinary phosphate. Serum phosphate should be maintained in the low normal range, as attempts to normalize serum phosphate are associated with gastrointestinal adverse events and development of secondary/tertiary hyperparathyroidism. Estimation of urinary phosphate helps in the assessment of compliance to therapy. Monitoring of FGF-23 is not useful as phosphate and calcitriol therapy may result in further elevation of serum FGF-23. With successful therapy, decrease in bone pain occurs within few weeks and normalization of ALP within 6–12 months. There is also increase in growth velocity by 1 year and improvement of deformities by 3–4 years of age.

70. *What are the alterations in serum iPTH in patients with XLH*?

Serum iPTH levels are usually normal/mildly elevated in patients with X-linked hypophosphatemic rickets–osteomalacia. Rise in iPTH is possibly due to FGF-23-mediated inhibition of $1,25(OH)_2D$. After initiation of phosphate therapy, secondary/tertiary hyperparathyroidism may ensue as a result of phosphate-mediated hypocalcemia and direct stimulatory effect of phosphate on parathyroid cell. This is deleterious as it will result in worsening of phosphaturia and consequently, hypophosphatemia. Overzealous treatment with calcitriol results in suppression of PTH and consequently hypercalciuria and nephrocalcinosis.

71. *What are the adjuvant therapies in XLH*?

Recombinant growth hormone has been tried to increase phosphate reabsorption in patients with X-linked hypophosphatemic rickets/osteomalacia; however, it has not been found to be beneficial. Calcimimetic agents like cinacalcet have been used to treat secondary hyperparathyroidism associated with phosphate therapy. Use of cinacalcet has been shown to be effective in reducing the doses of phosphate and calcitriol. Recently, use of intravenous iron therapy has also been shown to be useful. Monoclonal antibodies against FGF-23 have been shown to be effective in mouse models of X-linked hypophosphatemia.

72. *What are the endocrine manifestations of renal tubular acidosis*?

Renal tubular acidosis (RTA) is characterized by normal anion gap metabolic acidosis with hypokalemia in the presence of normal glomerular filtration rate. RTA may occur either due to the defect in reabsorption of HCO_3^- at proximal tubule (proximal RTA, type 2) or defect in excretion of H^+ ions at distal tubule (distal RTA, type 1). The endocrine manifestations of RTA include growth failure, polyuria, rickets–osteomalacia, and nephrocalcinosis–nephrolithiasis. In addition, type 4 RTA is characterized by normal anion gap metabolic acidosis with hyperkalemia and normal or modestly reduced glomerular filtration rate.

73. *What are the differences in clinical features of proximal and distal RTA*?

The characteristic clinical features of proximal and distal RTA are summarized in the table given below.

Parameters	**Proximal RTA (Type 2)**	**Distal RTA (Type 1)**
Pathogenesis	Defect in reabsorption of HCO_3^- at proximal tubule of kidney	Defect in excretion of H^+ ions at distal tubule of kidney
Growth failure	Often present	Often present
Rickets–osteomalacia	Often present	Often present
Nephrocalcinosis–nephrolithiasis	Absent	Often present
Other tubular defects	Common (Fanconi's syndrome)	Absent
Etiology	Primary	Primary
	Secondary:	Secondary:
	Wilson disease	Sjogren's syndrome
	Valproate Aminoglycoside Acetazolamide	Systemic lupus erythematosus

74. *Why is there rickets in RTA*?

Rickets–osteomalacia is a characteristic feature of both proximal and distal RTA, and the pathophysiological mechanisms for the development of rickets–osteoma-

lacia include metabolic acidosis, hypercalciuria, hypophosphatemia, and low $1,25(OH)_2D$. Metabolic acidosis leads to impaired bone mineralization and increased bone resorption. Activation of RANK ligand by systemic acidosis results in increased bone resorption, which leads to release of calcium, phosphate, and carbonate in order to counteract metabolic acidosis; however, it results in hypercalciuria. In addition, acidosis leads to decreased 1α-hydroxylase activity and consequently, reduced calcium and phosphate absorption from the intestine, and secondary hyperparathyroidism. Defective tubular reabsorption of phosphate in proximal RTA also contributes to hypophosphatemia. These alterations in mineral homeostasis result in decreased calcium–phosphate solubility product and impaired mineralization, thereby leading to rickets/osteomalacia (Fig. 5.15).

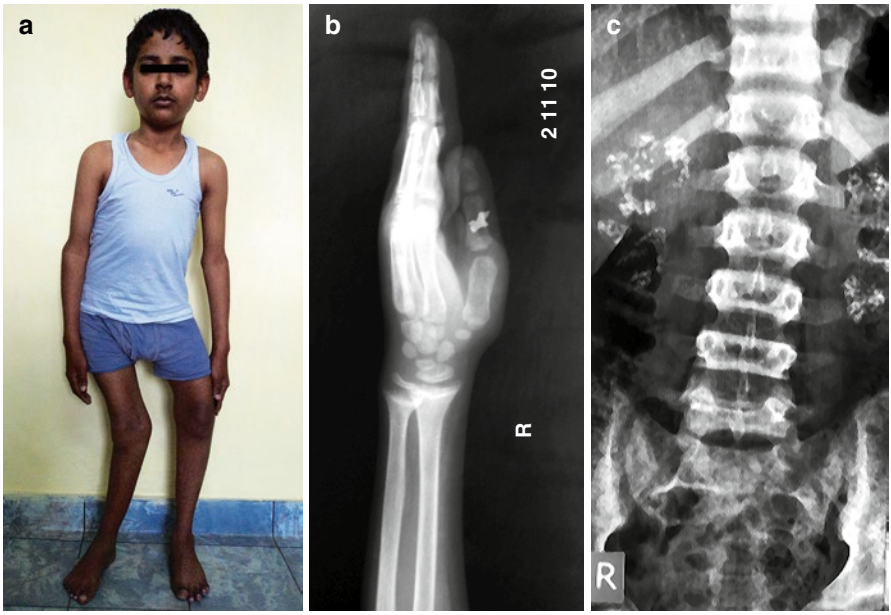

Fig. 5.15 (**a**) Windswept deformity in a child with rickets due to distal RTA. (**b**) X-ray of wrist shows classical features of rickets with metaphyseal fraying, cupping, and splaying. (**c**) X-ray of abdomen showing bilateral nephrocalcinosis

75. *What is the indication of ammonium chloride loading test in a patient with RTA*?

Ammonium chloride loading test is indicated in children with a strong clinical suspicion of distal RTA but with a normal blood pH. This is usually seen in patients who have incomplete or milder forms of distal RTA. The recommended dose of ammonium chloride is 0.1 g/Kg body weight administered over a period of 1h to avoid gastric irritation, and blood and urine samples are taken every hour for 4–6h. After administration of ammonium chloride, it is converted to urea in the presence of bicarbonate in the liver, thereby reducing the levels of

plasma bicarbonate. In a healthy individual, this results in excretion of hydrogen ions to maintain normal blood pH. However, in patients with distal RTA, excretion of hydrogen ions fails to occur, thereby leading to metabolic acidosis and alkaline urine (urine pH >5.5). A decrease in plasma bicarbonate of 3–5 mEq/L along with urine pH >5.5 suggests the diagnosis of distal RTA. The test should not be performed in the presence of liver disease, urinary tract infection, hypokalemia, or hypercalcemia.

76. *How to confirm the diagnosis of RTA*?

RTA should be suspected in a child in the presence of short stature, rickets–osteomalacia, polyuria, or nephrocalcinosis–nephrolithiasis. The evaluation of RTA includes renal function test, serum potassium, blood gas analysis, urine pH, and urinary anion ga+p. The diagnosis of RTA is confirmed by the presence of normal anion gap metabolic acidosis with normal renal function tests. If the baseline urinary pH is <5.5 then the diagnosis of proximal RTA is confirmed and if baseline urine pH is >5.5, bicarbonate loading test should be performed to differentiate between proximal and distal RTA. In bicarbonate loading test, oral sodium bicarbonate is administered at a dose of 2–4 mEq/Kg/day for 2–3 days, with the aim to normalize plasma pH and HCO_3^-. Fractional excretion of HCO_3^- of >10–15 % suggests proximal RTA and <5 % distal RTA. The differences in biochemical parameters between the two types of RTA are summarized in the table given below.

Parameters	Proximal RTA (Type 2)	Distal RTA (Type 1)
Plasma anion gap	Normal	Normal
Serum potassium	Low	Low
Serum HCO_3^- mEq/L	Usually 12–20	Usually <10
Urine pH	>5.5 (if HCO_3^- >20)	>5.5
	<5.5 (if HCO_3^- <15)	
Urine anion gap	Positive	Positive
Fractional excretion of HCO_3^-	>10–15 %	<5 %
Hypercalciuria	Present	Present
Hypocitraturia	Absent	Present
Aminoaciduria, glycosuria	May be present	Absent

77. *How to treat rickets–osteomalacia associated with RTA*?

The aim of therapy in a child with RTA is to correct metabolic acidosis in order to promote mineralization and prevent further progression of skeletal

deformities. Therefore, early diagnosis and optimal therapy with oral alkali, either as bicarbonate or citrate, is recommended. However, citrate is preferred over bicarbonate as it is better tolerated. The recommended dose is 1–2 mEq/Kg daily in divided doses in distal RTA and 10–20 mEq/Kg daily in proximal RTA (higher dose required due to massive urinary loss of bicarbonate). Short-term administration of potassium, calcitriol, and phosphate may be required in these patients. Correction of acidosis not only improves hypokalemia and hypercalciuria but also leads to increased $1,25(OH)_2D$ synthesis. Therefore, long-term use of calcitriol is not required in patients with RTA. In addition, it may worsen hypercalciuria in patients with distal RTA. Overtreatment with alkali should be avoided as it may worsen hypokalemia and hypercalciuria. During follow-up, serum potassium, pH, bicarbonate, and urinary calcium should be periodically monitored. In addition, renal ultrasonography should also be performed to detect nephrocalcinosis. The oral alkali preparations commonly used in the management of RTA are summarized in the table given below.

Preparation	Composition	Alkali content	Potassium content
Potassium citrate and citric acid syrup	Each 5 ml contains Potassium citrate 1100 mg	2 mEq base/ml	5 mEq potassium/ml
	Citric acid 334 mg		
Shohl's solution	Each litre contain citric acid 140 g	1 mEq base/ml	–
	Hydrated crystalline sodium citrate 90 g		
Sodium bicarbonate tablets	325 mg/tablet	325 mg tablet contains 4 mEq	–
	or		
	650 mg/ tablet	650 mg tablet contains 8 mEq	

78. *What are the endocrine causes of exuberant callus formation*?

The exuberant callus formation is the characteristic feature of osteogenesis imperfecta, glucocorticoid excess (exogenous or endogenous), Charcot's arthropathy, renal osteodystrophy, and multiple myeloma. The mechanism of exuberant callus formation remains elusive. However, unrestrained production of collagen by the osteoblast in response to poorly mineralized matrix (callus) possibly explains this phenomenon (Fig. 5.16).

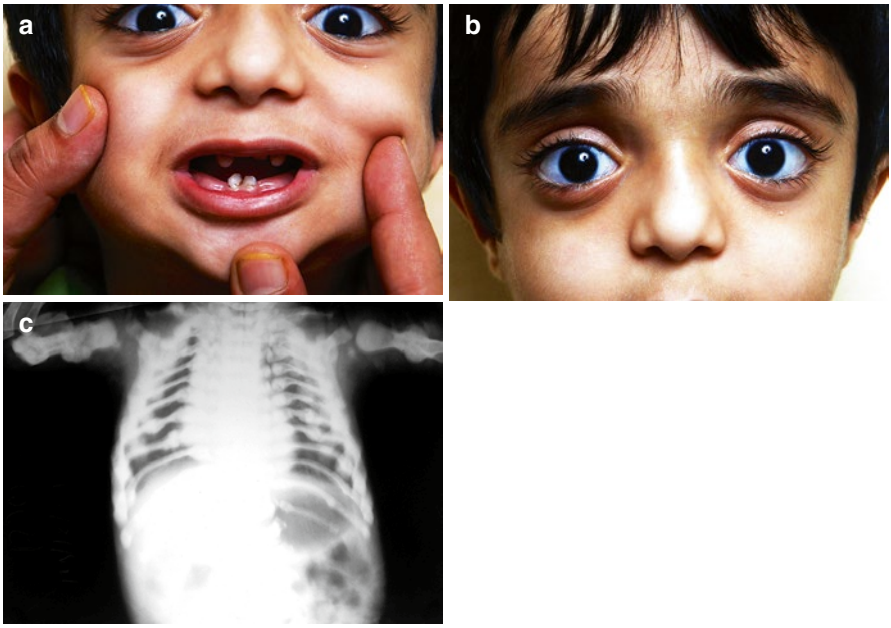

Fig. 5.16 A 3-year-old boy with (**a**) dentinogenesis imperfecta, (**b**) blue sclera, (**c**) X-ray of chest PA view showing multiple rib fractures with exuberant callus formation

Further Readings

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Precocious Puberty

6

6.1 Case Vignette

A 3-year-old girl was brought by her mother with the complaint of progressive development of breast for the last 6 months. It was not accompanied with appearance of pubic hair or history of vaginal bleed, though there was history of vaginal discharge occasionally. It was also noticed that she had sudden increase in her height over the last 6 months. There was no history of "waxing and waning" in the breast size. She did not have history of headache, visual disturbances, seizures, head injury, meningitis/encephalitis, cranial irradiation, pain in the abdomen, or palpable abdominal mass. She did not have symptoms suggestive of hypothyroidism. There was no history of any drug intake or use of estrogen or "hormone dust" exposure. On examination, her height was 103 cm (97^{th} percentile, +2SDS) and weight 15 Kg (75^{th} percentile), with a target height of 159 cm (25^{th} percentile). She did not have cafe-au-lait macule, adenoma sebaceum, shagreen patch, neurofibroma, or bony deformity. She had no goiter and deep tendon reflexes were normal. Visual field and acuity were normal. Her Tanner staging was $A_{-,}$ $P_{1,}$ B_3. Systemic examination was unremarkable. On investigations, hormonal profile revealed serum LH 2.3 mIU/ml ($N < 0.3$), FSH 3.9 mIU/ml, 17 β-estradiol 78.4 pg/ml ($N < 10$), T_4 6.7 μg/dl (N 4.8–12.7), TSH 1.38 μIU/ml (N 0.27–4.2), and prolactin 21.2 ng/ml (N 4.7–23.3). GnRH agonist stimulation test (triptorelin 0.1 mg/m^2) showed serum LH 56.3 mIU/ml at 3h and 17 β-estradiol 185.3 pg/ml at 24h. Her bone age was 7 years (Greulich and Pyle). Ultrasonography of the pelvis showed uterine length 4.1 cm and ovarian volume 3 ml (right) and 1.5 ml (left) with multiple follicles. CEMRI sella showed convex upper border of anterior pituitary and the rest of the other areas were normal. With this clinical and biochemical profile, a diagnosis of idiopathic gonadotropin-dependent precocious puberty (GDPP) was considered and the patient was initiated with depot leuprolide (3.75 mg monthly). At 3 months of follow-up, she did not have a flare and had regression of secondary sexual characteristics (B_3 to B_2). Serum LH, basal and stimulated (3h after the next dose of injection), was 1.7 mIU/ml and 14.7 mIU/ml,

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respectively. Basal serum estradiol was 23.7 pg/ml. The dose of depot leuprolide was increased to 7.5 mg once a month and advised to have a regular follow-up at three monthly intervals (Fig. 6.1).

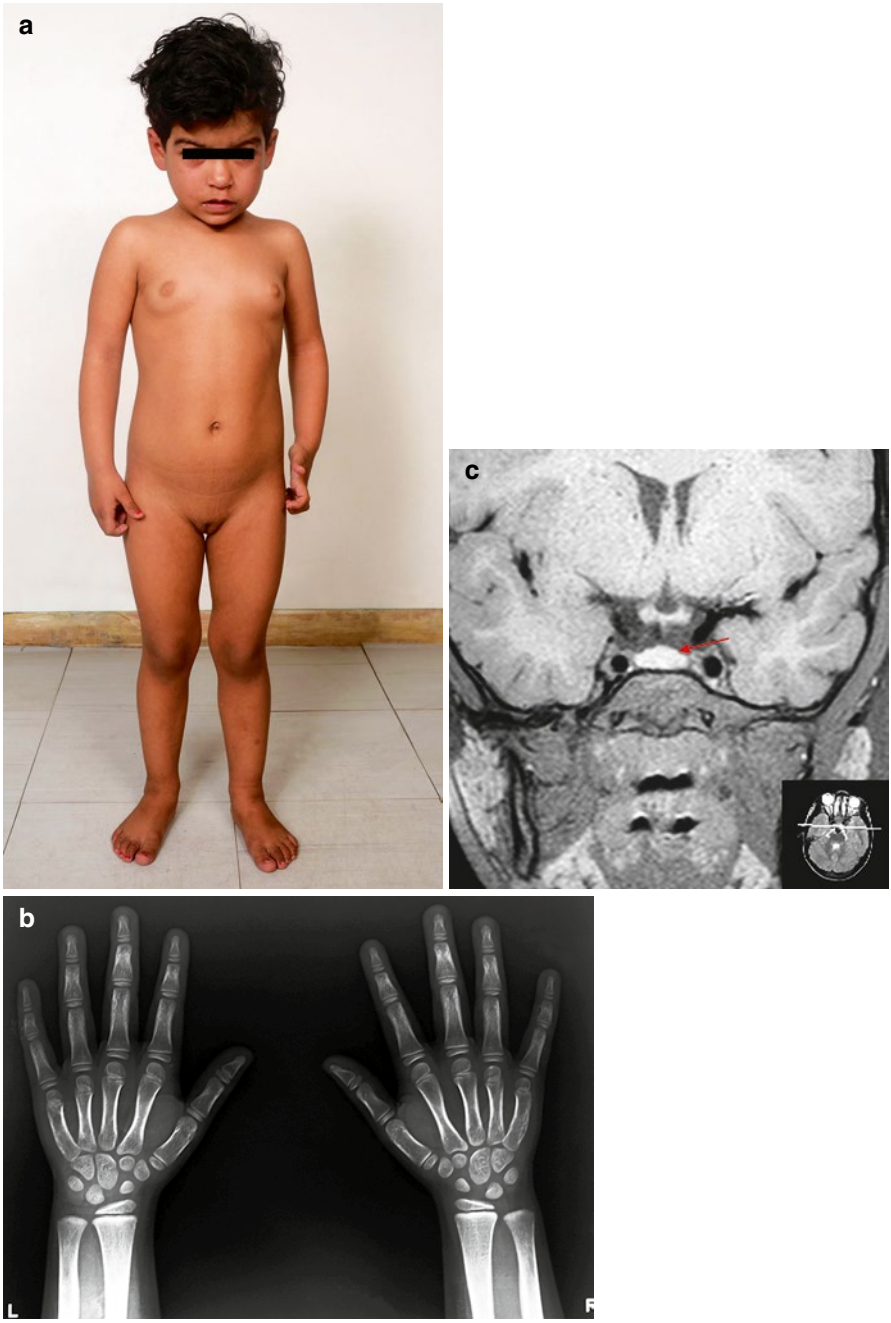

Fig. 6.1 (**a**) A 3-year-old child presented with thelarche (B_3), (**b**) X-ray of wrist showing bone age of 7 years, (**c**) CEMRI sella showing convex upper border of the pituitary (*red arrow*) suggestive of GDPP

6.2 Stepwise Analysis

The index patient presented with breast development (B_3) at 3 years of age, which is below the lower normal reference age for the onset of puberty (8 years), thereby, qualifying the criteria for the diagnosis of precocious puberty. The children with precocious puberty require further evaluation to prevent height loss and adverse psychosocial outcome. In addition, it also helps to exclude the presence of structural disease as a cause of precocious puberty, though less common in girls. The premature breast development in the index child could be due to gonadotropin-dependent precocious puberty (GDPP), gonadotropin-independent precocious puberty (GIPP), or premature thelarche (normal variant). Majority of children with premature thelarche present before the age of 4 years, and the breast development is usually up to stage B_3; however, it is nonprogressive and regresses spontaneously within 6 months to 6 years after the diagnosis. Other signs of sexual maturation like pubarche, menarche, and enlargement of uterus are absent. Further, growth velocity is normal in these children, and there is no advancement in bone age. Though the index patient presented at the age of 3 years with breast development (B_3), presence of growth spurt (height +2SDS), advancement in bone age (BA > CA,7 > 3 years), and enlargement of uterus (4.1 cm, prepubertal uterine length <3.5 cm) exclude the diagnosis of premature thelarche. Early age of onset of puberty (<2 years), dissociation between breast staging and menarche (<B_3 and menarche), waxing and waning size of breast, presence of cutaneous markers (cafe-au-lait macule, adenoma sebaceum, shagreen patch, neurofibroma), bony deformity, feature suggestive of hypothyroidism (goiter and delayed tendon reflexes), and palpable abdominal mass suggest the diagnosis of GIPP. However, the absence of these features neither excludes the diagnosis of GIPP nor confirms the presence of GDPP. Therefore, to differentiate between GDPP and GIPP, estimation of basal and stimulated LH along with gonadal steroids is required. Basal serum LH value of ≥0.3 mIU/ml or stimulated LH >8 mIU/ml (by chemiluminescence assay) after triptorelin is diagnostic of GDPP. In the index case, basal LH was 2.3 mIU/ml, and it was suggestive of activation of hypothalamo–pituitary–gonadal (HPG) axis. Further, the stimulated LH value in the index child was 56.3 mIU/ml; however, the stimulation test was not warranted in our patient, and it is only required if the basal LH is <0.3 mIU/ml. In addition, 17 β-estradiol cutoff of 80 pg/ml at 24h in response to GnRH agonist is also a surrogate indicator of activation of HPG-axis. The index child had stimulated 17 β-estradiol 185.3 pg/ml, further supporting the diagnosis of GDPP. MR imaging of the brain is recommended in all children with GDPP to localize any mass lesion in the hypothalamic region. The probability of organic lesion is much higher in boys than in girls (40–90 % vs. 8–33 %) in children with GDPP. Further, the probability is much lower in girls (approximately 2 %) when the puberty starts after the age of 6 years. In the index case, MRI brain did not reveal any organic lesion; however, there was convexity of the upper border of the pituitary gland (due to gonadotrope hyperplasia) suggestive of GDPP. The indications for treatment in a child with idiopathic GDPP include rapid progression of pubertal events over a period of 3–6 months (from one stage to the next), significant advancement of bone age (>2.5 SD for chronological age), or presence of psychosocial concerns. However, all children with GDPP having organic lesion must be treated irrespective of above mentioned criteria. The indication for

treatment in the index child was rapid progression of pubertal event (B_1 to B_3 during 6 months) and significant advancement of bone age (>2.5 SD) at presentation. GnRH agonists are the treatment of choice for children with GDPP. However, this therapy is preferred in children who have onset of pubertal events before the age of 6 years, because of their potential benefit in achievement of target adult height. Long-acting GnRH agonist depot preparations are preferred and can be administered either once a month or every three monthly with similar efficacy. The other treatment options include medroxyprogesterone acetate and cyproterone acetate, which can be used in children with GDPP who present after the age of 6 years as these drugs arrest the progression of pubertal development but have no apparent benefit on adult height potential. The index child was treated with leuprolide acetate depot once a month intramuscularly. The child did not report any flare after initiation of therapy. Regular follow-up is required initially at 3 monthly interval and include clinical assessment of secondary sexual characteristics, growth velocity, estimation of basal and stimulated LH, and basal testosterone/estradiol in boys or girls, respectively. The biochemical parameters are assessed on the day, when the next dose is administered. The GnRH agonist treatment is associated with regression of secondary sexual characteristics by 6–12 months of initiation of treatment with progressive decline in growth velocity and chronological age gradually approaches near to the bone age. The index patient had regression of breast (B_3 to B_2) at 3 months. However, basal serum LH was 1.71 mIU/ml and stimulated LH 14.7 mIU/ml (at 3h), necessitating the hike in dose of leuprolide depot. At 6 months of follow-up, Tanner staging was A_-, P_1, B_2, and her growth velocity was reduced to 4 cm/year. The adverse effects associated with the use of GnRH agonist therapy include initial flare, sterile abscess, and allergic reactions. Our patient did not experience any adverse event. The treatment is to be continued till the chronological age of 12 years. After discontinuation of GnRH agonist therapy, in girls, the gonadotropins start rising within few weeks to months with resumption of menses by 1 year, whereas in boys, the rise in gonadotropins occurs usually by 6 months to 1 year and testes may take a longer time to attain the adult testicular size.

6.3 Clinical Rounds

1. *What is precocious puberty*?

   Precocious puberty is defined as appearance of any secondary sexual characteristics at an age earlier than the established normal standards for children of the same gender and race. Although children with different race have different age of onset of puberty, a clinically useful definition of precocious puberty is development of any secondary sexual characteristics before the age of 8 years in girls or 9 years in boys.

2. *How were the age cutoffs for onset of normal puberty derived*?

   The normal age of onset and sequence of pubertal events were described in landmark studies by Tanner and Marshall in 1960s. One hundred and ninety-two British girls between 8 and 18 years of age were serially followed up with clinical photo-

graphs every 3 months, and pubertal staging was assessed from these photographs. It was observed that the first sign of puberty (either breast development or appearance of pubic hair) appeared in 95 % of girls between the age 8.5 and 13 years, with a mean age of 10.5 year and standard deviation of 1 year. Similarly, 228 British boys were followed up with clinical photographs every 3 months, and it was shown that 95 % of boys had onset of genital development (testes, penis, and scrotum) between 9.5 and 13.5 years of age, with a mean age of 11.5 years and standard deviation of 1 year. From these data, the cutoffs for the definition of abnormal onset of pubertal development were derived. However, the major shortcoming of this study was that the children recruited for the study were residents of children's home and belonged to lower socioeconomic strata. Further, the assessment of pubertal status was based on photographs rather than physical examination.

3. *How were the age cutoffs for precocious puberty defined*?

   The age of onset of puberty in a population is normally distributed (bell-shaped curve with a Gaussian distribution). In the studies by Tanner and Marshall, it was shown that the mean age of onset of puberty was 10.5 years in girls and 11.5 years in boys, with a standard deviation of approximately 1 year. Considering the normal range of age of pubertal onset as mean ± 2.5 SD, any sign of pubertal development before the age of 8 years in girls or before 9 years in boys (−2.5 SD from the mean, i.e., 10.5–2.5 years and 11.5–2.5 years, respectively) suggests precocious puberty.

4. *Does cutoff for the diagnosis of precocious puberty require redefinition*?

   In a large cross-sectional study involving 17,000 American girls between the age of 3 and 12 years [Pediatric Research in Office Settings (PROS) study], it was observed that the mean age of onset of Tanner 2 breast development was 9.96 ± 1.82 years in White girls and 8.87 ± 1.93 years in African–American girls, as opposed to mean age of 11.15 ± 1.1 years reported earlier by Tanner and Marshall. Based on these data, Lawson-Wilkins Pediatric Endocrine Society suggested that the cutoffs for defining precocious puberty should be lowered to 7 years in White girls and 6 years in African–American girls. However, the dissociation between the age of onset of pubertal development as evidenced by earlier age of onset of breast development without change in the age of menarche raised a question on the accuracy of assessment of breast staging (by visual inspection) in the PROS study. Thereafter, a recent study involving Black girls between the age 6 and 8 years and White girls between the age 7 and 8 years with precocious puberty showed that 88 % had "idiopathic precocious puberty." These girls with "idiopathic precocious puberty" were likely to have normal variant of puberty. However, in the same study, it was shown that 12 % of these girls had an organic cause of sexual precocity. Therefore, decreasing the age of evaluation of precocious puberty to <7 years in White and <6 years in Black girls will result in underdiagnosis of organic cause of precocious puberty in these girls. Despite limitations of the available data, the cutoffs of 8 years for girls and 9 years for boys are still widely used for defining precocious puberty in clinical practice.

5. *What is the sequence of normal pubertal development in females*?

Normal pubertal development is characterized by a predictable, progressive sequence of events which occur after reactivation of HPG-axis. The normative data for the sequence of events of pubertal development is derived from the landmark studies by Tanner and Marshall in 1960s. In girls, the first sign of puberty is increase in height velocity, closely followed by thelarche. However, in clinical practice, thelarche is regarded as the first sign of puberty as it is easily appreciable. Thelarche is followed by pubarche and menarche. The mean time interval between the onset of breast budding to the development of adult breast is 4.4 ± 2 years, and the mean interval between breast budding to menarche is 2.3 ± 1 years. In majority of girls, menarche occurs at Tanner breast stage B_4 and the peak height velocity at B_3. The mean age of onset of pubertal events in females as described by Tanner and Marshall are summarized in the table given below.

Pubertal events	Mean age at onset in years (range)	Mean age at completion in years (range)
Initiation of height spurt	10.5	14
Thelarche	11.15 (8–13)	15.33 (13–18)
Pubarche	11.69	14.41
Menarche	13.47 (10–16.5)	–
Peak height velocity	12.14 (9.5–14.5)	–

6. *What is the sequence of normal pubertal development in males*?

The first sign of puberty in boys is the enlargement of testes, followed by pubarche and penile enlargement. Testicular and penile growth is accompanied with progressive enlargement of scrotum along with increasing pigmentation and rugosity of scrotal skin. The growth spurt starts along with penile enlargement and the peak height velocity occurs during Tanner genital stage G_4 (at a testicular volume 10 ml) and pubic hair stage P_4. The mean time interval between the onset of testicular enlargement and penile enlargement is approximately 1 year, and development of pubic hair to stage P_5 marks the completion of puberty in boys. The mean age of onset of pubertal events in boys as described by Tanner and Marshall is summarized in the table given below.

Pubertal events	Mean age at onset in years (range)	Mean age at completion in years (range)
Testicular enlargement	11.4 (9.5–13.5)	15 (13.5–17)
Genital staging	G_2 at 11.5	G_5 at 15
Pubarche	12	16
Initiation of height spurt	12.5 (10.5–16)	16 (13.5–17.5)
Penile enlargement	12.5 (10.5–14.5)	14.5 (12.5–16.5)
Peak height spurt	14	–

7. *What are the shortcomings of genital staging proposed by Tanner*?

   The genital staging proposed by Tanner and Marshall considers the development of testis, penis, and scrotum together in a comprehensive manner. Although the development of testis and penis can be assessed objectively, the assessment of scrotal development (rugosity, darkening) is subjective. During normal puberty, the development of testis, penis, and scrotum occurs simultaneously, and staging of genitalia can be ascertained easily. However, in patients with GIPP, there is dissociation between testicular and penile development, and assignment to a particular genital staging is difficult.

8. *What is* "a*ccelerated*" *puberty*?

   Girls and boys who have onset of pubertal events at a normal age, but experience rapid progression of pubertal development (whether sequential or not), are said to have "accelerated" puberty. This may be due to CNS lesions and adrenal, ovarian, or testicular tumors, which have evolved after the onset of puberty. Although by definition these children do not have precocious puberty, rapid progression of pubertal events merits evaluation in these children.

9. *How is precocious puberty classified*?

   Precocious puberty is classified based on presence or absence of premature reactivation of hypothalamo–pituitary–gonadal (HPG) axis.

Classification based on HPG-axis
Gonadotropin-dependent precocious puberty (GDPP)/true or complete precocious puberty
Precocious puberty due to premature reactivation of HPG-axis
Development of secondary sexual characteristics is sequential and progressive
Thelarche followed by pubarche and menarche in girls and gonadarche followed by pubarche in boys
Gonadotropin-independent precocious puberty (GIPP)/pseudo or incomplete precocious puberty
Precocious puberty due to excess of sex steroids independent of reactivation of HPG-axis
Endogenous/exogenous sex steroids
Extrapituitary secretion of gonadotropins (hCG)
Development of secondary sexual characteristics is nonsequential and may or may not be progressive
Menarche at Tanner breast stage 2 or disproportionate penile enlargement in relation to testes
Normal variants of puberty
Isolated premature thelarche
Isolated premature pubarche
Isolated premature menarche

10. *What is isosexual precocious puberty*?

    Isosexual precocious puberty is defined as premature development of secondary sexual characteristics in concordance with chromosomal and gonadal sex,

e.g., thelarche and menarche in girls and gonadarche in boys. Pubarche does not distinguish between isosexual or heterosexual precocity as it is a common feature in both sexes. Isosexual precocious puberty may occur with or without premature reactivation of HPG-axis.

11. *What is heterosexual precocious puberty*?

Heterosexual precocious puberty is defined as premature development of secondary sexual characteristics in discordance with chromosomal and gonadal sex, e.g., clitoromegaly in girls and gynecomastia in boys. Heterosexual precocity is always independent of reactivation of HPG-axis.

12. *What are the causes of heterosexual precocious puberty*?

The causes of heterosexual precocious puberty in boys include feminizing adrenal and testicular neoplasms, and gain-of-function mutation of aromatase. These disorders are associated with increased secretion of estrogen or peripheral aromatization of androgens to estrogen. The causes of heterosexual precocious puberty in girls include congenital adrenal hyperplasia, androgen-secreting adrenal and ovarian neoplasms, aromatase deficiency, and glucocorticoid resistance syndrome.

13. *What are the features of estrogenization in a female*?

Breast development, female body contour (gluteofemoral adiposity), facial maturity, estrogenization of vaginal mucosa (dull, pink, moist, and thick vaginal mucosa as compared to prepubertal thin and red vaginal mucosa), acceleration of linear growth, rapid bone maturation, adult body odor, and behavioral changes typical of puberty are the features of estrogenization in a female.

14. *What are the features of virilization in a female*?

Clitoromegaly, acne, seborrhea, temporal hair recession, low-pitched voice, male-torso, accelerated linear growth, and rapid bone maturation are the characteristic manifestations of virilization. This is usually accompanied with hirsutism; however, isolated hirsutism is not considered as a manifestation of virilization.

15. *What are the clinical pointers toward etiological diagnosis of precocious puberty*?

The clinical pointers that suggest the etiological diagnosis of precocious puberty include café-au-lait macule and bony swellings (McCune–Albright Syndrome), lentigines (Carney's complex), adenoma sebaceum and shagreen patches (tuberous sclerosis), neurofibroma and optic glioma (neurofibromatosis type 1), gelastic seizures (tuber cinereum hamartoma), features of raised intracranial tension (suprasellar mass, e.g. germinoma, arachnoid cyst and pilocytic

astrocytoma), mucocutaneous pigmentation and nevi (Peutz–Jegher syndrome), waxing/waning of breast size (follicular cyst of ovary), hepatomegaly (hepatoblastoma), and hyperpigmentation (CAH and glucocorticoid resistance syndrome) (Fig. 6.2).

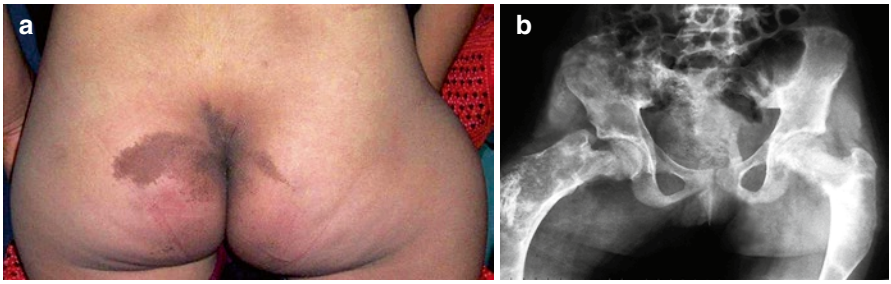

Fig. 6.2 (**a**) Café-au-lait macule in a girl with precocious puberty, (**b**) X-ray of pelvis showing deformity in upper end of the right femur (Shepherd's crook deformity) with ground-glass appearance suggestive of fibrous dysplasia

16. *What are the familial causes of precocious puberty*?

The familial causes of GDPP are neurofibromatosis type 1, tuberous sclerosis, gain-of-function mutation of kisspeptin gene and kisspeptin receptor (GPR54), and rarely, constitutional precocious puberty. The familial causes of GIPP are familial testotoxicosis, Peutz–Jegher syndrome, and congenital adrenal hyperplasia (21α-hydroxylase deficiency, 11β-hydroxylase deficiency, and 3β-hydroxysteroid dehydrogenase type 2 deficiency). Precocious puberty associated with McCune–Albright syndrome is not considered familial, as it is a result of postzygotic gain-of-function mutation of GNAS1 gene.

17. *What is the importance of gender in precocious puberty*?

Precocious puberty is more common in girls than in boys (3–5:1). Gonadotropin-dependent precocious puberty (GDPP) accounts for 70–80 % of patients with precocious puberty, while gonadotropin-independent precocious puberty (GIPP) contributes to the rest. Both GDPP and GIPP are more common in girls than in boys, with a ratio of 5:1 in GDPP and 2.5:1 in GIPP. Seventy percent of girls with GDPP have idiopathic precocious puberty, while 75 % of boys with GDPP have CNS pathology. Although organic lesion must be excluded in all children with GDPP, it is more likely to be present in boys. The etiology of GIPP in boys includes hCG-secreting tumors, testotoxicosis, and Leydig cell tumors, while in females, McCune–Albright syndrome, ovarian cyst, and exposure to environmental estrogen predominate. Congenital adrenal hyperplasia, virilizing adrenal tumors, feminizing tumors, and hypothyroidism may result in GIPP in both genders (Fig. 6.3).

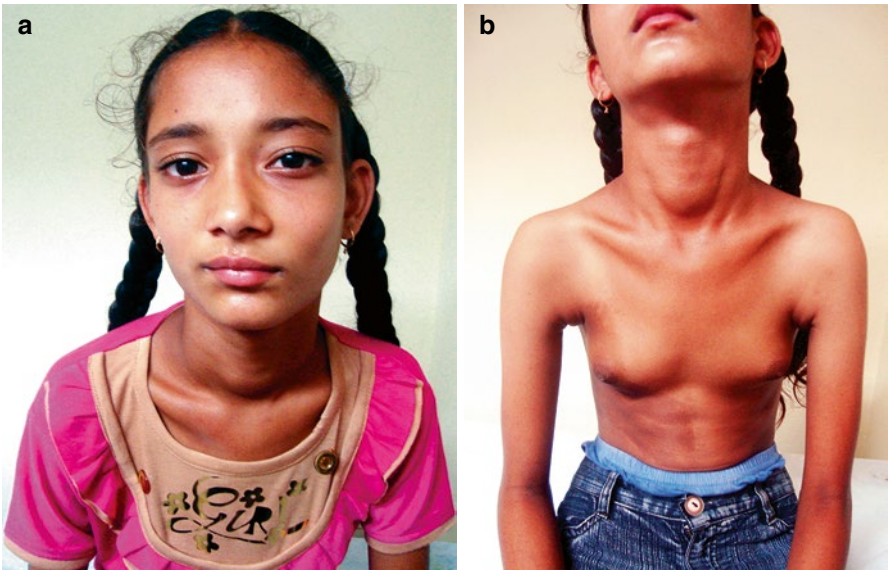

Fig. 6.3 A 6-year-old girl presented with thelarche. She also had features of thyrotoxicosis. Note the presence of (**a**) facial asymmetry (due to fibrous dysplasia), (**b**) goiter, and café-au-lait macule over abdomen and chest wall

18. *What is the importance of age in the etiological diagnosis of precocious puberty*?

    Onset of puberty during infancy, particularly in a girl, is usually due to McCune–Albright syndrome. Majority of children with GDPP due to hypothalamic hamartoma present between 2 and 4 years of age. Children with congenital adrenal hyperplasia and adrenal virilizing tumors may manifest with premature pubarche at 3–5 years of age. Fifty percent of children with idiopathic GDPP manifest between 6 and 8 years of age, while 25 % between 2 and 6 years and 18 % during infancy. Precocious puberty due to other CNS pathology, ovarian cyst, and hypothyroidism may present at any age (<8 years and <9 years in girls and boys, respectively).

19 *What are the causes of precocious puberty associated with decreased growth velocity*?

    Precocious puberty is usually associated with increased growth velocity as sex steroids potentiate "pubertal GH surge" and promote hepatic and local IGF1 generation. However, disorders like primary hypothyroidism, radiation-induced GDPP, coexisting isolated growth hormone deficiency, and malnutrition are associated with reduced growth velocity and retarded bone age, despite precocious puberty. Further, overzealous treatment with GnRH agonist for GDPP can also result in decreased growth velocity.

20. *A 3-year-old girl was noticed to have nonprogressive breast development by her mother. On evaluation, she did not have pubarche and had normal growth velocity without any advancement in bone age. How to proceed*?

In the index patient, the likely clinical diagnosis is isolated premature thelarche because it is nonprogressive, growth velocity is normal, and there is no advancement in bone age. The differences between isolated premature thelarche, and thelarche associated with GDPP and GIPP are described in the table given below.

Parameters	Isolated premature thelarche	Thelarche due to GDPP	Thelarche due to GIPP
Age of presentation	Usually <2 years	0–8 years	0–8 years
Breast development (Tanner stage)	Usually ≤ B_3	B_2–B_5	B_2–B_5
Other secondary sexual characteristics	Absent	May appear	May appear
Growth velocity	Normal for age	Increased	Increased
Bone age	Normal	Advanced	Advanced
Serum estradiol levels	Usually within prepubertal range, but may be modestly elevated	Elevated	Elevated
Serum FSH	Prepubertal/modestly elevated	Pubertal	Prepubertal
LH response to GnRH	Prepubertal	Pubertal	Prepubertal
Ovarian volume	Prepubertal (<1.6 ml)	Increased (>2.8 ml)	Increased (unilateral/bilateral)
Uterine length	Prepubertal (<3.5 cm)	>3.5 cm endometrial echo may be present	>3.5 cm endometrial echo may be present

Parents should be reassured as isolated premature thelarche is benign and nonprogressive, and usually regresses within 6 months to 6 years. Further, final adult height and subsequent pubertal development are normal in these girls; therefore, it does not require any treatment. However, regular surveillance is required as 10 % of girls with isolated premature thelarche who have onset of thelarche after 2 years of age may have underlying GDPP as a cause of premature thelarche.

21. *What are the mechanisms proposed for the development of isolated premature thelarche*?

The precise cause for isolated premature thelarche is not known. The various proposed mechanisms include transient activation of HPG-axis with predominant

FSH secretion, increased FSH sensitivity due to gain-of-function mutation of FSH receptor, and increased peripheral sensitivity to estradiol. However, it has been postulated that isolated premature thelarche may simply be an exaggerated manifestation of "mini-puberty," but this is debatable as premature thelarche does not occur in all girls, and there is a discordance in gonadotropin response to GnRH (FSH > LH) in girls with isolated premature thelarche.

22. *Why is thelarche nonprogressive in girls with isolated premature thelarche*?

The development of breast requires both estrogen and progesterone: estrogen for ductal and stromal development (B_3–B_4) and progesterone for differentiation of ductal elements into acini (B_5). In addition, prolactin, insulin, IGF1, and LH have a permissive role in breast development. In patients with isolated premature thelarche, serum estradiol levels are elevated, while serum progesterone levels are prepubertal; therefore, breast development is restricted up to Tanner stage B_2–B_3 only.

23. *What is the difference between adrenarche and pubarche*?

Adrenarche is a biochemical event characterized by an increase in adrenal androgens, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), and usually occurs by 6 years of age. Pubarche is a clinical event that begins around 8 years of age, and its onset is heralded by the appearance of pubic hair ($\leq P_3$), axillary hair, body odor, seborrhea, and acne. DHEA and DHEAS are weaker adrenal androgens and require conversion to testosterone and dihydrotestosterone to mediate their effect on pilosebaceous unit and consequently pubarche. The conversion of these weaker androgens to testosterone occurs in gonads and adipose tissue.

24. *What are the clinical disorders associated with discordance in adrenarche, pubarche, and gonadarche*?

Adrenarche is the consequence of activation of hypothalamo–pituitary–zona reticularis axis and results in secretion of DHEA and DHEAS. The presence of gonads is essential for the conversion of weaker androgens DHEA and DHEAS to testosterone and consequent pubarche. Gonadarche is due to activation of hypothalamo–pituitary–gonadal axis, thereby resulting in secretion of respective gonadal steroids. In a normal individual, adrenarche is followed by pubarche and gonadarche. However, there are many disorders which are associated with discordance in adrenarche, pubarche, and gonadarche, and these are summarized in the table given below.

Disorders	Adrenarche	Pubarche	Gonadarche
Premature adrenarche	+	+	–
GDPP (onset <6 years)	–	May be present in girls Present in boys	+
GDPP (onset >6 years)	+	+	+

Disorders	Adrenarche	Pubarche	Gonadarche
Turner's syndrome	+	–	–
Congenital adrenal hyperplasia (21α-hydroxylase)	+	+	–
Kallmann syndrome	+	Absent in girls Present in boys	–
Constitutional delay in growth and puberty	–	–	–

25. *What is isolated premature pubarche*?

Isolated premature pubarche is characterized by early appearance of pubic/axillary hair without other secondary sexual characteristics and is preceded by premature adrenarche. Growth velocity and bone age are usually appropriate or modestly increased for chronological age; however, the final adult height is within the target range. Serum adrenal androgens level are mildly elevated and LH response to GnRH is prepubertal. Children with small-for-gestational age are at risk for childhood obesity and later may manifest as premature pubarche. The exact mechanism remains elusive; however, increased secretion of adrenal androgens due to increased 17, 20 lyase activity in zona reticularis or increased sensitivity to circulating androgens has been proposed as the cause for isolated premature pubarche.

26. *What are the causes of premature pubarche*?

Besides isolated premature pubarche, a normal variant, the other causes of premature pubarche include late-onset congenital adrenal hyperplasia (e.g. 21α-hydroxylase deficiency), virilizing ovarian/adrenal neoplasms, glucocorticoid resistance syndrome, and exogenous androgen exposure.

27. *A 5-year-old girl presented with appearance of pubic hair. On examination, she was obese, had no breast development, and no signs of virilization. What is the likely diagnosis*?

The probable diagnosis in the index patient is premature pubarche due to obesity-related premature adrenarche. The pointers to the diagnosis are isolated appearance of pubic hair without any other secondary sexual characteristics, normal height velocity, and appropriate bone age. Premature pubarche in obese children is due to hyperinsulinemia and increased serum free IGF1 (due to insulin-mediated decreased IGFBP1) leading to increased adrenal androgen synthesis, and hence premature adrenarche.

28. *Should all patients with premature pubarche undergo biochemical evaluation*?

Assessment of bone age helps in predicting the need for biochemical evaluation in a child with premature pubarche. If the child has premature pubarche without any other signs of androgenization and bone age is not significantly advanced (i.e., bone age >2.5 SD for chronological age), the child possibly has premature

adrenarche/isolated premature pubarche. Fasting plasma insulin and glucose should be measured in children with obesity. If a child has premature pubarche along with other signs of androgenization (clitoromegaly in girls and penile enlargement in boys) or bone age is significantly advanced, child should be actively evaluated for pathological causes of premature pubarche. The evaluation includes 17α-hydroxyprogesterone (17-OHP), serum DHEAS, and total testosterone. A baseline serum 17-OHP of >2 ng/ml mandates ACTH stimulation test; serum 17-OHP level 10–100 ng/ml suggests a diagnosis of late-onset congenital adrenal hyperplasia, and serum 17-OHP level >100 ng/ml confirms the diagnosis of simple virilizing CAH due to 21α-hydroxylase deficiency. Serum testosterone >0.6 nmol/L and DHEAS >115 μg/dl suggest androgen excess, while a serum testosterone >6.94 nmol/L and DHEAS >700 μg/dl suggest virilizing ovarian and adrenal neoplasm, respectively. A scheme of workup for premature pubarche is illustrated below (Fig. 6.4).

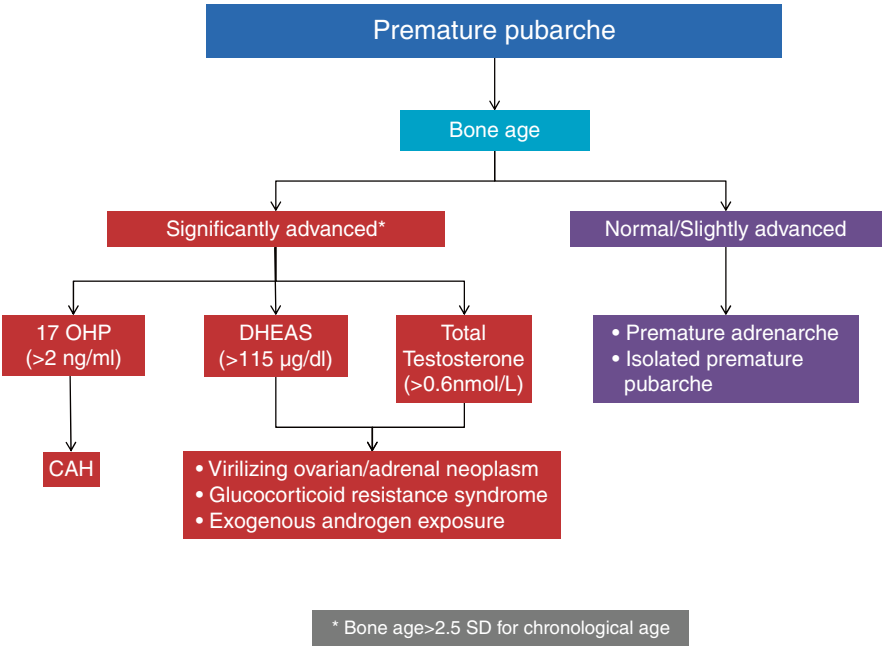

Fig. 6.4 Approach to a child with premature pubarche

29. *What is the role of metformin in premature pubarche*?

Girls with premature pubarche due to obesity-mediated hyperinsulinemia have an increased risk of polycystic ovarian disease (PCOD) and metabolic syndrome. Treatment with metformin in girls with premature pubarche has been shown to prevent the occurrence of early menarche and also reduces the risk of future development of PCOD and metabolic syndrome. Further, metformin decreases visceral adipose tissue and increases lean body mass, thereby leading to improvement in body composition of these adolescents.

30. *What are the causes of premature pubarche with genital ambiguity*?

Girls with CAH due to 21α-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase type 2 deficiency, and 11β-hydroxylase deficiency present with premature pubarche and genital ambiguity, while boys with CAH due to 3β-hydroxysteroid dehydrogenase type 2 deficiency manifest with premature pubarche along with genital ambiguity. However, boys with 21α-hydroxylase deficiency and 11 β-hydroxylase deficiency have premature pubarche with normal genitalia.

31. *A 3-year-old boy presented with history of brief intermittent staring followed by uncontrollable laughter and giggling. On evaluation, he had pubic hair tanner stage 2 and bilateral testicular enlargement of size 6 ml. MR imaging revealed an extrasellar mass in the hypothalamic region. What is the likely diagnosis*?

The most likely diagnosis in the index case is GDPP due to hypothalamic hamartoma. The unique features of precocious puberty associated with hypothalamic hamartoma include early age of presentation (2–4 years of age), absence of symptoms of mass effect, and gelastic seizures. Precocious puberty associated with hypothalamic hamartoma is more common in girls than in boys, but gelastic seizures are more frequent in boys. In the index case, appearance of pubic hair at 3 years of age without adrenarche is due to testicular production of testosterone. In girls, precocious puberty associated with hypothalamic hamartoma presents as GDPP with progressive and sequential development of secondary sexual characteristics, but may not have pubarche in those with onset below 6 years of age. After biochemical confirmation of GDPP, GnRH agonists are the treatment of choice for patients with hypothalamic hamartoma.

32. *What is hypothalamic hamartoma*?

Hypothalamic hamartoma is a bundle of disorganized neuronal tissue comprising of GnRH neurons. It arises due to faulty migration of GnRH neurons from

olfactory placode to hypothalamus during embryonic life. These hamartomas may be located at hypothalamus, tuber cinereum, or mammillary bodies. This occurs as a result of gain-of-function mutation of KAL1 gene with ectopic location of GnRH neurons, contrary to Kallmann syndrome which is due to loss-of-function mutation of KAL1 gene. In addition, TGF-α, a member of epidermal growth factor family, has also been implicated in its pathogenesis. Pallister–Hall syndrome, an autosomal dominant disorder, due to mutation of the GLP3 gene, can also be associated with hypothalamic hamartoma (Fig. 6.5).

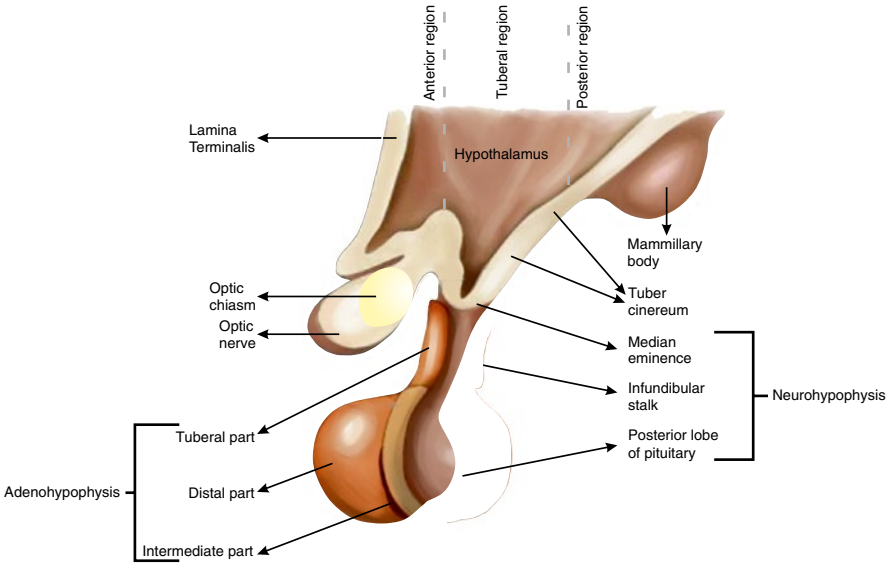

Fig. 6.5 Pictorial representation of anatomy of hypothalamo–pituitary region with focus on mammillary body, tuber cinereum, and hypothalamus; the common sites for the development of hamartoma

33. *How does hypothalamic hamartoma result in precocious puberty*?

Ectopically placed GnRH neurons (accessory hypothalamus) escape the inhibitory neuroendocrine regulation as opposed to eutopically placed GnRH neurons in the arcuate nucleus of hypothalamus. TGF-α, a member of epidermal growth factor family, has also been implicated in increasing the GnRH secretion from ectopic GnRH neurons. Further, hamartoma may exert mass effect on surrounding eutopic hypothalamic GnRH neurons; thereby, resulting in premature reactivation of GnRH pulse generator (Fig. 6.6).

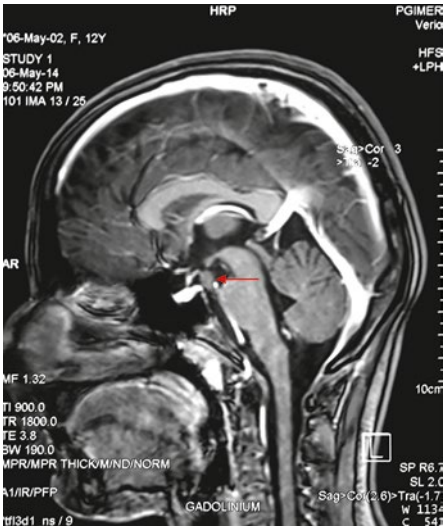

Fig. 6.6 Sagittal CEMR depicting small isointense well-defined lesion close to mammillary body suggestive of hypothalamic hamartoma (*red arrow*)

34. *What is gelastic seizure*?

Gelastic seizures are characterized by irresistible episodes of laughter accompanied with dysautonomia (sweating, flushing, and tachycardia) without impairment in sensorium. The most common cause of gelastic seizure is hypothalamic hamartoma. Patients with hypothalamic hamartomas of size >10 mm or located near mammillary bodies are predisposed for gelastic seizures. Although rare, lesions extending up to the floor of third ventricle (e.g., gliomas, meningioma, basilar artery aneurysms) may also result in gelastic seizure. These seizures are usually refractory to conventional antiepileptic therapy and may require surgical excision/stereotactic radiotherapy.

35. *A 6-year-old boy presented with increased penile length and appearance of pubic hair. On evaluation, his testicular volume was 2 ml bilaterally. Is it GDPP or GIPP*?

The index patient had increase in penile length and premature pubarche without testicular enlargement, thereby suggesting a diagnosis of GIPP. Gonadotropin-dependent precocious puberty (GDPP) is due to the reactivation of HPG-axis with a sequential and progressive evolution of secondary sexual characteristics like testicular enlargement followed by phallic enlargement in boys and thelarche followed by menarche in girls, as seen in normal puberty. During normal pubertal development, testicular growth (6–8 ml) precedes phallic enlargement in boys, and menarche appears at breast Tanner stage 4 in girls. However, in GIPP, there is discordance in the appearance of secondary sexual characteristics; penile enlargement can occur without testicular growth in boys and menarche may occur even at breast Tanner stage 2 in girls. This discordance is due to inappropriate exposure to sex steroids, both in tempo and quantity, as pubertal development in GIPP is independent of HPG-axis reactivation.

36. *What are the causes of testicular enlargement with GIPP*?

GIPP is independent of reactivation of HPG-axis; hence, it is not accompanied with testicular enlargement. However, certain clinical disorders can have bilateral symmetrical/asymmetrical testicular enlargement despite GIPP, and these disorders include testotoxicosis, primary hypothyroidism, McCune–Albright syndrome, and congenital adrenal hyperplasia with concurrent testicular adrenal rest tumor (TART) and hCG-secreting tumors like germinoma and hepatoblastoma. On the contrary, Leydig cell tumor may have unilateral testicular enlargement. All of these disorders, except primary hypothyroidism, are associated with pubarche and phallic enlargement.

37. *A 7-year-old boy presented with appearance of pubic hair. On examination, his testicular volume was 4 ml bilaterally, stretched penile length was 8 cm, and pubic hair staging was* P_3. *Hormonal profile revealed LH <0.1 μIU/ml and testosterone 33 nmol/L. What are the possibilities*?

The presence of testicular enlargement with penile growth and pubarche in a 7-year-old boy suggests a clinical possibility of GDPP. However, the index patient had disproportionate penile enlargement and pubic hair growth as compared to testicular growth, which is a clue to the presence of GIPP. Hormonal evaluation showed suppressed LH and elevated testosterone. In view of testicular enlargement and low diagnostic sensitivity (35 %) of basal LH in establishing the diagnosis of GDPP, GnRH stimulation test was performed. Absence of pubertal LH response to GnRH confirmed the diagnosis of GIPP. The disorders associated with LH-independent testosterone secretion (GIPP) include congenital adrenal hyperplasia, hCG-secreting tumors, adrenal or Leydig cell tumors, McCune–Albright syndrome, testotoxicosis, and exogenous testosterone therapy. The index patient had symmetrical testicular enlargement in the presence of GIPP, the differential diagnosis in the given scenario include CAH with testicular adrenal rest tumor, hCG-secreting tumors, McCune–Albright syndrome, and testotoxicosis. Further, ACTH-stimulated 17-OHP was normal, and serum hCG was 12,000 IU/L (N < 0.8), suggesting a diagnosis of hCG-secreting tumor. USG and CT scan of the abdomen did not reveal any abnormality and MRI brain demonstrated a mass lesion in the region of third ventricle and a possibility of germ cell tumor was considered, and the patient was subjected to chemo- and radiotherapy (Fig. 6.7). The correlation between pubic hair staging and testicular volume in normal children is shown in the table given below.

Pubic hair stage	**Testicular size (ml) mean ± SE**
P_1	1.9 ± 0.02
P_2	3.3 ± 0.06
P_3	5.3 ± 0.24
P_4	10.9 ± 0.34
P_5	15.6 ± 0.27

Adapted from Lall K, Singhi S, Gurnani M, Chowdhary B, Garg O. Normal testicular volume in school children. *Indian J Pediatr*. 1980;47(5):389–93.

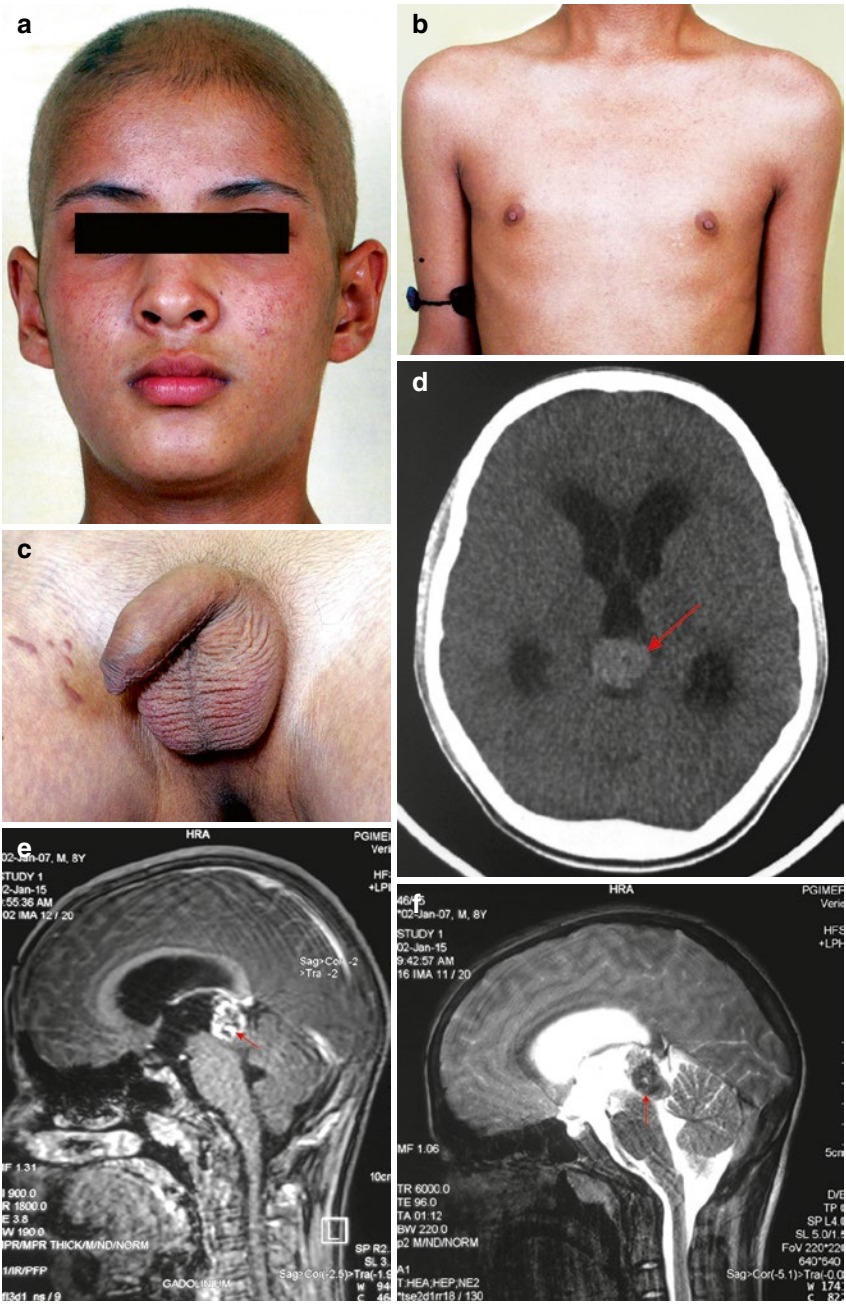

Fig. 6.7 (**a**) A 7-year-old boy with GIPP due to hCG secreting intracranial germinoma. (**b**) He did not have gynecomastia. (**c**) External genitalia showing disproportionate penile enlargement in relation to testicular volume (bilateral testicular volume 4 ml). (**d**) Axial CT head showing isodense lesion in the pineal region (*red arrow*) suggestive of germ cell tumor (**e**, **f**) Sagittal CEMR showing heterogeneously enhancing mass in the pineal region in T1 image (*red arrow*) and hypointense on T2 image (*red arrow*). Note the loss of scalp hair after chemotherapy

38. *What are the causes of premature testicular enlargement without precocious puberty*?

    The causes of premature testicular enlargement (symmetrical or asymmetrical) without precocious puberty include infiltrative disorders like amyloidosis, sarcoidosis, tuberculosis, lymphoma, and fragile X syndrome.

39. *What is fragile X syndrome*?

    Fragile X syndrome is an X-linked dominant disorder characterized by typical facies (long and narrow face, large ears, prominent forehead, and macrognathia), flat foot, and hyperextensible fingers. Intellectual disability is the key abnormality and is more common in affected males as compared to females. It may be accompanied with autistic behavior and social anxiety. Although enlargement of the testes may occur during prepubertal period, macroorchidism (testicular volume >30 ml) is observed only after the onset of puberty. The presence of macroorchidism and intellectual disability is the hallmark abnormalities of fragile X syndrome. Macroorchidism is related to increased connective tissue component rather than due to expansion of seminiferous tubules. The disorder is due to mutations in the *FMR* (fragile X mental retardation) gene. This gene is abundantly expressed in the brain and testes and encodes for a protein which is essential for neuronal synaptic plasticity and explains the intellectual disability in these patients; however, the mechanism of macroorchidism remains elusive (Fig. 6.8).

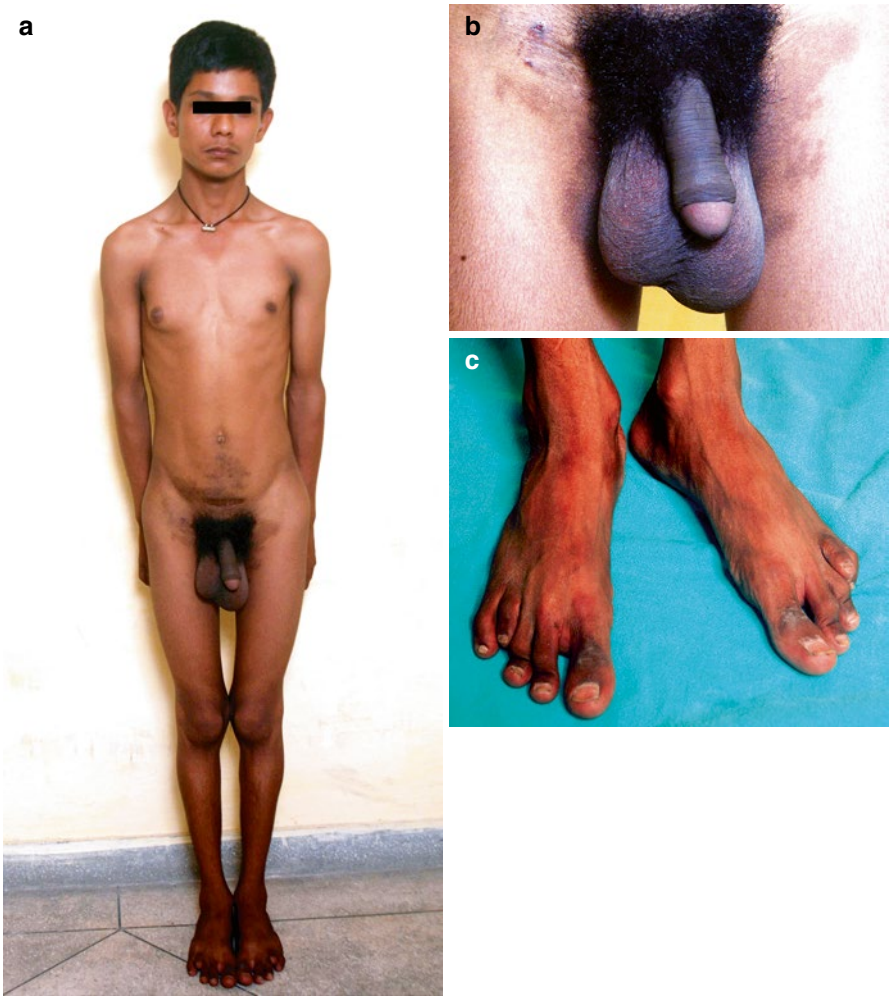

Fig. 6.8 (**a**) A 14-year-old boy presented with intellectual disability and bilateral testicular enlargement. He also had unilateral gynecomastia, (**b**) bilateral orchidomegaly (testicular volume >100 ml), and (**c**) short fourth metatarsals. A diagnosis of fragile X syndrome was considered

40. *What are the unique features of precocious puberty associated with primary hypothyroidism*?

    The unique features of precocious puberty associated with primary hypothyroidism include decreased growth velocity, absent/sparse pubic hair, and delayed bone age. Boys with precocious puberty associated with primary hypothyroidism have testicular enlargement without reactivation of HPG-axis, while the girls usually present with menarche and inappropriate progression of secondary sexual characteristics, e.g., menarche at Tanner breast stage 2. In addition, girls may also have galactorrhoea and multicystic ovaries. Absent/sparse pubic hair despite testicular enlargement is due to prepubertal levels of testosterone in boys and deleterious effect of decreased levels of thyroxine on pilosebaceous unit. Despite raised levels of gonadotropins (FSH), LH response to GnRH is prepubertal (GIPP) (Fig. 6.9).

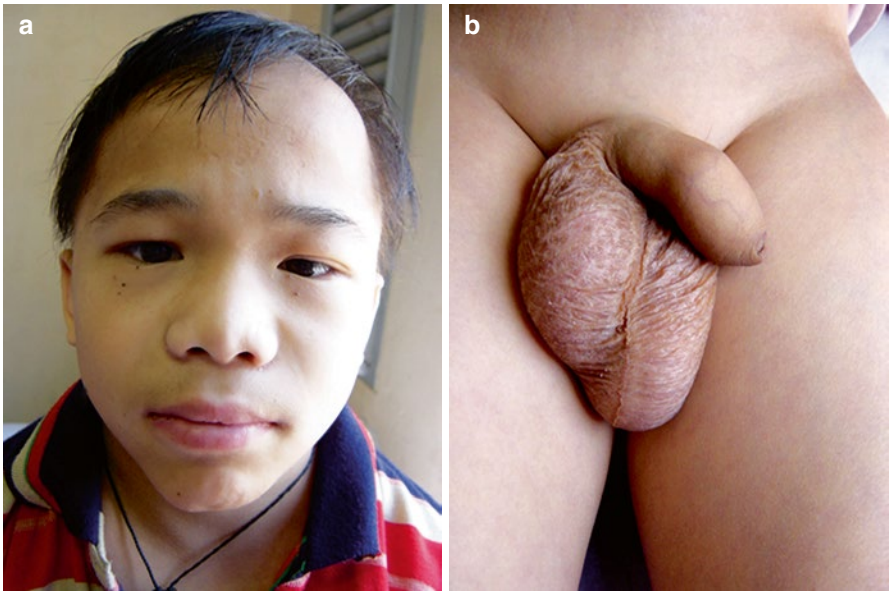

Fig. 6.9 (**a**) A 12-year-old boy with overt features of primary hypothyroidism. (**b**) Bilateral testicular enlargement without pubic hair

41. *A 2-year-old girl presented with breast development* (*B2*) *and one episode of vaginal bleed. Her MR imaging showed a sellar mass. What is the likely diagnosis*?

    Precocious puberty in a child with a sellar mass is almost always due to primary hypothyroidism, as patients with sellar mass usually have delayed puberty,

while intracranial lesions associated with precocious puberty are either suprasellar (astrocytoma) or extrasellar (germinoma, hamartoma) in location. Rarely, patients with intrasellar craniopharyngioma with suprasellar extension may also present with precocious puberty. The sellar or sellar–suprasellar mass in primary hypothyroidism is due to thyro-lactotrope hyperplasia and simulates pituitary tumor. Therefore, thyroid function test should always be performed in any patient with precocious puberty as optimal treatment with levothyroxine not only results in regression of secondary sexual characteristics but also prevents inadvertent pituitary surgery and treatment with GnRH agonist. The index patient had serum T_4 2.2 µg/dl, TSH 267 µIU/ml, and prolactin 110 ng/ml, and ^{99m}Tc thyroid scan showed ectopic thyroid. She was treated with levothyroxine. After 6 months of therapy, she had regression in breast size and resolution of sellar–suprasellar mass (Fig. 6.10).

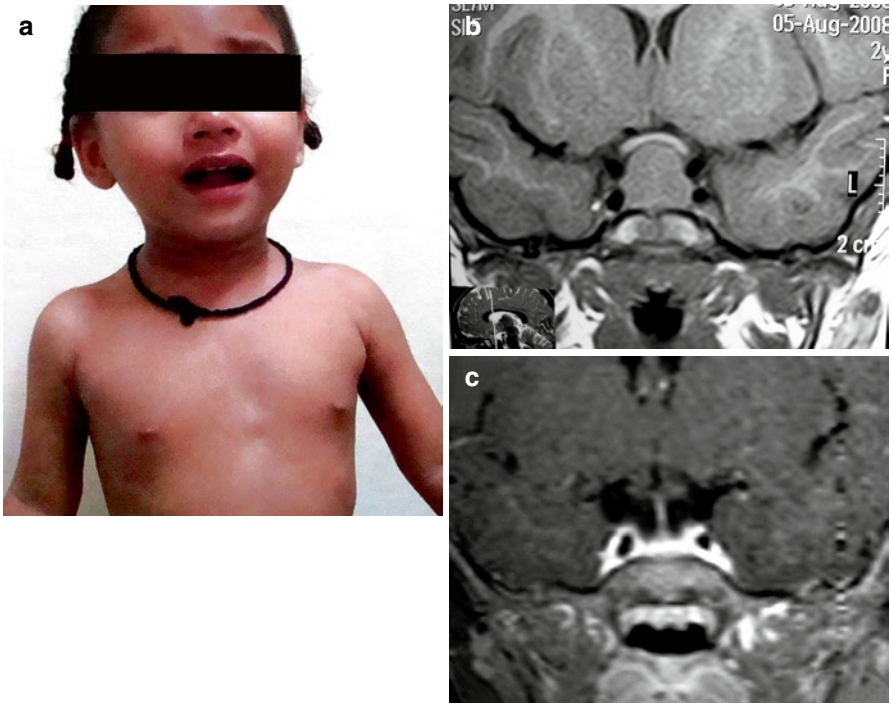

Fig. 6.10 (**a**) A 2-year-old child with primary hypothyroidism presented with breast development (B_2). (**b**) Coronal CEMR showing homogenous pituitary enlargement with splaying of optic chiasma suggestive of thyro-lactotrope hyperplasia. (**c**) Coronal CEMR showing marked reduction in the pituitary size after 6 months of L-thyroxine replacement in the same patient

42. *What are the mechanisms implicated in the development of precocious puberty in children with primary hypothyroidism*?

Numerous mechanisms have been postulated for the development of precocious puberty in children with primary hypothyroidism. Loss of negative feedback of thyroxine at the level of hypothalamus results in increased levels of thyrotropin-releasing hormone (TRH), which acts on GnRH receptor ("specificity-spillover") at pituitary, thereby leading to increase in gonadotropin secretion (Van Wyk–Grumbach phenomenon) particularly FSH. Further, delayed clearance of gonadotropins also contributes to increased levels of FSH. Despite increase in gonadotropins, LH response to GnRH is prepubertal. Increased serum TSH acts on FSH receptor at gonads ("specificity-spillover") and possibly augmented FSH sensitivity due to FSH receptor polymorphisms result in increased sex steroid production.

43. *A 3-year-old girl presented with breast development Tanner stage 2 and vaginal bleed. On examination, she had a café-au-lait macule. What is the likely diagnosis*?

Inappropriate progression of secondary sexual characteristics (menarche at breast Tanner stage 2) suggests a diagnosis of GIPP and the presence of café-au-lait macule points to a diagnosis of McCune–Albright syndrome (MAS). MAS is characterized by a triad of polyostotic/monostotic fibrous dysplasia, café-au-lait macule, and endocrinopathies, precocious puberty being the most common endocrinopathy. Precocious puberty in MAS is a result of development of follicular cyst in response to constitutive activation of Gsα-subunit of FSH receptor, and café-au-lait macule is due to constitutive activation of melanocyte-stimulating hormone (MSH) receptor. In patients with GIPP, there is inappropriate progression of secondary sexual characteristics because of rapid, inordinate, and excessive production of gonadal steroids resulting in menarche prior to breast Tanner stage 4–5. This is because development of breast requires exposure to relatively lower concentration of estradiol for a prolonged duration, while development of uterus and endometrium requires exposure to higher concentration of estradiol.

44. *What are the unique features of precocious puberty in girls with MAS*?

Precocious puberty associated with MAS is more common in girls and usually presents in infancy. The most common presenting manifestation of precocious puberty in girls with MAS is vaginal bleed, which may not always be accompanied with breast development. Café-au-lait macule is usually present at diagnosis; however, skeletal fibrous dysplasia may not be present initially and often evolve with time. Acyclical vaginal bleed and "waxing and waning" breast size are other atypical manifestations of precocious puberty associated with MAS, which occurs as a result of intermittent formation and involution of ovarian follicular cyst, despite constitutive activation of FSH receptor. Further, these

children should remain under regular surveillance as they may develop other endocrinopathies like hyperthyroidism and acrogigantism during follow-up.

45. *A 5-year-old boy presented with aggressive behavior. On examination, he had penile enlargement with bilateral testicular volume 6 ml. His father also had history of precocious puberty. Serum LH was undetectable and GnRH stimulated LH was 1.1 mIU/ml. What is the likely diagnosis*?

Bilateral testicular enlargement, disproportionate penile growth in relation to testicular enlargement, undetectable LH, and prepubertal LH response to GnRH with a family history of precocious puberty suggest the diagnosis of familial testotoxicosis. Although precocious puberty in testotoxicosis is gonadotropin-independent, there is an increase in testicular volume due to gain-of-function mutation of LH receptor. Testicular enlargement occurs as a result of increased intratesticular testosterone which directly promotes growth of seminiferous tubules. Patients with testotoxicosis can have spermatogenesis, which is rare in other causes of GIPP. A close differential diagnosis for testotoxicosis is precocious puberty associated with MAS; however, the absence of café-au-lait macule and fibrous dysplasia with presence of family history of precocious puberty favor a diagnosis of familial testotoxicosis in the index patient. Further, testicular enlargement is usually asymmetrical in MAS, while it is symmetrical in testotoxicosis.

46. *Why is precocious puberty in familial testotoxicosis only confined to boys*?

Familial testotoxicosis is due to the gain-of-function mutation of LH receptor and is inherited in an autosomal dominant pattern. Boys with familial testotoxicosis present with increased growth velocity, penile enlargement with symmetrical and moderate increase in testicular volume, premature pubarche, and accelerated skeletal maturation. On the contrary, girls harboring LH receptor mutations do not manifest with precocious puberty. This is because LH-induced (due to LH receptor activation) ovarian androgens are not aromatized to estrogen in the absence of FSH, as aromatase activity is FSH-dependent. In addition, FSH is essential for induction of LH receptors on theca cells. This also explains the lack of development of precocious puberty in girls with hCG-secreting tumors and the development of precocious puberty in McCune–Albright syndrome with gain-of-function mutation of Gsα subunit involving both FSH and LH receptor.

47. *What is* "*mixed precocious puberty*"?

Premature reactivation of HPG-axis in a patient with GIPP is termed as "mixed precocious puberty" (GDPP superimposed on GIPP). It is commonly seen in children with CAH, McCune–Albright syndrome, and testotoxicosis who have delayed initiation of treatment, particularly in those with advanced bone age

(>10 years in girls and >11 years in boys). This is because advanced bone age of >10 years in girls and >11 years in boys predicts imminent reactivation of HPG-axis. Abrupt lowering of persistently elevated sex steroids after initiation of therapy in a long-standing untreated patient with GIPP results in premature reactivation of a primed HPG-axis. The management of GDPP superimposed on GIPP includes GnRH agonist for mixed precocious puberty, in addition to specific treatment for the primary disorder (Fig. 6.11).

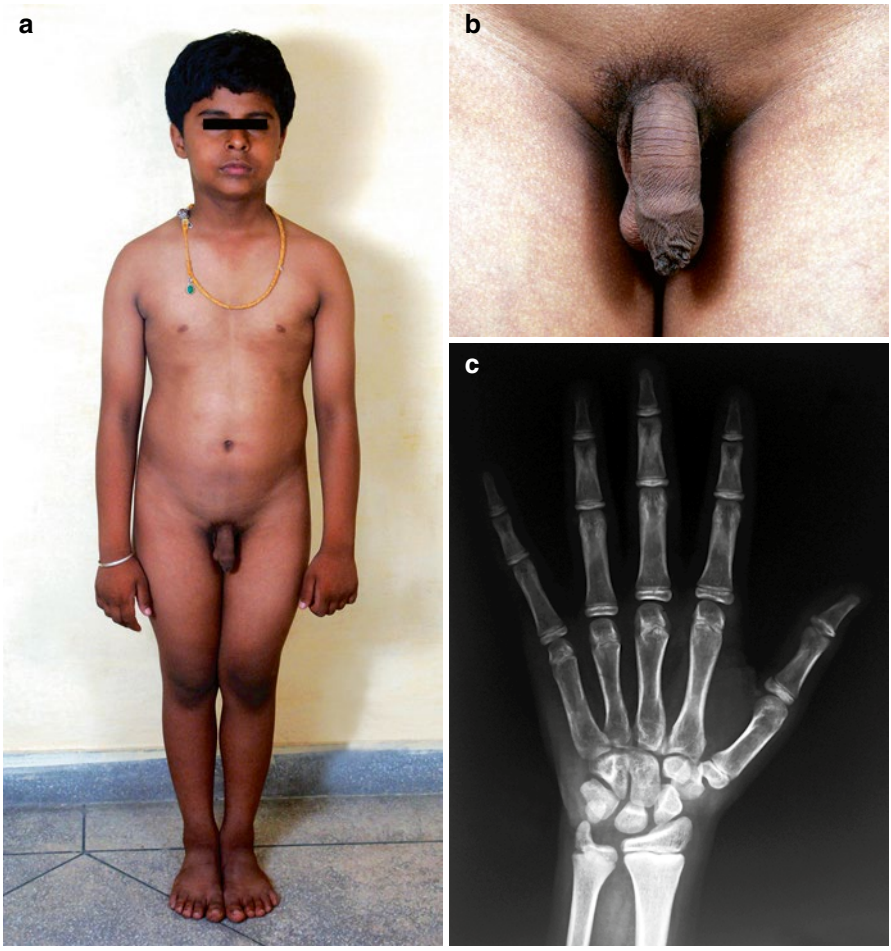

Fig. 6.11 (**a**) Mixed precocious puberty in a 7-year-old boy with congenital adrenal hyperplasia due to 21α-hydroxylase deficiency after initiation of glucocorticoid therapy. (**b**) External genitalia showing stretched penile length 10 cm, Tanner pubic hair stage 2, and bilateral testicular volume 6 ml. (**c**) X-ray of left hand AP view showing bone age of 13 years

48. *How to evaluate a boy with precocious puberty*?

In a boy with appearance of secondary sexual characteristics below 9 years of age, basal serum LH, testosterone, and LH response to GnRH should be performed. Basal LH ≥0.3 IU/L (ICMA) and testosterone >1.7 nmol/L (RIA) suggest a diagnosis of GDPP in boys. Peak LH >5.0 IU/L at 3h, after subcutaneous administration of aqueous leuprolide (20 μg/Kg), has a sensitivity and specificity of 83 % and 97 %, respectively, for the diagnosis of GDPP in boys. Further tests are required to establish the etiological diagnosis of precocious puberty including MR brain imaging or CT abdomen. An approach to a boy with precocious puberty is summarized in the algorithm given below (Fig. 6.12).

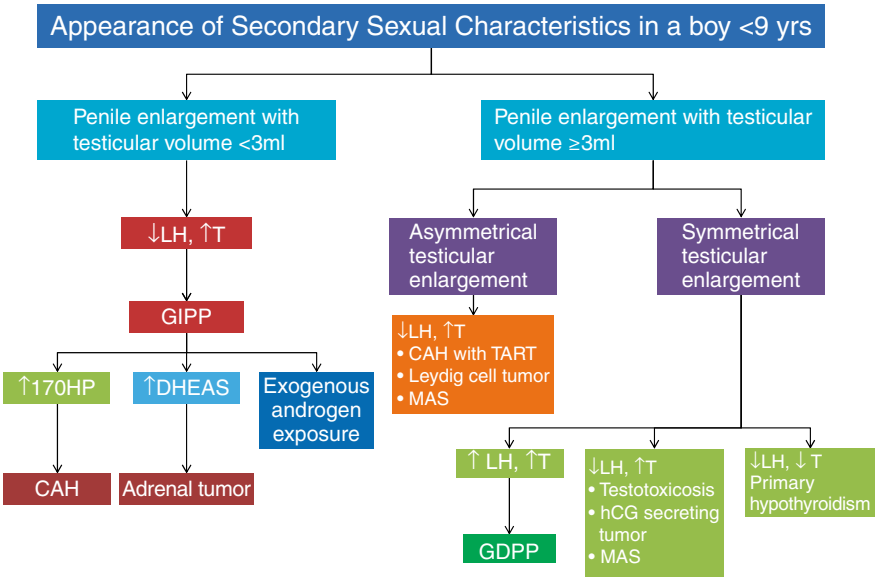

Fig. 6.12 Approach to a boy with precocious puberty

49. *Why is LH, but not FSH response to GnRH, used for the diagnosis of GDPP*?

FSH response to GnRH is present at all ages irrespective of reactivation of HPG-axis, while LH response to GnRH is present only after reactivation of HPG-axis. Therefore, rise in LH, but not FSH, reflects the onset of GnRH pulse generator activity. This is because of stringent neuroendocrine regulation of LH as compared to FSH at the level of hypothalamus, which is mediated by opioids, GABA, and dopamine. Hence, in clinical practice, basal and stimulated LH is estimated to establish the diagnosis of GDPP.

50. *How to evaluate a girl with isosexual precocious puberty*?

An approach to a girl child with precocious puberty is summarized in the algorithm given below (Fig. 6.13).

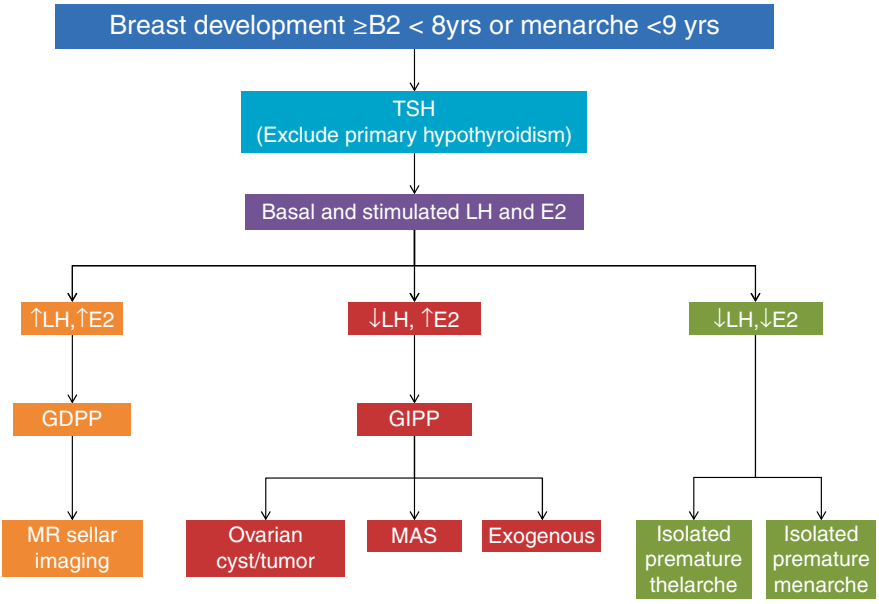

Fig. 6.13 Approach to a girl with precocious puberty

51. *What are the diagnostic cutoffs for basal and stimulated gonadotropins/estradiol for differentiating between GDPP and GIPP in a female child*?

In a girl presenting with precocious puberty, basal serum LH, estradiol, and LH response to GnRH should be performed. Basal LH ≥0.3 IU/L (ICMA) has a sensitivity and specificity of 35 % and 100 %, respectively, for the diagnosis of GDPP, and basal serum estradiol ≥10 pg/ml has a sensitivity and specificity of 39 % and 100 %, respectively. Basal serum estradiol >100 pg/ml suggests a diagnosis of precocious puberty due to ovarian cyst/tumor. Various GnRH agonists (e.g., leuprolide, triptorelin) have been used for the assessment of LH response to GnRH. Peak LH ≥5.0 IU/L at 60 or 120 min, after subcutaneous administration of aqueous leuprolide (20 μg/Kg) and/or serum estradiol ≥50 pg/ml at 24h, is suggestive of GDPP. Peak LH ≥8 IU/L at 3h after subcutaneous administration of aqueous triptorelin acetate (0.1 mg/m^2, maximum of 0.1 mg) and/or serum estradiol ≥80 pg/ml at 24h suggests a diagnosis of GDPP. However, it should be remembered that these cutoffs may not be applicable to children <3 years of age because of lack of cutoffs for defining precocity during mini-puberty in this age

group. The sensitivity and specificity of various diagnostic cutoffs for basal and stimulated LH and estradiol are summarized in the table given below.

Parameters	Cutoffs	Sensitivity (%)	Specificity (%)
Basal			
LH	≥0.3 IU/L	35	100
E_2	≥10 pg/ml	39	100
Leuprolide (20 μg/Kg)			
LH at 60/120 min	≥5 IU/L	78	100
E_2 at 24h	≥50 pg/ml	84	100
Triptorelin (0.1 mg/m2)			
LH at 3h	≥8 IU/L	76	100
E_2 at 24h	≥80 pg/ml	79	100

52. *What are the ultrasonographic parameters used to determine the onset of puberty in a girl child*?

Ultrasonography is a noninvasive, inexpensive, and widely available diagnostic modality with a good sensitivity and specificity for the evaluation of precocious puberty in girls. Assessment of uterine length, uterine body/cervix ratio, uterine echogenicity, and ovarian volume are the sonographic parameters to determine the onset of puberty. The various age-based cutoffs are mentioned in the table given below.

Parameters	Neonate	Prepubertal	Pubertal
Uterine length (cm)	3.5[a]	<3.5	5–8
Uterine body/cervix ratio	1:2	1:1	2–3:1
Endometrial echo	Echogenic	Not apparent	Echogenic
Uterine shape	–	Tubular shaped	Pear shaped
Ovarian volume (cm^3)	1	≤1.6	≥2.8

[a]Uterine length is greater in neonates as compared to prepubertal girls because of transplacental transfer of maternal estrogen

53. *What is the role of ultrasonography in the evaluation of precocious puberty in girls*?

Uterine length >3.5 cm, uterine volume >2 ml, presence of endometrial echo, and ovarian volume ≥2.8 ml on pelvic ultrasonography in a girl less than 8 years of age suggest the diagnosis of precocious puberty. These imaging characteristics are the evidence of estrogen exposure and denote onset of puberty, but do not differentiate between GDPP from GIPP. Increased ovarian volume is the best index for the diagnosis of precocious puberty, whereas increased uterine length (>3.5 cm) is the best discriminator between isolated premature thelarche and premature thelarche associated with precocious puberty. In addition, ovarian follicular cyst size <9 mm may be present in normal prepubertal girls

as well as in girls with GDPP; however, a follicular cyst size >9 mm suggests a diagnosis of GIPP. The presence of multiple cysts (six or more) suggests GDPP, while a single large cyst is a feature of GIPP. Breast ultrasonography is recommended to differentiate between lipomastia and true breast budding, as in current practice, referral for premature thelarche is not uncommon with increasing prevalence of childhood obesity (Fig. 6.14).

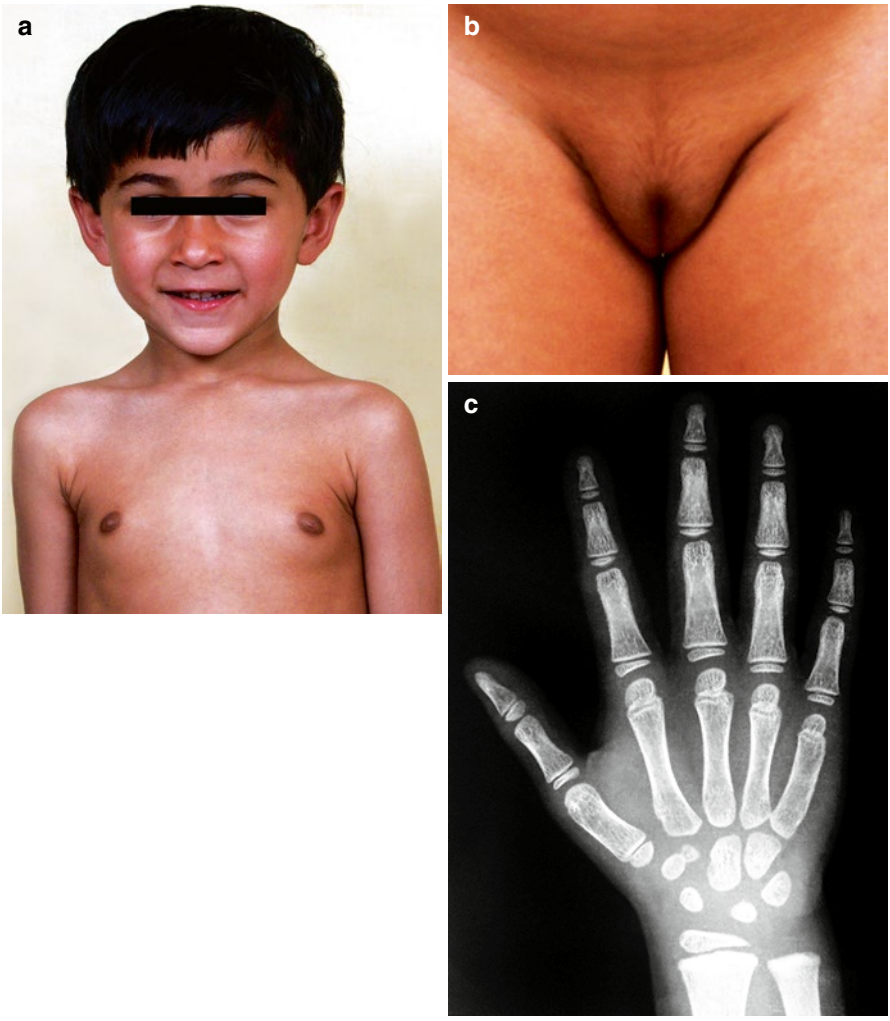

Fig. 6.14 (**a**) A 4-year-old girl with thelarche, B_3 (**b**) Tanner pubic hair stage 2, (**c**) X-ray of left hand showing bone age 5 years and 9 months (**d**) ^{99m}Tc MDP scan was normal. She had a follicular cyst of 12 mm suggestive of GIPP. Presence of pubarche denotes production of ovarian androgens by follicular cyst

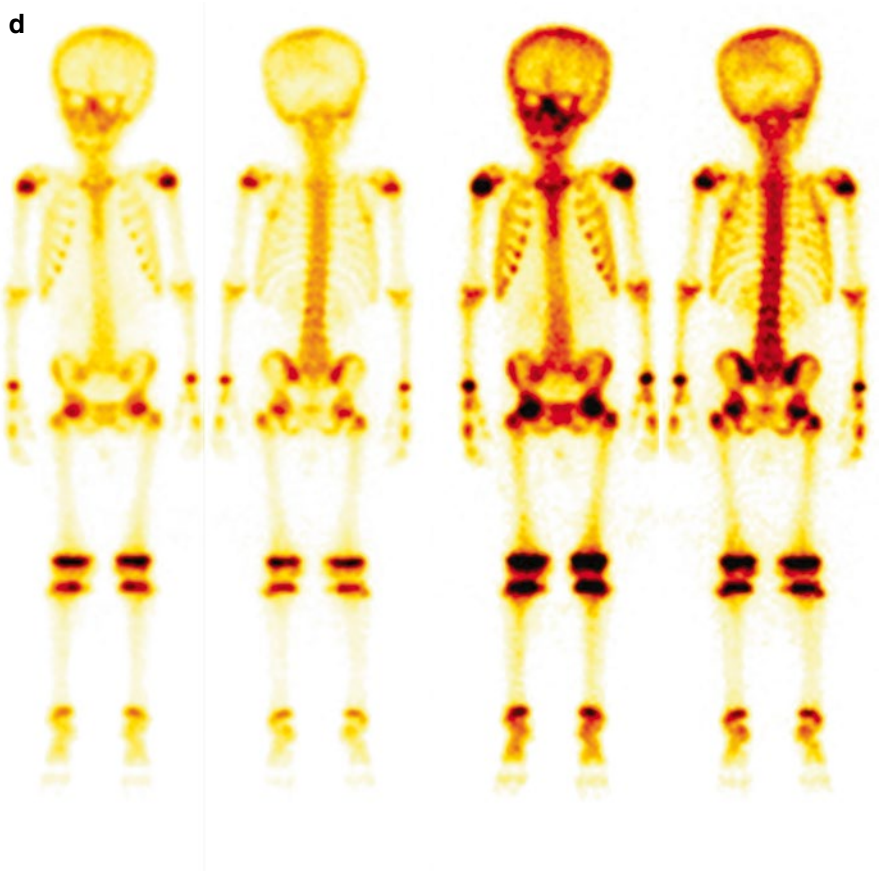

Fig. 6.14 (continued)

54. *What is the role of ultrasonography in the evaluation of precocious puberty in boys*?

    Ultrasonography of the testes is useful for determination of exact size of testes, particularly at Tanner stage 2 (testicular volume 3–4 ml or testicular length >25 mm). An ultrasonography testis is also useful in evaluation of disorders associated with asymmetrical testicular growth including Leydig cell tumor, MAS, and CAH with testicular adrenal rest tumor (TART). Leydig cell tumors are usually very small (2–3 mm) and can be missed on routine palpation of testes. Further, gynecomastia versus lipomastia can also be differentiated by ultrasonography of the breast.

55. *Is neuroimaging indicated in all children with GDPP*?

    Contrast-enhanced MRI brain is indicated in all children with GDPP. The probability of having an intracranial pathology is higher in boys than in girls. In

addition, girls with age of onset of precocity <6 years, patients with coexisting neurological manifestations (e.g., gelastic seizures, visual impairment), and those with rapid progression of puberty (progression from one stage to the next within 3–6 months) are also likely to have a CNS pathology as the cause of precocity. A pituitary height >6 mm with convex upper border suggests the presence of GDPP of any etiology. The common CNS lesions associated with precocious puberty include hypothalamic hamartoma, optic glioma associated with NF1, and suprasellar lesions (e.g., germinoma, pilocytic astrocytoma, arachnoid cyst, and rarely craniopharyngioma) (Figs. 6.15, 6.16, and 6.17).

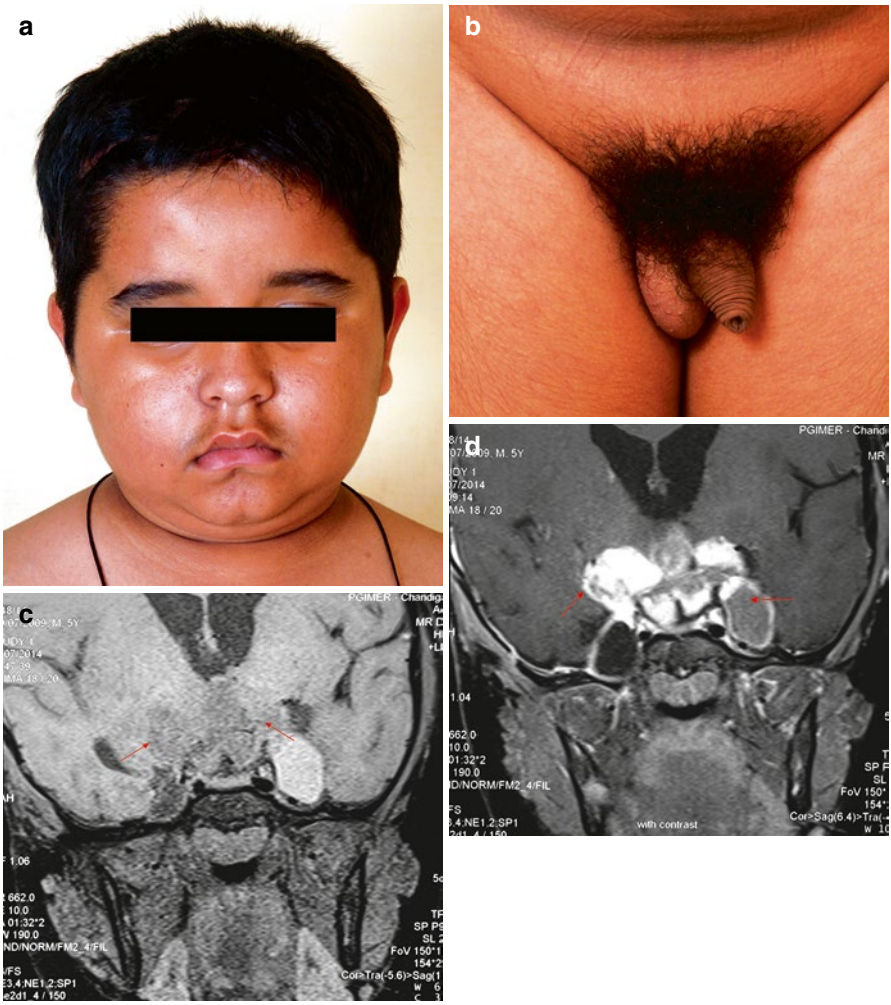

Fig. 6.15 (**a**) A 5-year-old boy with GDPP. (**b**) External genitalia showing Tanner pubic hair stage P_4 and bilateral testicular volume 10 ml. (**c**, **d**) Coronal T1 pre- and post-contrast MR showing a complex solid cystic suprasellar lesion (*red arrows*). Histopathology of tumor tissue was consistent with pilocytic astrocytoma

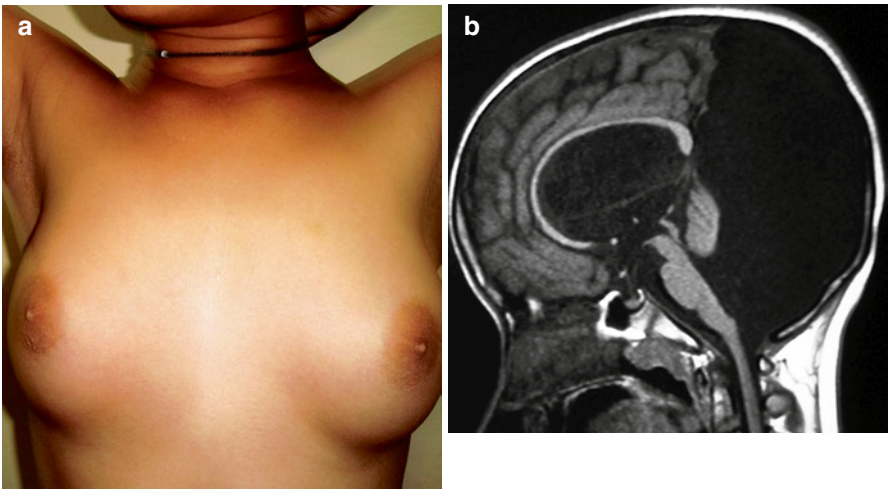

Fig. 6.16 (**a**) An 8-year-old girl with GDPP. (**b**) Sagittal CEMR shows a large retrocerebellar cystic lesion causing cerebellar vermian rotation and communicating with fourth ventricle suggestive of Dandy–Walker syndrome

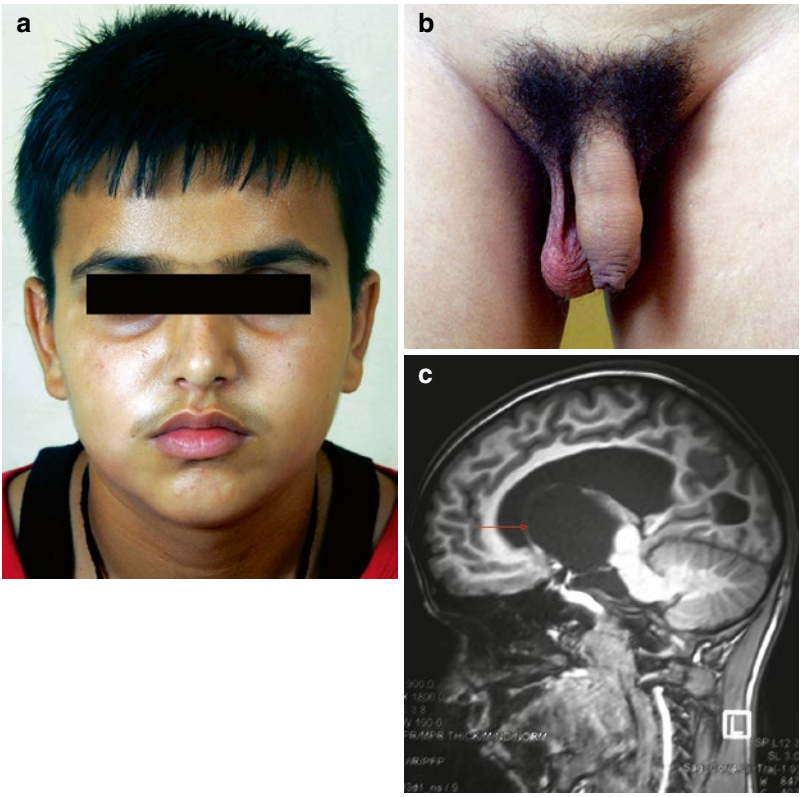

Fig. 6.17 (**a**) An 8-year-old boy with GDPP due to suprasellar arachnoid cyst. (**b**) External genitalia showing Tanner pubic hair stage 3 and bilateral testicular volume 6 ml. (**c**) Sagittal T1 MRI showing third ventricle arachnoid cyst (*red arrow*)

56. *How to treat a child with GDPP associated with an intracranial pathology*?

    Neurosurgical intervention is the treatment of choice in most patients with GDPP due to intracranial pathology, except in those with uncomplicated hypothalamic hamartoma. Despite curative surgery, medical therapy needs to be continued for the management of precocious puberty, as HPG-axis remains active once stimulated.

57. *How to treat a child with precocious puberty due to hypothalamic hamartoma*?

    Hypothalamic hamartoma is a slowly growing heterotopic mass comprised of disorganized neuronal tissue. These "tumors" cease to grow after the age of 8–12 years, as the development of brain tissue is complete by this age. GnRH agonist therapy is the treatment of choice for precocious puberty associated with hypothalamic hamartoma. Although there is regression of secondary sexual characteristics, size of hamartoma does not change with GnRH agonist therapy. Surgical intervention is required only in those with refractory seizures or mass effects.

58. *Do all children with GDPP require therapy*?

    Children with GDPP due to an organic cause should always be treated. Rapid progression of pubertal events over a period of 3–6 months, significant advancement of bone age (>2.5 SD for chronological age), or presence of psychosocial concerns are the indications of therapy in children with idiopathic GDPP. However, all children with idiopathic GDPP who do not require therapy should be kept under regular surveillance.

59. *A 5-year-old girl child presented with thelarche. On evaluation, she had Tanner breast stage B_3, height of 108 cm (50^{th} percentile), and bone age of 6.5 years, and MRI brain was normal. Biochemical evaluation confirmed a diagnosis of GDPP. How to proceed further*?

    Rapid advancement of puberty, accelerated height velocity, and significant advancement in bone age merit therapy in children with idiopathic GDPP. The index patient had idiopathic GDPP and at presentation, information regarding the rapidity of progression of secondary sexual characteristics and growth velocity were not available. In such a scenario, bone age may be a simple tool in deciding the need for therapy. Bone age of the child was 6.5 years and it was not significantly advanced (bone age <2.5 SD over chronological age), and hence a decision was made to closely follow up the child for progression of pubertal events and height velocity.

60. *How to decide whether bone age is significantly advanced over chronological age or not*?

In the above-said child, chronological age was 5 years and bone age was 6.5 years. From the table given below, it can be seen that at the age of 5 years, 1SD for bone age is 8.6 months for girls. A bone age >2.5 SD above chronological age is considered as significantly advanced.

i.e., 2.5 SD is 8.6 × 2.5 = 21.5 months = 1.79 years

Patient's chronological age + 2.5 SD = 5 years +1.79 year = 6.79 years

Since the child's bone age was 6.5 years (<2.5 SD advancement over chronological age), it was not considered significant.

The table given below shows standard deviation for bone age at various chronological ages in boys and girls from age of 1 year to 17 years.

Age (months)	Boys SD (months)	Girls SD (months)
12	2.1	2.7
18	2.7	3.4
24	4	4
30	5.4	4.8
36	6	5.6
42	6.6	5.5
48	7	7.2
54	7.8	8
60	8.4	8.6
66	9.1	8.9
72	9.3	9
84	10.1	8.3
96	10.8	8.8
108	11	9.3
120	11.4	10.8
132	10.5	12.3
144	10.4	14
156	11.1	14.6
168	12	12.6
180	14	11.2
192	15	15
204	15.4	15.4

With permission from Gilsanz V, Ratib O. *Hand bone age*. Berlin: Springer; 2005.

61. *How to treat a child with idiopathic GDPP*?

Aim of treatment in a child with idiopathic GDPP is to herald the progression of secondary sexual characteristics, prevent adverse psychosocial consequences,

and improve the final adult height. Therapeutic options for the medical management of GDPP include GnRH agonists, medroxyprogesterone acetate (MDPA), and cyproterone acetate. However, GnRH agonist is the treatment of choice as it is highly effective, safe, and has a beneficial effect on final adult height, particularly if the treatment is initiated before 6 years of age. Further, suppression of HPG-axis is reversible after discontinuation of GnRH therapy.

62. *What are the commonly used GnRH agonists for the management of GDPP*?

Leuprolide acetate depot and triptorelin acetate depot are the commonly used GnRH agonist for the management of GDPP. The recommended dose of leuprolide acetate depot is 140–300 μg/Kg/month intramuscularly, while that of triptorelin acetate depot is 60 μg/Kg/month intramuscularly. Three monthly formulations of these agonists are also available and are equally effective as compared to monthly preparations. Adverse events associated with use of GnRH agonist include initial flare, sterile abscess at injection site (3–10 %), hot flushes, and local rash. An initial "flare" response occurs within first 1–2 weeks of administration of GnRH agonist and manifests clinically as vaginal bleed in females and priapism in males. It occurs as a result of stimulation of GnRH receptors and consequent secretion of gonadotropins and gonadal steroids. This flare response can be prevented by the concurrent administration of MDPA.

63. *What are the advantages of GnRH agonist over MDPA in the management of GDPP*?

GnRH agonists are the treatment of choice for GDPP, because they effectively inhibit HPG-axis. Treatment with GnRH agonist results in regression of secondary sexual characteristics, improvement in final adult height, and possibly optimal psychosocial development. However, final adult height may not improve in children aged >6 years at initiation of therapy, possibly because of significant advancement of bone age at presentation. In this scenario, MDPA is an alternative option to prevent the progression of secondary sexual characteristics, especially in resource-constraint settings. Therapy with MDPA does not have a beneficial effect on final adult height possibly because of incomplete suppression of HPG-axis and deleterious effect of MDPA on epiphyseal growth plate (glucocorticoid-like effect). Prolonged therapy with MDPA results in cushingoid appearance and inhibition of HPA-axis.

64. *What is the role of GnRH antagonist in the management of precocious puberty*?

Long-acting preparations of GnRH antagonist can be used for the management of GDPP. GnRH antagonists have the advantage of immediate inhibition of HPG-axis, without any "flare" response. However, with prolonged therapy, there is a progressive increase in the dose requirement, as therapeutic effects of these drugs are offset by increased levels of GnRH. This occurs as a result of reduced feedback inhibition at hypothalamus due to decrease in gonadal steroids.

65. *How to monitor a child with precocious puberty on GnRH agonist therapy*?

Children with GDPP receiving GnRH agonist should be monitored for pubertal status, height velocity, and any treatment-related adverse events every three monthly. Hormonal evaluation should be performed after 8–12 weeks of initiation of therapy and every 3–6 monthly, thereafter. Bone age should also be assessed annually in these children.

66. *What is the predicted clinical response to GnRH agonist in a child with precocious puberty*?

Optimal therapy with GnRH agonist results in reversal of almost all features of precocity in girls including regression of breast size and pubic hair, cessation of menses, and reduction in ovarian and uterine volume. However, appearance of pubic hair while on therapy with GnRH agonist does not necessarily mean failure of therapy, as these agents have no effect on adrenal androgen synthesis. In boys, there is a decrease in testicular volume, thinning of pubic hair, resolution of acne and seborrhea, and improvement in aggressive behavior. These clinical improvements are evident within 6 months of initiation of therapy. Height velocity steadily decreases in the initial years of therapy, accompanied with retarded progression of bone age. In a study, it was shown that mean height velocity decreased from pretreatment value of 8.4 cm/year to 5.9 cm/year after 1 year of therapy with GnRH agonist, 5.3 cm/year after 2 years and 4 cm/year thereafter. Bone maturation was slowed, and the mean increase in bone age was 0.5 year per year.

67. *What are the predictors of optimal adult height in a child with GDPP at the initiation of GnRH agonist therapy*?

The predictors of optimal adult height in a child with GDPP at initiation of GnRH agonist therapy include younger age (<6 years), greater height at initiation of therapy, higher predicted adult height (based on bone age), and greater target height.

68. *What is the role of GH therapy in children with precocious puberty treated with GnRH Agonist*?

Children with GDPP who have significant advancement of bone age irrespective of age of presentation may be considered for rhGH therapy along with GnRH agonist to attain final adult height, though evidence for the use of rhGH therapy is limited.

69. *What is the predicted biochemical response to GnRH agonist therapy in a child with precocious puberty*?

Therapy with GnRH agonist results in an initial "flare," characterized by rise in FSH, LH, and gonadal steroids within 1–3 days of administration. This may manifest clinically as priapism in boys and vaginal bleed in girls. The initial flare is followed by inhibition of pulsatile secretion of FSH and LH within

15 days, while the suppression of gonadal steroids to prepubertal levels occurs by 2–4 weeks in girls and 6 weeks in boys.

70. *What are the hormonal parameters to be monitored in a child with precocious puberty on GnRH agonist therapy*?

Basal LH and estradiol/ testosterone (prior to the next dose) and stimulated LH (after 3h of GnRH agonist depot administration) are used for the assessment of response to GnRH agonist. However, the cutoffs for defining the adequacy of therapy are not well validated, as different studies have used different GnRH agonist preparations and variable assays to estimate LH and gonadal steroids. Further, it is not clear whether to monitor basal LH and gonadal steroids/stimulated LH and gonadal steroids or both during therapy with GnRH agonist. Nevertheless, a basal LH in prepubertal range (<0.3 IU/L), testosterone (<0.7 nmol/L), estradiol (<5 pg/ml), and a stimulated LH at 3h <3.3 IU/L following GnRH agonist depot is considered as optimal response to therapy. If these criteria are not fulfilled, either dose or frequency of GnRH agonist administration should be increased.

71. *What is the rationale of estimation of stimulated LH after administration of depot preparation of GnRH agonist*?

Short-acting preparations of GnRH agonist (aqueous formulations) are used to assess LH response for the diagnosis of GDPP and to monitor the efficacy of therapy. Long-acting preparations of GnRH agonist (depot formulations) are used in the management of GDPP. However, these long-acting preparations can also be used to assess the efficacy of therapy by estimation of LH levels after 3h of administration. This is possible because of the presence of small quantity of free form of GnRH agonist in these depot preparations.

72. *When to discontinue therapy with GnRH agonist*?

The exact time to discontinue therapy with GnRH agonist is not well defined in children with GDPP. Retrospective analyses suggest that GnRH agonist can be stopped at chronological age of 11 years, as there is no further significant height gain despite continuation of GnRH agonist. Rather, continuation of GnRH agonist beyond 11 years of age has been shown to result in loss of 2.5 cm of final adult height, as growth after 11 years of age is dependent on gonadal steroids. In children with precocity who are treated for psychosocial concerns, therapy can be stopped at an age appropriate for the pubertal development for that particular race (e.g., 10–12 years).

73. *What is the pattern of recovery of HPG-axis after discontinuation of GnRH agonist therapy for precocious puberty*?

Use of GnRH agonist is associated with reversible suppression of HPG-axis and the axis recovers within a year of discontinuation of therapy. In girls, progression of pubertal signs starts within 3–6 months after discontinuation of

therapy, menses resumes within 1–1.5 years, and cycles become ovulatory in majority of girls 2 years after the onset of menses. The pattern of recovery of HPG-axis is similar in boys, but the progression of puberty is slower as compared to girls. Although testicular volume starts increasing by 1 year after discontinuation of GnRH agonist therapy, testicular volume remains smaller as compared to age-matched peers for the next 2–5 years. Fertility is not impaired in both sexes with the use of GnRH agonist.

74. *Does GnRH agonist therapy interfere with accrual of peak bone mass*?

Although there are theoretical concerns regarding decreased bone mineral density with the prolonged use of GnRH agonist, data from various studies have shown that GnRH agonist therapy does not influence the accrual of peak bone mass. Optimal supplementation with vitamin D and calcium should be ensured in all patients receiving GnRH agonist.

75. *What are the treatment options for GIPP*?

Treatment for patients with GIPP depends on the underlying cause. Surgical excision is the treatment of choice for GIPP due to adrenal, ovarian, and testicular neoplasms. Ovarian follicular cyst spontaneously regresses and does not require surgical treatment in majority of children. However, medroxyprogesterone acetate (MDPA) may be used, if required during interim period. Optimal therapy with glucocorticoids and fludrocortisone results in regression of secondary sexual characteristics in children with congenital adrenal hyperplasia. Girls with GIPP associated with McCune–Albright syndrome can be treated with MDPA, ketoconazole, and aromatase inhibitors, whereas boys with testotoxicosis can be treated with MDPA, ketoconazole, spironolactone, and aromatase inhibitors. If GDPP is superimposed on GIPP, therapy with GnRH agonist may be added.

76. *How to treat precocious puberty associated with McCune–Albright syndrome*?

Therapy for precocious puberty associated with McCune–Albright syndrome (MAS) is only partially effective. Treatment options include MDPA, ketoconazole, and aromatase inhibitors. MDPA is the most commonly used drug and it acts by inhibiting ovarian steroidogenesis. The recommended dose of MDPA is 100–150 mg/m^2/month. Therapy with MDPA results in regression of secondary sexual characteristics; however, it does not have an effect on skeletal maturation. It has glucocorticoid-like activity; therefore, prolonged therapy with MDPA results in cushingoid appearance and inhibition of HPA axis. Ketoconazole acts by inhibiting cytochrome P450-dependent enzymes involved in steroidogenesis. Adverse effects of ketoconazole include transaminitis and hypocortisolemia. Aromatase inhibitors like testolactone and letrozole are partially effective for GIPP associated with MAS, and efficacy of these agents progressively decline after a period of 6 months to 1 year; however, the beneficial effects on skeletal maturation and growth velocity persist despite decrease

in efficacy of these drugs. Other aromatase inhibitors like fadrozole and anastrozole or selective estrogen receptor modulator tamoxifen has not been found to be useful in the management of GIPP associated with MAS. Fulvestrant, a pure antiestrogen, has been shown to be effective in some of these children.

77. *What are the long-term complications associated with precocious puberty in girls*?

Few studies have shown an increased incidence of obesity and polycystic ovarian disease in children with GDPP on follow-up; however, long-term data do not support this observation. Fertility is not impaired in these individuals, and women with GDPP are not predisposed for early menopause. The risk of estrogen-dependent malignancies is not increased in these women. Patients with isolated premature pubarche are at a higher risk for the development of polycystic ovarian disease due to coexisting hyperinsulinemia

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Delayed Puberty

7

7.1 Case Vignette

A 19-year-old male presented with complaints of failure to develop secondary sexual characteristics. He was born at term through normal vaginal delivery, and his developmental milestones were normal. He was average in scholastic performance and studied up to tenth standard. He had history of learning disabilities but no behavioral abnormalities. He was tallest among his peers during late teenage. He noticed appearance of pubic and axillary hair by 15 years of age, but failed to develop facial or body hair or increase in penile length or size of the testes. There was no history of head trauma, surgery for midline defects, chronic systemic illness, testicular trauma, mumps, or drug abuse. He had no history of abnormality in smell, visual deficits, headache, seizure disorder, or other neurological deficits. There was no family history of delayed puberty, infertility, or gynecomastia. He did not receive any medical treatment prior to visit to this hospital. On examination, his height was 170 cm (height -1 SDS, height age 15 years, target height 173 cm), weight was 55 Kg (weight age 15 years), and blood pressure was 110/70 mmHg. Anthropometry showed eunuchoidal proportions with upper segment/lower segment ratio (US: LS, 80:90 cm) 0.88 and arm span exceeding height by 10 cm. There was no gynecomastia. Tanner stage of pubertal development was A_+, P_2, and both testes were present within poorly developed scrotal sac and soft in consistency and measured 1 ml each. The stretched penile length was 8 cm. His sense of smell was normal. He had genu valgum but no midline defects, synkinesia, nystagmus, ataxia, and visual deficits. On investigations, complete blood count and liver and renal function tests were normal. Hormonal profile revealed serum LH 0.29 mIU/ml (N 1.7–8.6), FSH 0.69 mIU/ml (N 1.5–12.4), testosterone 0.44 nmol/L (N 9.9–27.8), estradiol 12.3 pg/ml (N 7.6–42.6), prolactin 9.6 ng/ml (N 4–15.2), T_4 7.32 μg/dl (N 4.8–12.7), TSH 1.9 μIU/ml (N 0.27–4.2), and 0800h cortisol 447 nmol/L (N 171–536). His bone age was 15 years. CEMRI sella and olfactory region did not display any abnormality. LH response to triptorelin at 4h was 2.8 mIU/ml. Serum testosterone at baseline was 0.45 nmol/L, and in response to hCG, it increased to 1.2 nmol/L (after 24h of last injection of hCG). Based on available

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clinical and biochemical profile, a diagnosis of congenital idiopathic hypogonadotropic hypogonadism (IHH) was considered, and he was initiated with testosterone enanthate 100 mg intramuscularly every fortnightly. The doses of testosterone were increased gradually to 200 mg every fortnightly over a period of 2 years. On testosterone therapy, he developed gynecomastia. He is planned for gonadotropin therapy for induction of spermatogenesis after the attainment of virilization (Fig. 7.1).

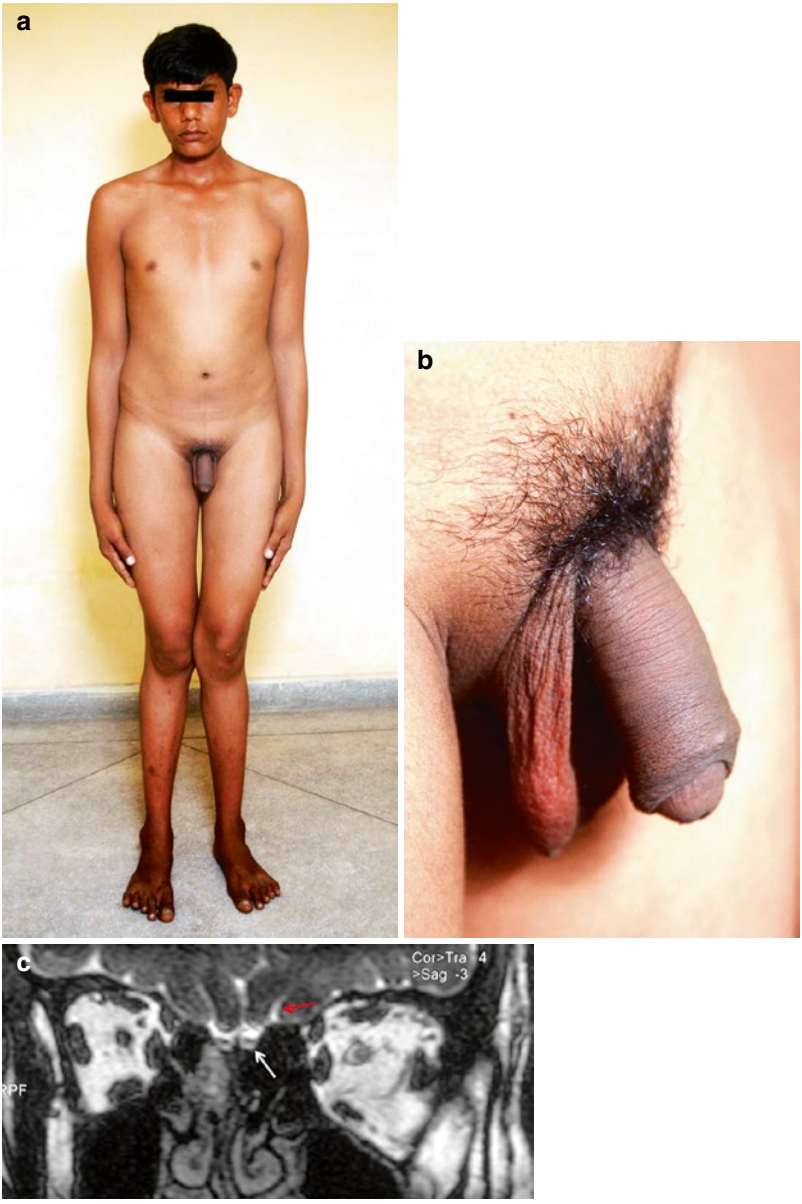

Fig. 7.1 (**a**) A 19-year-old male presented with delayed puberty (**b**) testicular volume of 1 ml with stretched penile length of 8 cm. Patient is on testosterone therapy. (**c**) T2W MR image shows normal olfactory bulb (*white arrow*) and sulci (*red arrow*)

7.2 Stepwise Analysis

The index case presented at the age of 19 years with delayed pubertal development. Delayed puberty in boys is defined as lack of pubertal development by the age of 14 years which is in correspondence with 2.5 SD above the mean for the population. The main differentials for the delayed pubertal development between the age of 14–18 years are constitutional delay in growth and puberty (CDGP) and hypogonadism. However, the adolescents with CDGP enter into puberty by the age of 18 years; hence, the possibility of CDGP after this age is virtually excluded. Our patient presented at the age of 19 years with poor development of secondary sexual characteristics; therefore, the diagnosis of hypogonadism as a cause of delayed puberty was considered. Development of secondary sexual characteristics results from both adrenarche and gonadarche which may overlap or come in succession. Though the first sign of puberty in boys is testicular enlargement (TV $\geq$ 4 ml), only in 1 % of boys' pubarche precede the gonadarche. On the contrary, in 20 % of girls, pubarche precedes the thelarche. Patients with hypogonadism usually have normal onset of adrenarche, but pubarche is delayed as was seen in our patient who had appearance of pubic hair at the age of 15 years without evidence of gonadarche. This is because the weaker adrenal androgens require conversion to potent androgens in functional testes for induction of pubarche. The presence of anosmia, midline defects, synkinesia, eunuchoidal proportions, small soft testes, skeletal anomalies, and neurological deficits (nystagmus and ataxia) usually suggests the diagnosis of IHH. Further, the manifestations of IHH vary according to the age of presentation; infants present with micropenis and cryptorchidism, adolescents with delayed or arrested puberty and gynecomastia, and adults with infertility. Long-leggedness, gynecomastia, small firm testes, learning disabilities/behavioral abnormalities, and some degree of virilization favor the diagnosis of Klinefelter's syndrome which is considered as prototype of hypergonadotropic hypogonadism. Our patient had eunuchoidal proportions, skeletal deformities (genu valgum), and small soft testes which support the diagnosis of hypogonadotropic hypogonadism. Low LH and low testosterone below the reference range confirm the diagnosis of hypogonadotropic hypogonadism. LH response to short-acting GnRH agonist (triptorelin) and testosterone response to hCG were prepubertal in our patient, further substantiate the diagnosis of hypogonadotropic hypogonadism. However, these dynamic tests help in differentiating between CDGP and IHH and are not required if the patient is above the age of 18 years. High LH, FSH, and low testosterone indicate hypergonadotropic hypogonadism and require further evaluation by karyotyping to establish the diagnosis of Klinefelter's syndrome. Hypogonadotropic hypogonadism can be due to hypothalamic or pituitary lesion or due to familial or sporadic genetic mutations. The index patient was diagnosed to have isolated hypogonadotropic hypogonadism, as other pituitary hormone profile was normal and MR brain imaging was unremarkable. Patients of IHH with anosmia or hyposmia are termed as Kallmann syndrome. Defective migration of olfactory neurons from olfactory placode to bulb results in impaired development of olfactory bulb and consequent anosmia. This is evident in MRI as olfactory bulb aplasia/hypoplasia and absent olfactory sulci. Since our patient did not have hyposmia/anosmia, he was considered to have normosmic variant of IHH. The aims of

treatment in a patient with IHH include induction and maintenance of secondary sexual characteristics and to improve the fertility prospects. For induction of secondary sexual characteristics, testosterone therapy is initiated with a low dose of testosterone esters (testosterone enanthate 50–100 mg) intramuscularly every month which is gradually built up to 200–250 mg every fortnightly over a period of 2–3 years. Improvement in libido, mood, and quality of life is observed over a period of 3–6 months, whereas increase in body hair, muscle mass and strength, and deepening of voice take longer time over a period of 1–2 years. Serum testosterone should be measured midway between the two injections after 3 months of initiation of treatment to assess the adequacy of therapy; however, it may also be required to measure serum testosterone just prior to the next injection to decide about the dosing interval. The adverse effects associated with testosterone therapy include gynecomastia, aggressive behaviour, priapism, mood swings, acne, and androgenic alopecia. hCG has also been tried for the induction of puberty which is associated with stable level of serum testosterone, minimal fluctuation in hypogonadal symptoms, and initiation of spermatogenesis; however, frequent injections and cost preclude its routine use in clinical practice. In addition, limited data is available regarding the use of gonadotropins as a primary therapy in induction of secondary sexual characteristics. The index patient was initiated with 100 mg testosterone every monthly for 3 months, and later the dose frequency was increased to fortnightly. At 6 months of follow-up, his serum testosterone was 5 nmol/L and he had improvement in generalized well-being. The dose was further increased to 150 mg fortnightly. Gonadotropin therapy is indicated when fertility is desired. hCG is initiated at a dose of 1,000–2,000 IU twice or thrice a week with monthly monitoring of serum testosterone to achieve and sustain testosterone in eugonadal range. If the target is not achieved the doses can be increased up to 5,000 IU thrice a week. Once the serum testosterone level is maintained >9 nmol/L, semen analysis should be performed at monthly interval. If spermatogenesis is not initiated despite continuation of hCG for 6–12 months after achieving the serum testosterone in normal range, hMG should be added at a dose of 75 IU thrice weekly. If the sperm count is still <1 million/ml, the doses of hMG should be increased to 150 IU thrice weekly. The predictors of response to gonadotropin therapy include initial larger testicular volume, prior history of gonadotropin therapy, and absence of prior androgen therapy. Prior androgen therapy may be associated with less favorable outcome, because optimal concentration of intratesticular testosterone is not achieved with exogenous testosterone therapy, as intratesticular testosterone is required to inhibit the secretion of AMH from Sertoli cells, which in turn exerts the suppressive effect on germ cell growth and proliferation. The overall response rate to gonadotropin therapy in terms of spermatogenesis and fertility has been shown to vary from 50 to 90 %.

7.3 Clinical Rounds

1. *What is delayed puberty?*

   Delayed puberty is defined as lack of development of secondary sexual characteristics at an age corresponding with the established normal standards for children of the same gender and race. In clinical practice, absence of testicular enlargement by the age of 14 years in boys or lack of breast development by 13 years in girls is used to define delayed puberty. In addition, children with normal age of onset of puberty, but without progression of pubertal events over a period of 2 years (arrested puberty), or those with significant delay in the progression of pubertal events (>5 years between thelarche and menarche in girls or >5 years between onset of testicular enlargement and complete genital development in boys) are also considered to have delayed puberty.

2. *How were the age cutoffs for delayed puberty defined?*

   The age of onset of puberty in a population is normally distributed (bell-shaped curve with a Gaussian distribution). In the studies by Tanner and Marshall, it was shown that the mean age of onset of puberty was 10.5 years in girls and 11.5 years in boys, with one standard deviation of approximately 1 year. Considering the normal range of age of pubertal onset as mean ±2.5 SD, lack of any sign of pubertal development after the age of 13 years in girls or after 14 years in boys (+2.5 SD from the mean, i.e., 10.5 + 2.5 years in girls and 11.5 + 2.5 years in boys) suggests delayed puberty.

3. *Why is pubarche not used to define the onset of normal puberty?*

   Reactivation of hypothalamic–pituitary–gonadal (HPG) axis is manifested by thelarche in girls and testicular enlargement in boys, and these are considered as first signs of puberty. Pubarche is a clinical manifestation of adrenarche and denotes the maturation of zona reticularis which is independent of reactivation of HPG-axis. Therefore, pubarche is not used to define the onset of normal puberty.

4. *What is kisspeptin?*

   Kisspeptin is a neuropeptide secreted from the arcuate nucleus and anteroventral and periventricular (AVPV) nuclei of the hypothalamus. The arcuate nucleus comprises of KNDy (pronounced as "candy") neurons which co-secrete kisspeptin (K), neurokinin B (N), and dynorphin (Dy), whereas AVPV nuclei comprise of Kiss-1 neurons, which secretes only kisspeptin. Both KNDy neurons and Kiss-1 neurons synapse with GnRH neurons. Kisspeptin acts on its

receptor GPR-54, which is present on GnRH neurons in the hypothalamus. Kisspeptin signaling is involved in initiation of puberty, regulation of gonadotropin secretion by sex steroids, and preovulatory LH surge (Fig. 7.2).

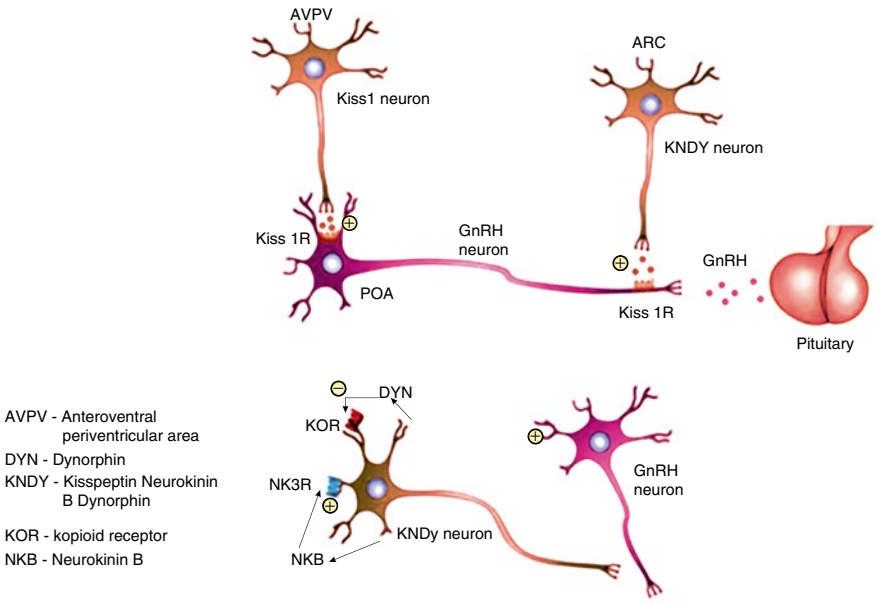

Fig. 7.2 "Kisspeptin-GnRH" axis

5. *How does kisspeptin regulate the onset of puberty?*

Kisspeptin is believed to be the "gateway to puberty." Release of kisspeptin from KNDy neurons (arcuate nucleus) results in initiation of puberty. The secretion of kisspeptin by KNDy neurons is modulated by neurokinin B and dynorphin, which has stimulatory and inhibitory effects on release of kisspeptin, respectively. Further, the expression of kisspeptin is negatively regulated by MKRN3 gene product (makorin RING-finger protein 3) and polycomb group (a protein complex). Kisspeptin acts through its receptor GPR-54 present on GnRH neurons of the hypothalamus and results in activation of pulsatile GnRH secretion.

6. *How does kisspeptin regulate the preovulatory LH surge?*

   Kisspeptin is synthesized in both, arcuate nucleus (KNDy neurons) and AVPV nuclei (Kiss-1 neurons). However, kisspeptin from KNDy neurons is responsible for the initiation of puberty, whereas kisspeptin from Kiss-1 neurons is involved in preovulatory LH surge. This effect is mediated by the stimulatory effect of estrogen at Kiss-1 neurons present in AVPV nuclei. On the contrary, AVPV nuclei are devoid of Kiss-1 neurons in males.

7. *What is the role of leptin in the initiation of puberty?*

   It has been shown that a critical body weight/body fat is essential for the onset of puberty. This is evidenced by the presence of delayed/absent puberty in girls with low body fat and early puberty in obese girls. Adipose tissue signals to hypothalamus through the adipokine leptin, which stimulates KNDy neurons. This results in the release of kisspeptin, which in turn activates HPG-axis. The key role of leptin in the induction of puberty is evidenced by the occurrence of isolated hypogonadotropic hypogonadism in individuals with congenital leptin deficiency and initiation of puberty in these individuals with recombinant leptin therapy. Despite these evidences, leptin is considered to have a permissive role, rather than a primary role as evidenced by timely onset of puberty in patients with congenital generalized lipodystrophy, who are deficient in leptin.

8. *What is "mini-puberty"?*

   Reactivation of Hypothalamic–pituitary–gonadal (HPG) axis during neonatal period occurs as a result of withdrawal of inhibitory effect of placental estrogen on HPG-axis and is termed as "mini-puberty." It is characterized by postnatal surge of gonadotropins and sex steroids that initiate at approximately second week of life. In boys, gonadotropins and testosterone peak at 3 months and decline by 6–9 months of age. In girls, FSH and LH peak at 3 months; although LH levels decline by 6–9 months of age, FSH remains elevated for about 3–4 years of age. Serum estradiol levels widely fluctuate during mini-puberty in girls, whereas serum testosterone is stable in boys. The wide fluctuation in serum estradiol during mini-puberty is possibly due to cyclical maturation of ovarian follicles (Fig. 7.3).

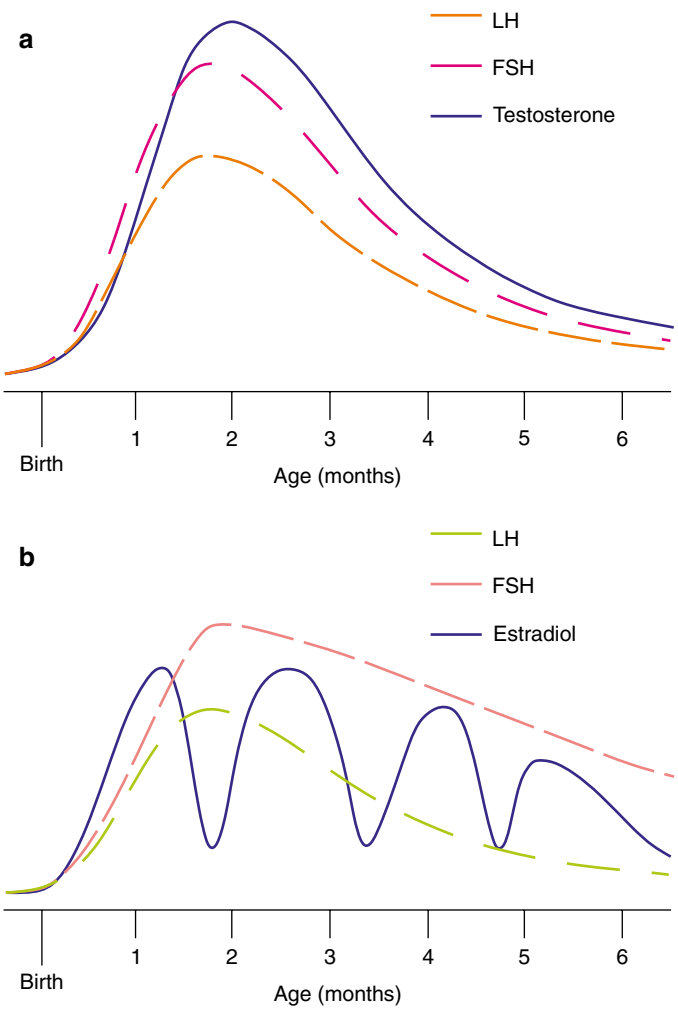

Fig. 7.3 (**a**) Gonadotropins and serum testosterone during mini-puberty in boys, (**b**) gonadotropins and serum estradiol during mini-puberty in girls

9. *What is the importance of mini-puberty?*

The physiological importance of mini-puberty is better described in boys. The postnatal surge of gonadotropins and testosterone leads to growth and proliferation of Leydig cells, Sertoli cells, and germ cells, thereby resulting in increase in testicular volume (by approximately 1 ml) and penile size and also contributing to postnatal testicular descent. Further, mini-puberty also helps in priming of pilosebaceous units and development of male psyche. The role of mini-puberty in girls remains elusive.

10. *What are the disorders associated with absence of mini-puberty?*

    Mini-puberty is absent in children with congenital hypogonadotropic hypogonadism and complete androgen insensitivity syndrome; however, it is present in children with partial androgen insensitivity syndrome. This underscores the importance of androgens in priming of GnRH-gonadotropin axis for the onset of mini-puberty. In addition, infants with CAH and androgen excess of any etiology (e.g., congenital adrenocortical carcinoma) may not experience mini-puberty because of suppression of gonadotropins by excess androgen.

11. *What are the gender dimorphisms acquired during peripubertal period?*

    Negative feedback between most of the target glands and hypothalamic–pituitary axis is usually established by 2–3 years of age. However, estrogen-mediated positive feedback for LH in girls is acquired only during peripubertal period and is responsible for initiation of ovulatory cycles. Further, the gender difference in serum prolactin level, i.e., higher levels in women as compared to men, is also acquired during peripubertal period consequent to estrogen production.

12. *How to define hypogonadism?*

    Hypogonadism refers to a clinical syndrome characterized by impaired gonadal function resulting in decreased gonadal steroidogenesis and/or gametogenesis. However, in clinical practice, those with isolated germ cell dysfunction but with normal gonadal steroidogenesis are not considered as having hypogonadism. Similarly, a well-feminized female with normal circulating levels of estradiol, but with chronic anovulation (e.g., polycystic ovarian disease), is not considered to have hypogonadism.

13. *How to classify hypogonadism?*

    Hypogonadism is classified based on the site of primary defect in the hypothalamic–pituitary–gonadal (HPG) axis. Disorders caused by abnormalities of hypothalamus and pituitary gland are termed as hypogonadotropic hypogonadism, whereas those with a primary defect at the level of gonads are termed as hypergonadotropic hypogonadism (primary gonadal failure).

14. *What are the causes of delayed puberty?*

    The most common cause of delayed puberty is CDGP, followed by hypogonadotropic hypogonadism and hypergonadotropic hypogonadism. The prevalence of various causes of delayed puberty is summarized in the table given below.

Disorders	**Boys**	**Girls**
CDGP	65 %	30 %
Hypogonadotropic hypogonadism		
Functional	20 %	20 %
Permanent	10 %	20 %
Hypergonadotropic hypogonadism	5–10 %	25–30 %

The causes of functional hypogonadotropic hypogonadism include anorexia nervosa and chronic systemic illness like celiac disease, Crohn's disease, nephrotic syndrome, and rheumatoid arthritis. The causes of permanent hypogonadotropic hypogonadism include congenital isolated hypogonadotropic hypogonadism, congenital multiple pituitary hormone deficiency, and tumors/cysts in sellar–suprasellar region. Turner syndrome, Klinefelter syndrome, and post-chemotherapy/gonadal irradiation are the common causes of hypergonadotropic hypogonadism (Fig. 7.4).

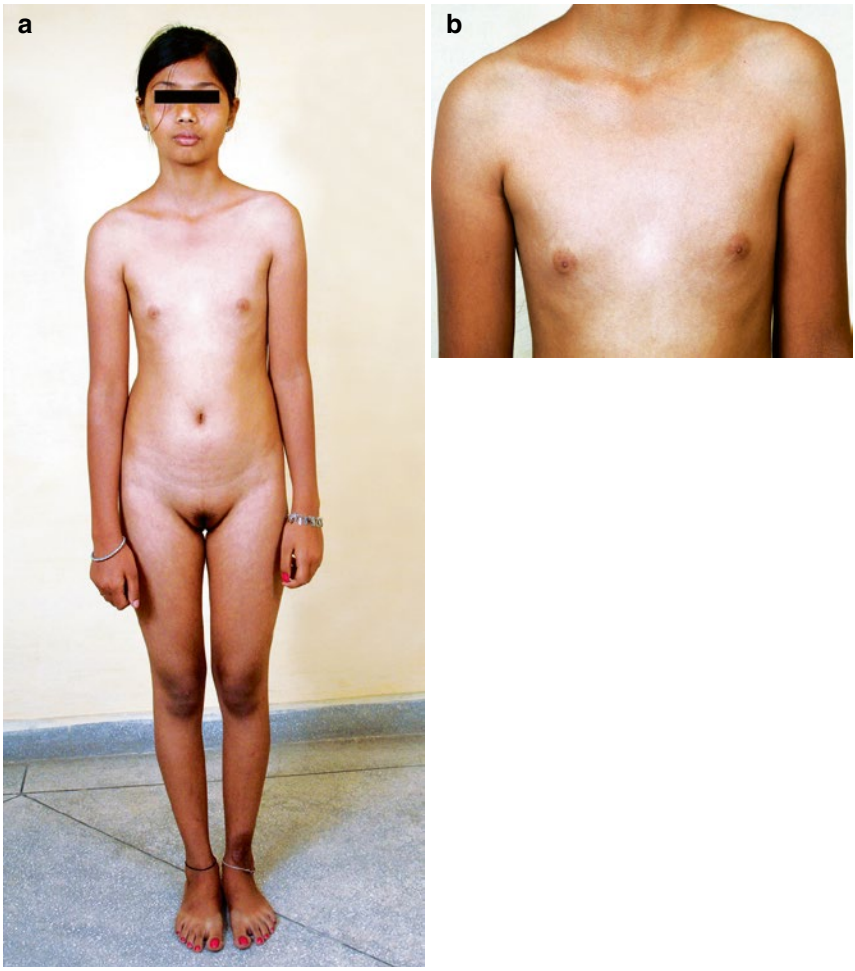

Fig. 7.4 (**a**) An 18-year-old girl with poor development of secondary sexual characteristics due to hypogonadotropic hypogonadism, (**b**) Tanner breast stage B_1 in the same patient

15. *What are the acquired causes of hypogonadotropic hypogonadism?*

    Any patient with adult-onset hypogonadotropic hypogonadism should be investigated for sellar–suprasellar pathology like tumor and infiltrative disorders. A history of head injury, cerebrovascular accident (e.g., subarachnoid hemorrhage), snake bite, and postpartum lactational failure (pituitary apoplexy/necrosis) should be actively sought as the cause for hypogonadotropic hypogonadism. In addition, functional hypogonadotropic hypogonadism can be associated with any chronic systemic illness.

16. *What is congenital isolated hypogonadotropic hypogonadism?*

    Congenital isolated hypogonadotropic hypogonadism is defined as absence or arrested puberty due to impaired GnRH and/or gonadotropin secretion without structural abnormalities in hypothalamic–pituitary region or other pituitary hormone deficiency. Approximately 30 % of individuals with congenital isolated hypogonadotropic hypogonadism have identified genetic mutation as a cause of hypogonadism, while the rest are idiopathic.

17. *How to suspect congenital isolated hypogonadotropic hypogonadism during early childhood?*

    The presence of micropenis, cryptorchidism, midline/skeletal defects, and anosmia/hyposmia in a child, with or without family history of delayed puberty, should raise a suspicion of IHH.

18. *What are the clinical clues to differentiate between prepubertal and postpubertal onset of hypogonadism in an adult male?*

    The presence of micropenis, cryptorchidism/small testicular volume (testicular volume <4 ml), scant pubic and axillary hair, eunuchoidal habitus (due to delayed epiphyseal closure), and high-pitched voice suggests the prepubertal onset of hypogonadism. Postpubertal onset of hypogonadism is suggested by the presence of normal body proportions, normal- or small-sized testes with soft consistency, regression of pubic and axillary hair, and reduced shaving frequency (Fig. 7.5).

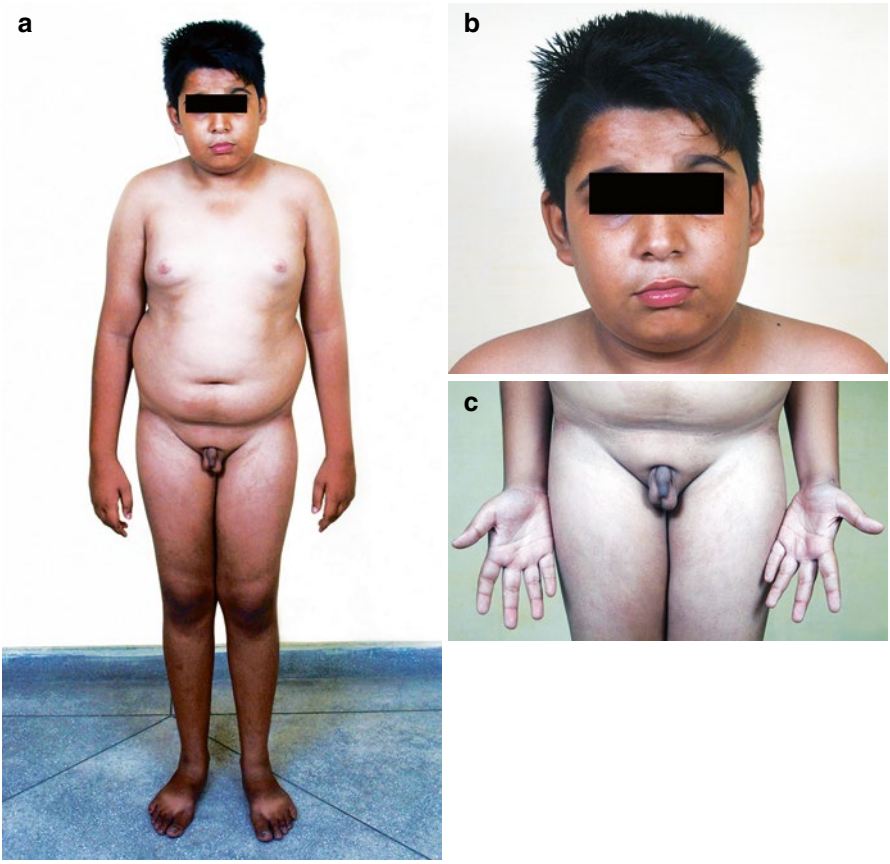

Fig. 7.5 (**a**) An 18-year-old boy with absent secondary sexual characteristics due to isolated hypogonadotropic hypogonadism, (**b**) immature facies and absent facial hair, and (**c**) genitalia showing Tanner pubic hair stage P_1, micropenis, and small testes. Note bilateral lipomastia in the same patient

19. *What is Kallmann syndrome?*

Kallmann syndrome is characterized by the coexistence of anosmia/hyposmia in a patient with congenital isolated hypogonadotropic hypogonadism. On the basis of genetic analysis, Kallmann syndrome is defined by the presence of mutations of genes involved in the development and migration of both olfactory and GnRH neurons (i.e., KAL1, FGF8, FGFR1, PROK2, PROKR2, NELF, CHD7, HS6ST1, WDR11, and SEMA3A gene). Mutations in KAL1 gene are invariably associated with anosmia/hyposmia. However, mutations in FGFR1, FGF8, PROKR2, CHD7, and WDR11, if associated with anosmia/hyposmia, result in Kallmann syndrome; whereas if not, then these are referred as normosmic IHH. These genes might be playing a minor role in the development and migration of olfactory neurons (Fig. 7.6).

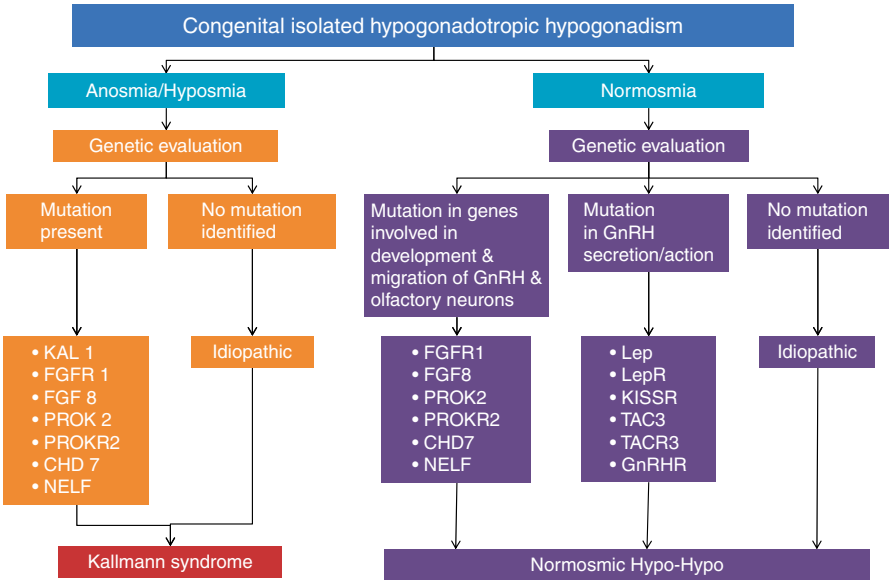

Fig. 7.6 Etiology of congenital isolated hypogonadotropic hypogonadism

20. *What are the genes implicated in the development and migration of both olfactory and GnRH neurons?*

    The genes responsible for the development and migration of olfactory and GnRH neurons include KAL1, FGF8, FGFR1, PROK2, PROKR2, NELF, CHD7, HS6ST1, WDR11, and SEMA3A.

21. *What are the genes implicated only in GnRH secretion?*

    The genes implicated in GnRH secretion include LEP, LEPR, KISS1/KISS1R, TAC3, TACR3, and PCSK1. Therefore, inactivating mutations of these genes results in congenital isolated hypogonadotropic hypogonadism (normosmic).

22. *What is anosmin?*

    Anosmin is a 680-amino-acid protein which is coded by KAL1 gene present on short arm of X chromosome. Anosmin is involved in growth, development, proliferation, and migration of olfactory and GnRH neurons to their appropriate destination.

23. *Why is anosmia associated with Kallmann syndrome?*

    During embryogenesis, olfactory neurons and GnRH neurons share a common origin at olfactory placode. These neuronal fibers migrate together through cribriform plate to the olfactory bulb where olfactory fibers terminate, while GnRH neurons continue to migrate to the arcuate nucleus of hypothalamus.

Therefore, any insult during development or migration of these neurons result in anosmia and hypogonadotropic hypogonadism.

24. *How to test for anosmia?*

Absence of history of anosmia/hyposmia does not exclude the possibility of olfactory abnormalities in a patient with isolated hypogonadotropic hypogonadism; therefore, formal clinical testing for anosmia should be performed in all patients. Oil of clove, oil of peppermint, and asafoetida can be used for bedside evaluation of anosmia, whereas University of Pennsylvania Smell Identification Test (UPSIT) is an objective test for anosmia.

25. *What are the nonreproductive abnormalities associated with Kallmann syndrome apart from anosmia?*

A variety of nonreproductive abnormalities are associated with Kallmann syndrome. The neurological abnormalities include bimanual synkinesia (mirror movements), neurosensory deafness, cerebellar ataxia and oculomotor abnormalities, and skeletal abnormalities which include clinodactyly, syndactyly, camptodactyly, and short fourth and fifth metacarpals and metatarsals. Other associations include cleft lip/palate, high-arched palate, ocular hypertelorism, dental agenesis, and unilateral renal agenesis.

26. *What is synkinesia?*

Non-suppressible involuntary movements accompanied with voluntary movements are known as synkinesia or mirror movement. Synkinesia is a physiological phenomenon during childhood due to incomplete brain myelination and can be associated with a variety of disorders like Kallmann syndrome, Klippel–Feil disease, corpus callosum agenesis, Joubert syndrome, stroke, and Parkinson's disease. Approximately 40 % of patients with Kallmann syndrome manifest synkinesia, which is classically seen in upper limbs, predominantly involving hands (bimanual synkinesia), and is confined only to X-linked variant of Kallmann syndrome (*KAL1* mutation).

27. *What is the mechanism of synkinesia?*

Synkinesia can be considered as a manifestation of midline defect in patients with Kallmann syndrome. Various theories have been proposed to explain the phenomenon of synkinesia and include partial failure of decussation of

corticospinal fibers, lack of inter-hemispheric inhibition between the two motor cortices, and functional defects in motor planning and execution (Fig. 7.7).

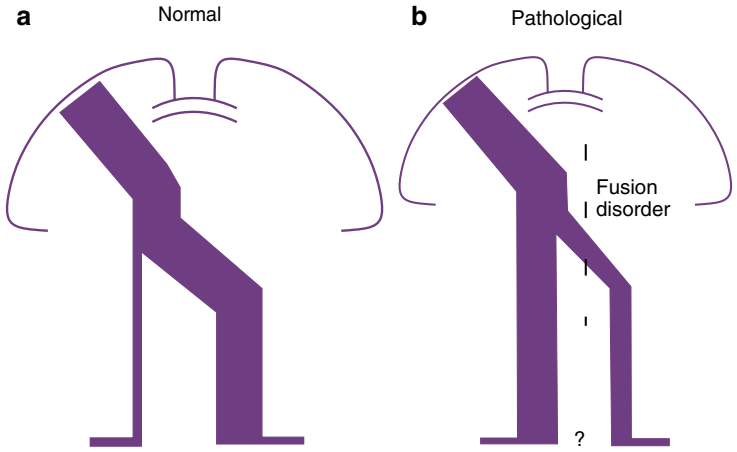

Fig. 7.7 (**a**) Normal pattern of decussation of corticospinal fibers, (**b**) partial failure of decussation of corticospinal fibers in patients with Kallmann syndrome results in synkinesia

28. *Can clinical phenotype guide the selection of genetic testing for Kallmann syndrome?*

    Although Kallmann syndrome can be associated with a wide variety of nonreproductive abnormalities, the presence of certain phenotypic characteristics points toward a specific mutation. The presence of synkinesia should prompt evaluation for KAL1 gene mutation, dental agenesis, and skeletal abnormalities for FGF8/FGFR1 and hearing loss for CHD7.

29. *What are the causes of Kallmann syndrome with short stature?*

    Kallmann syndrome is typically associated with tall stature due to delayed epiphyseal closure as a result of gonadal steroid deficiency. However, those with FGFR1 mutations have short stature, despite hypogonadotropic hypogonadism.

30. *A 10-year-old obese boy was brought with complaint of small size of penis. What to do next?*

    A diagnosis of micropenis is inadvertently made in obese children, as penis is buried in surrounding fat, giving an impression of apparently small-sized

penis. Therefore, accurate measurement of stretched penile length should be done prior to subjecting a child for evaluation of micropenis. The stretched penile length of the child was 5 cm, which was within 2.5 SD for his age, and therefore, parents were reassured. The normative data for stretched penile length at various ages and cutoff for the diagnosis of micropenis are given in the table below.

Age	Mean ± SD (cm)	Micropenis (Mean-2.5 SD, cm)
New born: 30 weeks	2.5 ± 0.4	1.5
New born: 34 weeks	3.0 ± 0.4	2.0
New born: term	3.5 ± 0.4	2.5
0–5 months	3.9 ± 0.8	1.9
6–12 months	4.3 ± 0.8	2.3
1–2 years	4.7 ± 0.8	2.6
2–3 years	5.1 ± 0.9	2.9
3–4 years	5.5 ± 0.9	3.3
4–5 years	5.7 ± 0.9	3.5
5–6 years	6.0 ± 0.9	3.8
6–7 years	6.1 ± 0.9	3.9
7–8 years	6.2 ± 1.0	3.7
8–9 years	6.3 ± 1.0	3.8
9–10 years	6.3 ± 1.0	3.8
10–11 years	6.4 ± 1.1	3.7
Adult	13.3 ± 1.6	9.3

Adapted from *Indian Journal of Pediatrics* 2000; 67:455–460

31. *What are the causes of micropenis?*

Micropenis is defined as a stretched penile length <−2.5SD for that particular age. The causes of micropenis include hypogonadotropic hypogonadism, hypergonadotropic hypogonadism, disorders of androgen biosynthesis and action, and isolated growth hormone deficiency (Fig. 7.8).

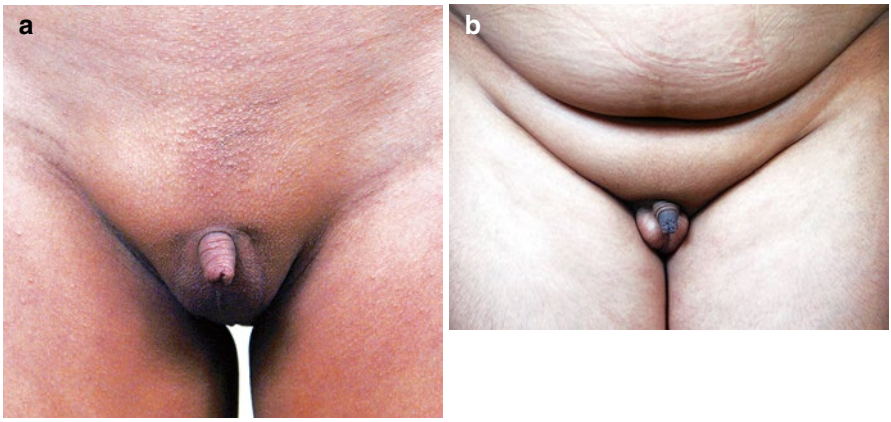

Fig. 7.8 (**a**) Micropenis in a patient with hypogonadotropic hypogonadism, (**b**) buried penis in an obese boy

32. *What are the hormones responsible for prepubertal penile growth?*

    During intrauterine period, there is an increase in penile size by approximately 2 cm in second and third trimesters due to the activation of fetal hypothalamic–pituitary–testicular (HPT) axis. This is mediated by the effects of testosterone and dihydrotestosterone. During infancy, postnatal surge of testosterone as a consequence of mini-puberty contributes to penile growth. In addition, growth hormone also has a permissive role in penile growth during intrauterine and prepubertal period as evidenced by the presence of micropenis in newborns and children with growth hormone deficiency.

33. *What are the causes of obesity with congenital hypogonadotropic hypogonadism?*

    Prader–Willi syndrome (PWS) and Laurence–Moon–Bardet–Biedl syndrome (LMBB) are characterized by obesity and hypogonadotropic hypogonadism. In addition, patients with inactivating mutations of LEP, LEPR, PROK2, and PROKR2 can also present with obesity and hypogonadotropic hypogonadism (Fig. 7.9).

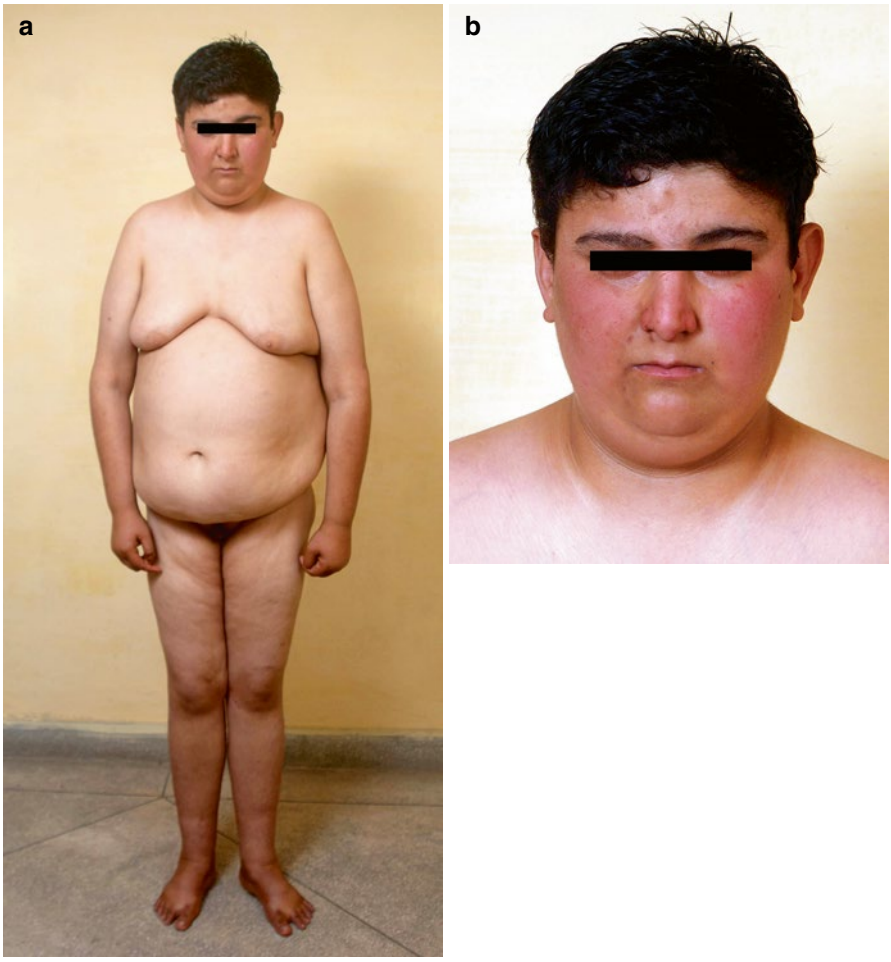

Fig. 7.9 (**a**) A 21-year-old patient with Prader–Willi syndrome with gynecomastia, (**b**) immature facies and absent facial hair suggestive of hypogonadism

34. *How to investigate a child with delayed puberty?*

The first-line investigations in a child presenting with delayed puberty include hemogram, renal and liver function tests, celiac serology, and thyroid function test. After exclusion of chronic systemic disorders, hormonal evaluation including LH, FSH, testosterone/estradiol, and prolactin should be performed. In addition, IGF1 and serum cortisol should be estimated, if there is a clinical suspicion of multiple pituitary hormone deficiency. Bone age assessment

should also be done as it may help in differentiating between CDGP and hypogonadotropic hypogonadism. Further evaluation is guided by the results of hormonal tests and include karyotype, MRI brain/sella, inhibin B, LH response to GnRH, and testosterone response to hCG (Fig. 7.10).

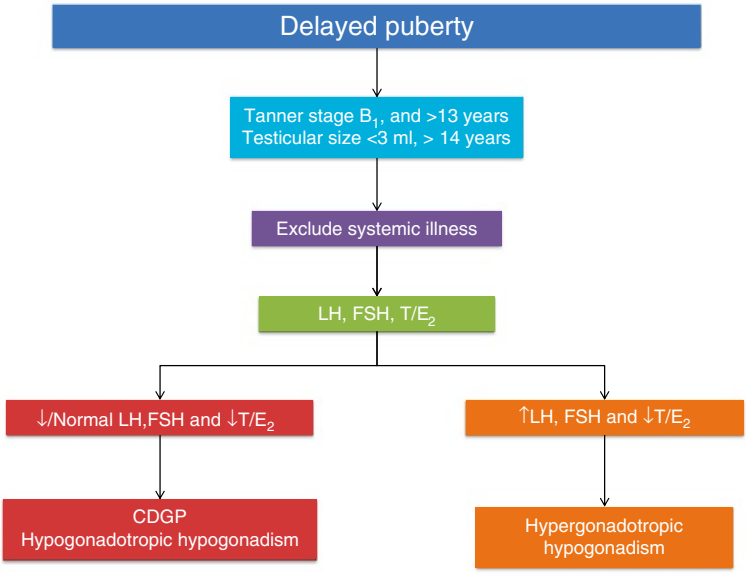

Fig. 7.10 Approach to a child with delayed puberty

35. *Should all patients with hypogonadotropic hypogonadism undergo MRI brain?*

The need for MR brain imaging in patients with hypogonadotropic hypogonadism should be individualized. MRI brain should be performed in a patient with hypogonadotropic hypogonadism, if associated with anosmia/hyposmia, multiple pituitary hormone deficiency, hyperprolactinemia, or symptoms of mass effect.

36. *What are the neuroimaging characteristics of Kallmann syndrome?*

Hypoplasia/agenesis of olfactory bulb and/or olfactory sulci and non-visualization of olfactory tracts are the characteristic neuroimaging abnormalities in patients with Kallmann syndrome. In addition, corpus callosum agenesis and cerebellar abnormalities have also been described. Olfactory bulbs and tracts are best visualized by coronal images, whereas olfactory sulci in axial images (Fig. 7.11).

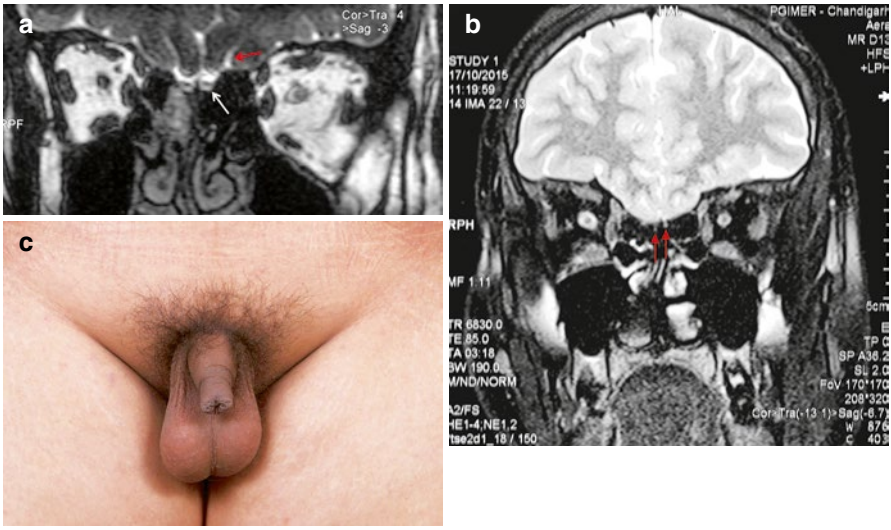

Fig. 7.11 Coronal T2W MRI brain showing: (**a**) normal olfactory bulb (*white arrow*) and sulci (*red arrow*) in a patient of normosmic IHH, (**b**) hypoplastic olfactory bulb (*red arrows*) and absent sulci in a patient with anosmia due to Kallmann syndrome

37. *How to define reversible congenital isolated hypogonadotropic hypogonadism?*

    Reversible congenital isolated hypogonadotropic hypogonadism in a male is defined as sustained maintenance of serum testosterone levels in the normal adult range (>9 nmol/L), irrespective of testicular volume after discontinuation of hormonal therapy including GnRH, gonadotropins, or androgens. The criteria to define reversibility in females with IHH are not clear.

38. *What is the mechanism of reversibility in hypogonadotropic hypogonadism?*

    The mechanism of reversibility in patients with isolated hypogonadotropic hypogonadism remains elusive; however, various theories have been proposed to explain this phenomenon. The reversal may be due to the presence of mutations which results in delayed maturation of GnRH neurons (rather than mutation which prevent the development of these neurons). Plasticity of GnRH neurons (ability of neurons to adapt to the environment) has also been proposed to explain the reversibility of IHH. The plasticity of GnRH neurons may be amplified by therapy with gonadal steroids.

39. *A 15-year-old boy presented with delayed puberty. His height was 156 cm (at 3rd percentile, with target height of 173 cm, 25th percentile) and he had a testicular volume of 2 ml bilaterally, pubic hair Tanner stage P_2, and no axillary hair. Systemic examination was normal. His bone age was 11 years, and routine investigations, thyroid function tests, and celiac serology were normal. His LH was 0.1 μIU/ml and testosterone was 0.3 nmol/L. What is the diagnosis?*

    The differential diagnosis in this scenario includes CDGP and isolated hypogonadotropic hypogonadism, and it is difficult to differentiate between these two disorders either clinically or biochemically. However, on prospective follow-up, if the child does not enter into puberty by the age of 18 years, the diagnosis of isolated hypogonadotropic hypogonadism is almost certain. The cutoff of 18 years is based on the fact that 97.5 % of normal children complete their pubertal development (B_2 to menarche in girls and G_2 to G_5 in boys) within 5 years after the onset of puberty (i.e., thelarche 8–13 years, testicular enlargement 9–14 years). The probability of CDGP is more likely in the index patient as he is short and his father had a history of delayed puberty. Further, triptorelin stimulation test was performed to differentiate between CDGP and IHH, and LH response of 16 mIU/ml was observed which excluded IHH.

40. *What is CDGP?*

    CDGP is a normal variant of growth and puberty which is characterized by a decline in growth velocity between 2 and 3 years of age, followed by normal height velocity during prepubertal period (along the third percentile) and delayed but spontaneous pubertal growth and development before the age of 18 years. It is accompanied by delay in skeletal maturation (BA < CA); however, the bone age correlates with height age. CDGP is the most common cause of delayed puberty and is more common in boys. A family history of delayed puberty is present in 50–80 % of these individuals. The final adult height is usually within the target height range and fertility is normal. The exact cause for initial decline in height velocity and delay in onset of puberty remains elusive, but the possible mechanisms include transient dysfunction of GHRH–GH–IGF1-axis and delayed reactivation of HPG-axis, respectively (“lazy pituitary syndrome”) (Fig. 7.12).

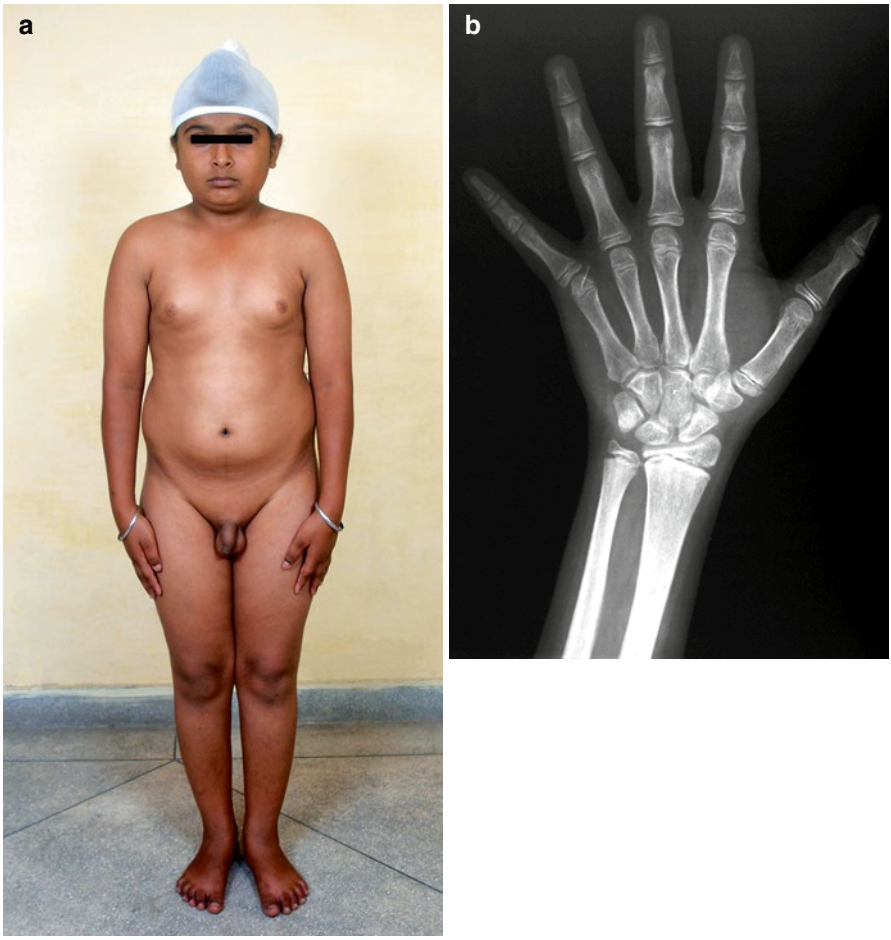

Fig. 7.12 (**a**) A 14-year-old boy with short stature and delayed puberty with bilateral testicular volume of 3 ml. Note bilateral lipomastia in the same patient. (**b**) X-ray wrist shows bone age of 12 years; (**c**) growth chart shows height age 10 years and weight age 13 years suggestive of CDGP

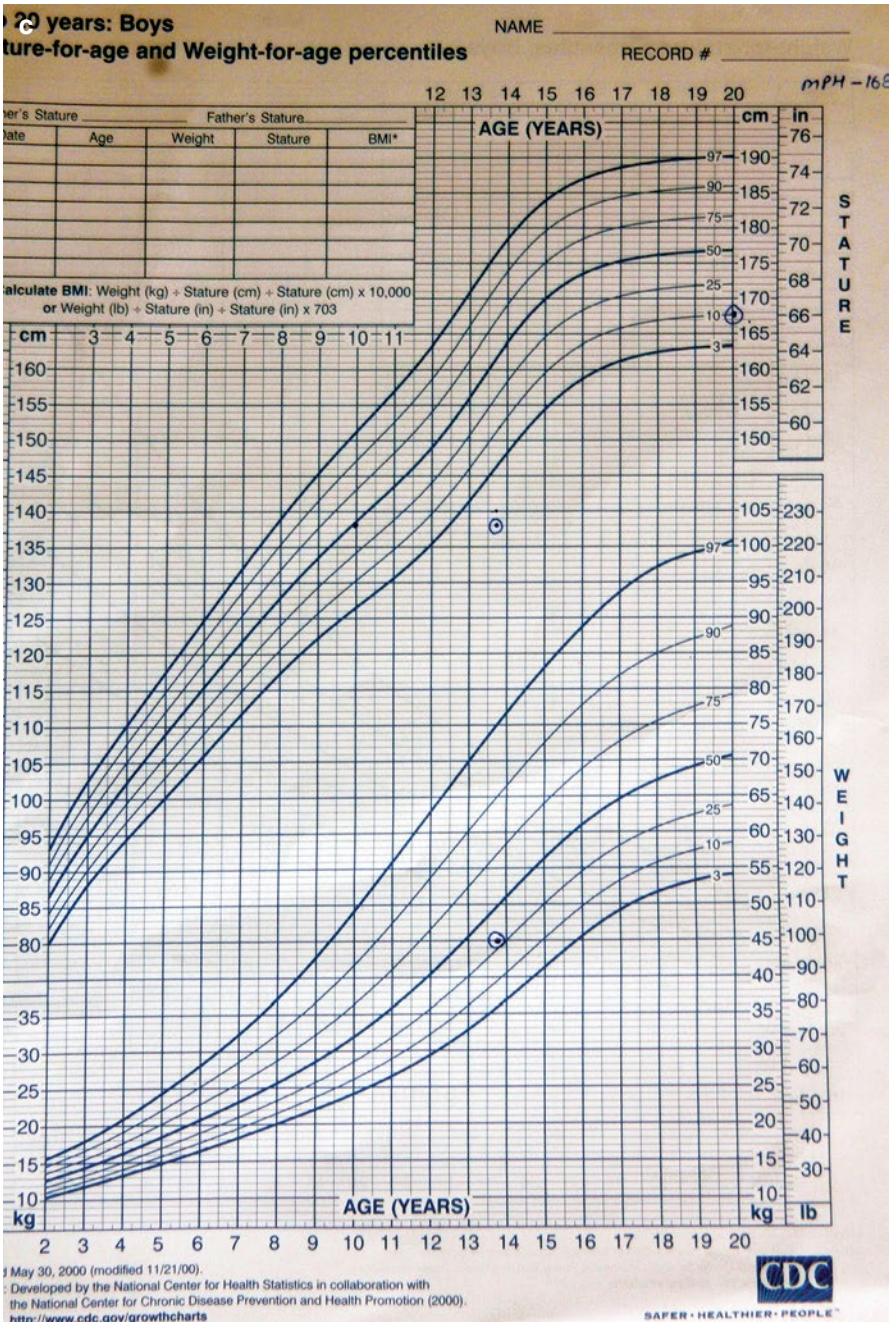

Fig. 7.12 (continued)

41. *Does CDGP and congenital idiopathic hypogonadotropic hypogonadism represent a spectrum of a common disorder?*

A strong family history of delayed puberty is present in majority of children with CDGP. Similarly, 10 % of patients with congenital idiopathic hypogonadotropic hypogonadism (IHH) also have a family history of delayed puberty. It has also been shown that CDGP and congenital IHH may coexist in the same family pedigree. This suggests that CDGP and congenital IHH represent a spectrum of aberration in pubertal development, with CDGP and permanent congenital IHH at two extremes, with reversible IHH in between. Recently, a study has shown that individuals with CDGP had higher prevalence of inactivating mutations of TAC3 gene, which is classically associated with congenital IHH.

42. *A 15-year-old boy presented with poor development of secondary sexual characteristics and short stature. His father also had a history of delayed puberty. What is the most likely diagnosis?*

The most likely diagnosis in the index child is constitutional delay in growth and puberty (CDGP). It is difficult to differentiate between CDGP and congenital idiopathic hypogonadotropic hypogonadism; however, there are some clinical pointers which help to differentiate between the two disorders, and these are enlisted in the table given below.

Parameters	**CDGP**	**Congenital IHH**
Presenting manifestation	Growth failure and delayed puberty	Delayed puberty
Family history	Strong family history of "late bloomer"	Familial clustering is known
Neonatal manifestations	Absent	Micropenis, cryptorchidism, and midline defects
Anosmia/hyposmia	Absent	May be present
Neuroskeletal abnormalities	Absent	May be present
Height velocity	Normal	Normal
Pubarche	Delayed	Normal/delayed
Testicular volume >4 ml	May be present	Less common
Bone age >12 years for boys or >11 years for girls	Unlikely	Likely
Final adult height	Within range for target height	Exceeds target height

Although the presence of eunuchoidal body proportions is a classical feature of congenital IHH, patients with CDGP can also have eunuchoidal body proportions. This is because of poor spine growth due to delay in exposure to gonadal steroids.

43. *What are the diagnostic tests to differentiate between CDGP and congenital IHH?*

The diagnostic tests that help to differentiate between CDGP and congenital IHH include basal LH and testosterone. With the advent of ultrasensitive gonadotropin assays, the utility of GnRH stimulation test is limited only in those situations where basal gonadotropin levels are in prepubertal range. The utility of the various diagnostic tests is depicted in the figure given below. The measurement of inhibin B and α-subunit has been used for the differentiation between CDGP and IHH; however, the data are limited (Fig. 7.13).

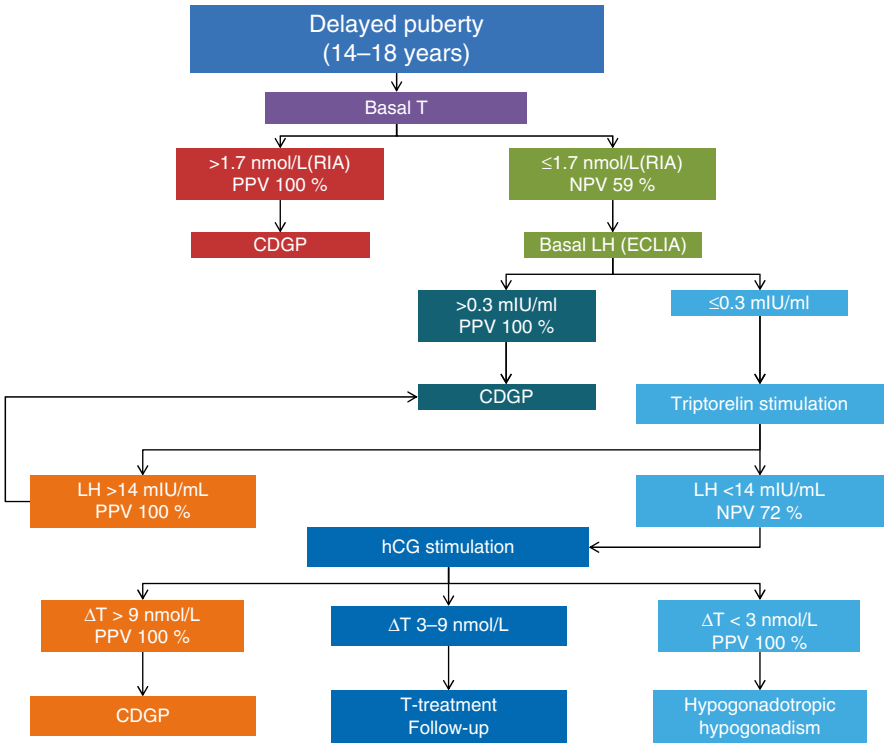

Fig. 7.13 Approach to a child with delayed puberty

44. *How is short-term testosterone therapy beneficial in children with CDGP?*

Short-term low-dose testosterone therapy is used in children with CDGP who have completed 14 years of age and have significant psychosocial concerns regarding their growth and/or pubertal development. The commonly used regimen is testosterone enanthate or cypionate 50–100 mg intramuscularly every

month for a period of 3 months. With this therapy, there is an increase in testicular volume by 3–4 ml in 6–9 months, progressive appearance of secondary sexual characteristics, and acceleration of growth velocity from 4 cm/year to 9–10 cm/year. The increase in testicular volume during testosterone replacement therapy is attributed to increase in FSH secretion by low-dose testosterone. The growth spurt which occurs after testosterone therapy is due to gonadal steroid-mediated increase in GH-IGF1 secretion. Following withdrawal of testosterone, there is reactivation of HPG axis due to loss of negative feedback at hypothalamus and pituitary leading to further progression of puberty. If testicular enlargement does not occur within 3 months after discontinuation of testosterone therapy, another short course of testosterone may be administered. If there is no testicular enlargement even after 1 year of therapy, the diagnosis of congenital IHH should be considered.

45. *When to induce puberty in boys with congenital IHH?*

Puberty should be initiated at a chronological age of 14 years in boys with congenital IHH, and this cutoff is based on the definition of delayed puberty. In addition, boys with congenital IHH with bone age of ≥12 years can also be considered for pubertal induction.

46. *How to induce puberty in boys with congenital IHH?*

Normal puberty is a slow and progressive process which is completed over a period of 2–5 years; therefore, pubertal development should be accomplished slowly over a period of 2–5 years. Various treatment modalities used for the induction of puberty include pulsatile GnRH, hCG with/without FSH, and testosterone therapy.

47. *What are the merits and demerits of pubertal induction with GnRH in boys with congenital IHH?*

Pulsatile GnRH therapy is the most physiological way to induce puberty and it results in virilization, testicular growth, and spermatogenesis. GnRH is administered in a pulsatile manner at an interval of 90–120 min either subcutaneously or intravenously via a pump. GnRH therapy is effective in nearly 75 % of patients with congenital IHH. However, this therapy is expensive and cumbersome.

48. *What are the merits and demerits of pubertal induction with hCG?*

Normal pubertal development is orchestrated by synergistic actions of gonadotropins. LH acts on Leydig cells and increases the level of circulating testosterone, resulting in virilization. LH in concert with FSH initiates the onset of

spermatogenesis at puberty, and this effect of LH is mediated by increase in intratesticular testosterone. However, once initiated, spermatogenesis can be maintained by LH alone. Therapy with hCG results in testicular growth along with virilization and spermatogenesis, particularly in those who have evidence of endogenous FSH secretion (e.g., testicular volume ≥4 ml). Further, hCG therapy leads to stable levels of serum testosterone without peaks and troughs as compared to exogenous testosterone therapy because of regulated production of testosterone by Leydig cells. The recommended dose of hCG is 500–2,000 IU subcutaneously/intramuscularly thrice a week, with an aim to maintain serum testosterone in mid-normal adult reference range. However, hCG therapy is expensive, requires frequent injections, and is associated with development of gynecomastia and anti-hCG antibodies.

49. *Why is gynecomastia more common with hCG therapy than with testosterone?*

Gynecomastia is more common with hCG as compared to testosterone therapy. This is because hCG directly stimulates aromatase activity in Leydig cells, resulting in increased testicular production of estradiol, in addition to peripheral aromatization of testosterone. Exogenous testosterone therapy leads to gynecomastia due to aromatization of testosterone to estradiol in adipose tissues. Testosterone-mediated gynecomastia is more common in obese subjects and possibly in those with increased sensitivity to estradiol and/or FSH (as FSH increases aromatase activity). Circulating estradiol levels may not necessarily be elevated in all patients because local aromatase activity in the breast tissue also contributes to gynecomastia.

50. *A 16-year-old boy presented with delayed puberty and was diagnosed to have congenital IHH. He was initiated on intramuscular testosterone 50 mg every month. After 6 months, the dose was increased to 100 mg every month. Three months later, he presented with painful gynecomastia. How to proceed further?*

The index patient developed gynecomastia after initiation of testosterone therapy. Testosterone-mediated gynecomastia is frequently painful because of rapid enlargement of breast. Treatment strategies include reduction in either dose and/or frequency of testosterone administration or use of selective estrogen receptor modulators/aromatase inhibitors. Selective estrogen receptor modulators like tamoxifen have been widely used in the treatment of peripubertal gynecomastia and are most effective in those with recent-onset gynecomastia. There are anecdotal case reports regarding use of aromatase inhibitors like anastrozole for the treatment of testosterone-mediated gynecomastia. In the index patient, dose of testosterone was reduced to 50 mg every month.

51. *What is the utility of FSH in the induction of puberty in boys with congenital IHH?*

The aim of therapy in a patient with congenital IHH is not only to induce virilization but also to initiate and maintain spermatogenesis. Although the most common agent used to induce virilization is testosterone, it does not initiate spermatogenesis. Therapy with hCG induces virilization in majority of patients with congenital IHH and can initiate spermatogenesis in 20–30 % of patients, who have residual endogenous FSH activity. However, patients with complete deficiency of gonadotropins (both FSH and LH) as evidenced by small testes (testicular volume <4 ml) should be treated with hCG along with FSH, either sequentially or simultaneously, to increase testicular size and initiate spermatogenesis. The recommended dose of FSH is 75–300 IU administered subcutaneously or intramuscularly two to three times a week. However, it should be remembered that isolated FSH therapy does not result in virilization or spermatogenesis.

52. *When to initiate combined gonadotropin therapy in boys with congenital IHH?*

Both LH and FSH act in concert to induce spermatogenesis; FSH induces the expression of LH receptor on Leydig cells and LH provides a support for germ cells by increasing the intratesticular testosterone. However, it is not clear whether to initiate combined gonadotropin therapy, at induction of puberty or when fertility is desired. The data regarding combined therapy with gonadotropins are scarce. It has been shown that early use of combined therapy (at 15–20 years) is more effective for initiation of spermatogenesis, as compared to its use in older subjects (at 25–30 years). Therefore, early use of combination therapy with hCG and FSH may be useful, especially in boys with testicular volume of <4 ml, during mid-late adolescence for optimal pubertal development including spermatogenesis.

53. *What are the predictors of response to gonadotropin therapy in a male with congenital IHH?*

The predictors of response to gonadotropin therapy in a patient with congenital IHH include testicular volume (>4 ml), absence of cryptorchidism and micropenis, higher serum inhibin-B level (>35 pg/ml) at presentation, and early initiation of gonadotropin therapy. Few patients (10 %) with underlying genetic mutations associated with reversible IHH may also respond better (Fig. 7.14).

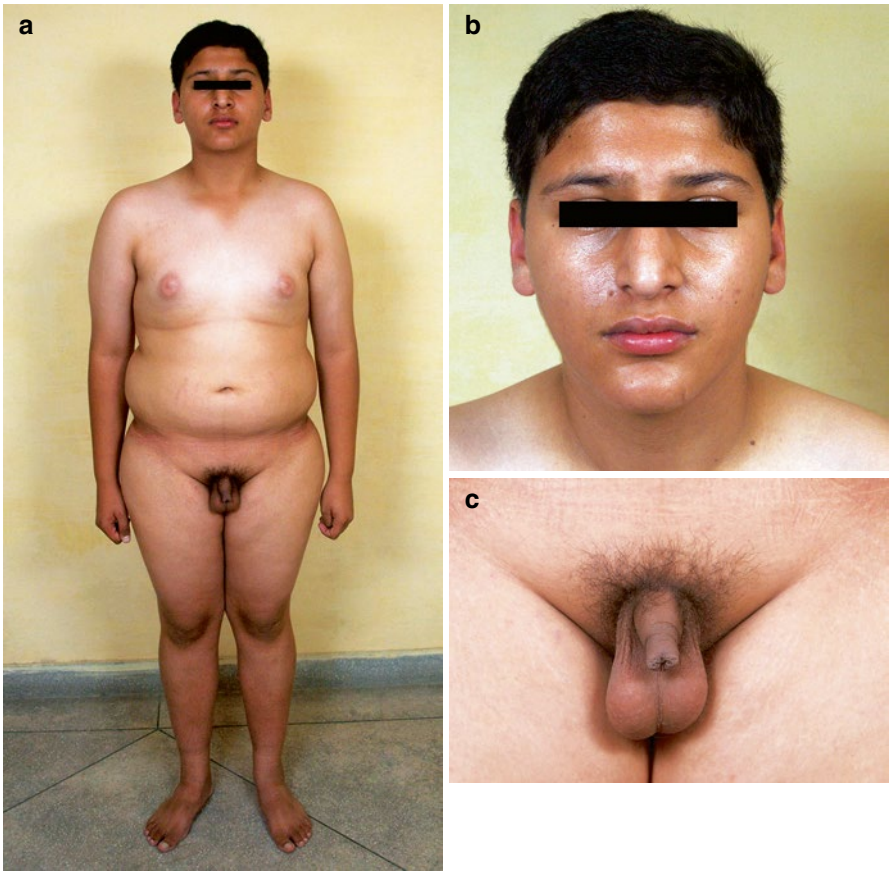

Fig. 7.14 (**a**) An 18-year-old adolescent with delayed puberty and bilateral lipomastia, (**b**) immature facies and absence of facial hairs, (**c**) pubic hair Tanner stage 2, and testicular volume 8 ml suggestive of partial hypogonadotropic hypogonadism

54. *What is the most common therapy to induce puberty in boys with congenital IHH?*

Exogenous testosterone is the most common therapy used to induce puberty in boys with congenital IHH. Various formulations of testosterone are available including oral, intramuscular, transdermal, buccal, and nasal spray; however,

intramuscular preparations of testosterone like enanthate, propionate, or cypionate are preferred for induction of puberty because of the vast experience with their use. Therapy is initiated at a dose of 50–100 mg monthly, and the dose is gradually increased by 50 mg, every six months. Therapy is initiated at a low dose to minimize the risk of priapism, aggressive behavior, and acne and to prevent premature closure of epiphysis. Once a dose of 100–150 mg is reached, the frequency of administration can be increased to fortnightly. The adult replacement dose of testosterone is 200–250 mg intramuscularly every 2–3 weeks. After initiation of therapy, boys should be monitored for growth and progression of pubertal development. Monitoring of serum testosterone levels is not recommended during induction of puberty because of wide variation in reference range of serum testosterone during pubertal development in healthy boys. However, monitoring of serum testosterone should be performed once the adult replacement dose is initiated, with a target to maintain serum testosterone in the mid-normal adult range.

55. *What are the merits and demerits of testosterone therapy?*

Pubertal induction with testosterone is inexpensive and has the convenience of monthly/fortnightly injections as compared to gonadotropins/GnRH. In addition, there is extensive experience of pubertal induction with intramuscular testosterone therapy as compared to other modalities. However, therapy with testosterone only induces virilization and does not initiate spermatogenesis. Further, testosterone therapy is associated with adverse effects like priapism, acne, aggressive behavior, mood disorders, and gynecomastia. Therapy with intramuscular preparations is associated with supraphysiological levels of serum testosterone in the initial few days, followed by low levels before the next injection, resulting in wide swings in the concentration of serum testosterone, which manifests as disturbing fluctuations in sexual function, energy level, and mood.

56. *How does intratesticular testosterone facilitate spermatogenesis?*

In normal men, intratesticular testosterone concentration is 100- to 200-folds higher than serum testosterone levels. High levels of intratesticular testosterone directly promote the growth of seminiferous tubules in concert with FSH. In addition, high concentration of intratesticular testosterone also results in inhibition of AMH from Sertoli cells, which has a suppressive effect on germ cell growth and proliferation.

57. *How to induce fertility in men with congenital IHH?*

In a male with congenital IHH desiring fertility, testicular volume is the key determinant of further management. In patients with a testicular volume >4 ml, therapy with hCG should be initiated and serum testosterone should be

monitored and maintained in the eugonadal range. After 6–12 months of initiation of hCG therapy with serum testosterone in eugonadal range, absence of spermatozoa in ejaculate mandates the addition of FSH (hMG/ rFSH). The combination therapy should be continued for at least next 1–2 years. However, in patients with a testicular volume <4 ml, therapy may be initiated with hCG and FSH simultaneously to improve the fertility outcome. Assisted reproductive technologies may be considered in those who fail to achieve spermatogenesis despite optimal therapy.

58. *How to induce puberty in girls with congenital IHH?*

Puberty should be initiated at the age of 12–13 years in girls. Treatment modalities to induce puberty in girls with congenital IHH include pulsatile GnRH and estrogen therapy. Pubertal induction with estrogen is preferred because of oral route of administration and once-daily dosing. Many preparations of estrogen are commercially available; however, preparations containing 17β-estradiol are preferred, because it is the predominant estrogen in premenopausal women. 17β estradiol (e.g., estradiol valerate, micronized estradiol) is initiated at a dose of 0.25 mg/day and titrated upward every six months to an adult replacement dose of 2 mg/day. Progesterone should be added once breakthrough bleed occurs or after at least 2 years of estrogen therapy. Later, the treatment should be maintained with estrogen and progesterone cyclically.

59. *How to clinically differentiate between congenital IHH and Klinefelter's syndrome in a boy with delayed puberty?*

The presence of anosmia/hyposmia, synkinesia, craniofacial midline defects, micropenis, cryptorchidism, and eunuchoidal proportions (arm span > height and lower segment > upper segment) favors a diagnosis of congenital IHH in a boy with delayed puberty, whereas the presence of long-leggedness, gynecomastia, small firm testis, and learning disabilities suggests hypergonadotropic hypogonadism, especially Klinefelter's syndrome.

60. *Does the presence of gynecomastia differentiate between hypogonadotropic hypogonadism and hypergonadotropic hypogonadism during adolescence?*

No. Although gynecomastia is considered as a typical feature of hypergonadotropic hypogonadism (particularly Klinefelter's syndrome), 30–40 % of patients with hypogonadotropic hypogonadism can also have gynecomastia. Elevated LH levels in patients with Klinefelter's syndrome induce the activity of aromatase in Leydig cells, leading to altered testosterone/estradiol ratio in favor of estradiol, and consequent gynecomastia. Gynecomastia in patients with hypogonadotropic hypogonadism is possibly due to partially preserved LH secretion.

61. *What is Klinefelter's syndrome?*

Klinefelter's syndrome (KFS) is characterized by small testes, gynecomastia, long-leggedness, and learning disabilities in a phenotypic male with the presence of one Y chromosome and two or more X chromosomes. The most common karyotypic abnormality in patients with KFS is 47,XXY (80%), while the rest have mosaicism (46,XY/47,XXY) and higher-grade chromosomal aneuploidies (48,XXXY and 49,XXXXY). However, detection of low-grade mosaicism in a male without any phenotypic features does not merit a diagnosis of Klinefelter's syndrome.

62. *What are the variants of Klinefelter's syndrome?*

The most common variant of KFS is 47,XXY (80%), while the rest have mosaicism (e.g., 46,XY/47,XXY) and higher-grade chromosomal aneuploidies (e.g., 48,XXXY). Hypergonadotropic hypogonadism is present in patients with both classical KFS and in those with higher-grade chromosomal aneuploidies; however, there are important differences in the clinical manifestations between these variants of KFS. The prevalence of congenital malformations (skeletal and cardiac anomalies), learning disabilities, and mental retardation are more in patients with higher-grade chromosomal aneuploidies. In addition, patients with higher-grade chromosomal aneuploidies are taller than those with classical KFS, except patients with 49, XXXXY who have short stature.

63. *What are the defects related to SHOX overdosage in Klinefelter's syndrome?*

Patients with KFS have two or more X chromosomes and at least one Y chromosome. Each sex chromosome has two pseudoautosomal regions (PAR 1 and PAR 2). PAR1 contains at least 24 genes, whereas there are only 4 genes in PAR2. SHOX is a gene present in PAR1 in both X and Y chromosomes. Genes present in PAR 1 region of X chromosome do not undergo inactivation during lyonization. Therefore, patients with KFS have overdosage of SHOX genes, which results in tall stature (long-leggedness) and skeletal abnormalities (scoliosis, kyphosis, pectus excavatum, and clinodactyly).

64. *How to differentiate between hypogonadotropic hypogonadism and hypergonadotropic hypogonadism?*

The presence of anosmia, synkinesia, midline defects, skeletal anomalies, cryptorchidism, micropenis, small soft testes, and eunuchoidal proportions points to the diagnosis of hypogonadotropic hypogonadism (idiopathic),

whereas long-leggedness, small firm testes, gynecomastia, learning disabilities, and moderate degree of spontaneous virilization suggest the diagnosis of hypergonadotropic hypogonadism (Klinefelter's syndrome). Further, the serum gonadotropins are low or low normal in hypogonadotropic hypogonadism, whereas both serum LH and FSH are elevated (FSH > LH) in hypergonadotropic hypogonadism.

65. *How to suspect Klinefelter's syndrome during early childhood?*

Although there are no specific phenotypic features to diagnose KFS in newborns; clinodactyly, cleft palate, inguinal hernia, micropenis, and undescended testis are more common in newborns with KFS. The clinical features which suggest a diagnosis of Klinefelter's syndrome in early childhood include long-leggedness, docile behavior, developmental delay in speech, and learning disabilities. Long-leggedness is usually apparent by the age of 5–8 years. Recognition of KFS in childhood is important because it may help in appropriate management of learning disabilities at an earlier age and timely initiation of testosterone therapy to prevent LH-mediated testicular damage.

66. *What is the trimodal presentation of KFS?*

Majority of the patients with KFS (>75 %) are never diagnosed in their lifetime. Patients with KFS are commonly diagnosed during three phases of life: incidentally during intrauterine period (prenatal karyotyping), in childhood with tall stature and learning disabilities, and in adulthood with infertility.

67. *How to explain the variability in phenotypic manifestations in patients with Klinefelter's syndrome?*

Patients with classical KFS exhibit variability in phenotypic manifestations, and this is possibly related to difference in number of CAG repeats in the androgen receptor. Boys with KFS who have longer CAG repeats manifest with late onset and slow progression of pubertal development, gynecomastia, tall stature, low bone mineral density, and poor response to androgen replacement. However, it has been shown that testicular degenerative process is relatively slower in these subjects. Skewed inactivation of X chromosome was also considered as a cause for variability in phenotypic manifestations; however, this hypothesis has been refuted in recent studies. In addition, patients with mosaic Klinefelter's syndrome may also have variable phenotypic manifestations (Fig. 7.15).

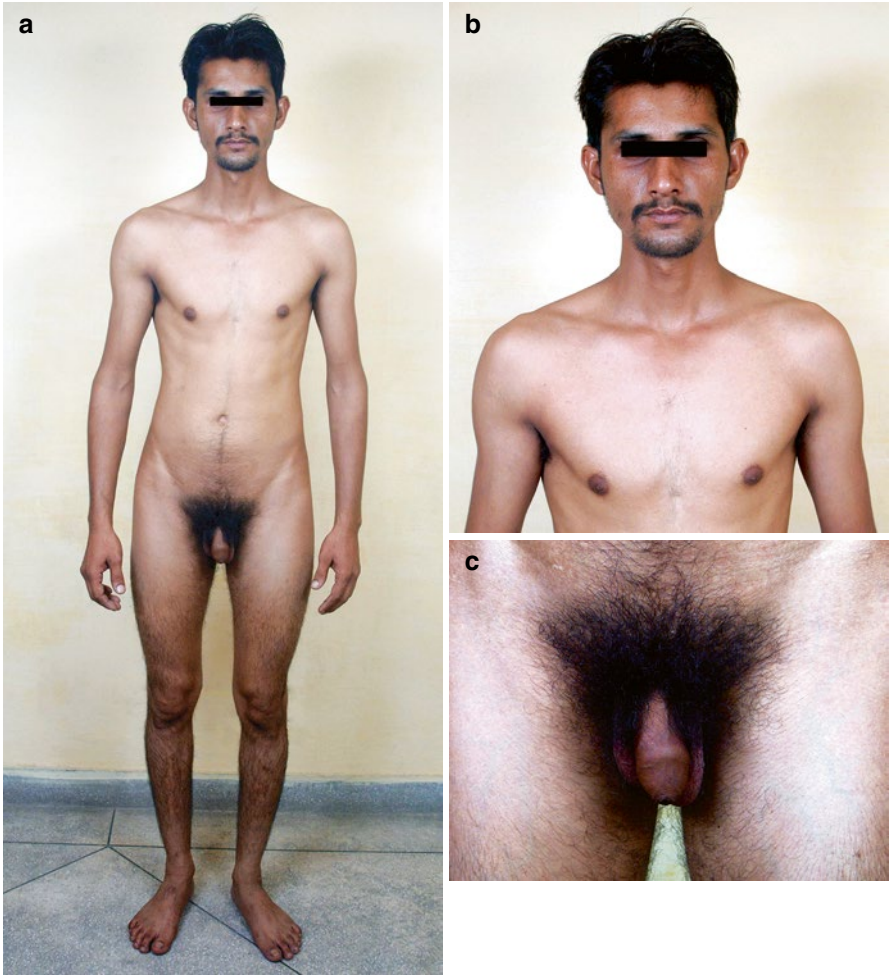

Fig. 7.15 (**a**) A 30-year-old well-virilized man presented with primary infertility. (**b**) He had no gynecomastia, (**c**) pubic hair Tanner stage P_4, and testicular volume 2 ml. His karyotype was 46XY/47XXY

68. *What is the natural history of testicular dysfunction in patients with KFS?*

One of the hallmark feature of KFS is testicular failure and this process starts in utero as evidenced by reduced number of germ cells in fetuses with 47,XXY. The degenerative process continues during childhood and accelerates during adolescence. Adults with KFS have extensive fibrosis and hyalinization of seminiferous tubules, absent/impaired spermatogenesis, and hyperplasia of Leydig

cells and interstitium. The morphology and function of testes in patients with KFS at various stages of life are described in the table given below.

	Seminiferous tubules	Sertoli cells	Germ cells	Leydig cells	Serum testosterone
Fetus	Normal	Normal	Reduced	Normal	–
Mini-puberty	Normal	Normal	Reduced	Normal	Reduced
Childhood	Normal	Normal	Reduced	–	–
Puberty	Hyalinization and fibrosis	Reduced	Reduced	Pseudohypertrophy	Initially normal, later decline
Adulthood	Hyalinization and fibrosis	Reduced	Reduced/ absent	Pseudohypertrophy	Reduced

69. *What is the influence of puberty on testicular function in patients with KFS?*

Although the damage to testes initiates in utero in patients with KFS, there is accelerated testicular damage during midpuberty. The onset of puberty is normal in most patients with Klinefelter's syndrome, but majority have incomplete development of pubertal events. With reactivation of HPG-axis at the onset of puberty, there is an increase in testicular volume (approximately up to 6 ml) along with rise in serum testosterone levels. However, the rise in serum testosterone is accompanied with accelerated hyalinization and fibrosis of seminiferous tubules and degeneration of Sertoli cells. This results in regression of testicular volume to a mean size of 3 ml. The cause for accelerated testicular damage during puberty is not clear; however, elevated levels of gonadotropins, increased intratesticular estradiol levels, and alteration in intratesticular testosterone/estradiol ratio have been implicated.

70. *Why is there testicular failure in patients with Klinefelter's syndrome?*

Ten to 15 % of the genes present in X chromosome are expressed in testes. Overdosage of genes from the extra X chromosome/s is the likely mechanism for testicular failure in patients with KFS. The genes which are implicated in testicular failure are most probably located in the non-PAR region of X chromosome (which escape lyonization) and are expressed in testes. Overdosage of genes from the PAR region is unlikely to be the cause of testicular failure in patients with KFS, as evidenced by normal testicular function in individuals with 47, XYY who also have three copies of genes in the PAR region.

71. *What are the common malignancies in patients with Klinefelter's syndrome?*

Patients with Klinefelter's syndrome are at high risk for the development of breast cancer, lung cancer, mediastinal germ cell tumors, and non-Hodgkin's lymphoma. The risk for breast cancer is increased by 50-fold, while that of mediastinal germ cell tumors is 500-fold. Although the exact mechanism for increased cancer risk is not clear, the most likely explanation is overdosage of genes present in X chromosome which are not lyonized. In addition, abnormal estradiol/testosterone ratio may also contribute to the development of breast cancer.

72. *What are the peculiarities of germ cell tumors associated with Klinefelter's syndrome?*

The most common site of germ cell tumors (GCTs) is gonad in both sexes (95 %), while the rest are present in mediastinum, retroperitoneum, and central nervous system. However, in patients with KFS, there are only anecdotal case reports of testicular GCTs, and the majority of GCTs are present in the anterior mediastinum. Although the incidence of GCTs is only 1.5 in 1,000 in patients with KFS, almost 20 % of all mediastinal germ cell tumors are associated with KFS. GCTs occur much earlier in patients with KFS (childhood and adolescence) as compared to normal population. Children with GCTs classically present with precocious puberty along with gynecomastia, whereas adults present with respiratory symptoms including chest pain, dyspnea, and dry cough. It is also recommended that patients with mediastinal/intracranial germinoma should undergo karyotype analysis. High levels of gonadotropins and overdosage of genes present in the extra X chromosome have been implicated in the increased risk of GCTs in patients with KFS.

73. *When to suspect germ cell tumors in a patient with Klinefelter's syndrome?*

Rapid development/worsening of gynecomastia or presence of respiratory symptoms including chest pain, dyspnea, and dry cough in a patient with KFS should lead to suspicion of GCTs. In addition, development of precocity in a child with KFS also merits evaluation for hCG-secreting GCTs. Estimation of serum β-hCG and alpha-fetoprotein and mediastinal imaging are required to confirm the diagnosis of GCTs.

74. *When to initiate testosterone therapy in patients with KFS?*

Patients with KFS diagnosed during adolescence/adulthood and have low serum testosterone or have elevated LH (>7.7 IU/L) with normal serum testosterone should be treated with exogenous testosterone. The rationale for the initiation of testosterone therapy in those with normal testosterone with elevated LH is to prevent/delay gonadotropin-mediated testicular damage. However, the benefits of this approach have not been proven. Patients who are diagnosed to have KFS during childhood should be monitored with testosterone and LH during puberty

and may be considered for therapy if serum LH starts rising. However, data regarding the use of testosterone therapy during peripubertal period are scarce in patients with KFS as the diagnosis is commonly made in early adulthood.

75. *What are the fertility prospects in a patient with KFS?*

Less than 10 % of patients with classical KFS have spermatozoa in their ejaculate, and donor sperm was the only fertility option for majority of patients with KFS in the past. However, it is now known that even in patients of KFS with azoospermia, there are patchy areas of spermatogenesis in the testis, irrespective of testicular size, serum FSH, inhibin B, and AMH levels. The advent of testicular sperm extraction (TESE) has led to improvement in sperm retrieval rates to 40–50 % in patients with KFS and up to 70 % with the use of micro-TESE. TESE involves excision of a small piece of testicular tissue followed by in vitro extraction of sperm, whereas micro-TESE involves selective excision of seminiferous tubules with active spermatogenesis (identified as swollen seminiferous tubules) followed by in vitro extraction of sperm. After sperm extraction, intracytoplasmic sperm injection (ICSI) is performed. However, even with these newer technologies, the live birth rates vary from 20 to 46 %.

76. *What are the risks associated with fertility in males with KFS?*

KFS is a disorder that occurs due to meiotic nondisjunction of sex chromosomes. Therefore, there are concerns for having hyperhaploid spermatozoa rather than haploid sperms (24,XX or 24,XY instead of 23,X or 23,Y) during meiosis, which may result in chromosomal abnormalities in fetus like 47,XXX or 47,XXY. In addition, there is a higher risk of autosomal abnormalities in chromosome 13, 18, and 21. Chromosomal analysis of sperms from patients with KFS revealed sex chromosomal abnormalities in 4.4 % and autosomal abnormalities in 2 % of sperms. Chromosomal analysis of embryos of KFS couples showed sex chromosomal abnormalities in 13.2 % and autosomal abnormalities in 15.6 % of embryos, as compared to 3.1 % and 5.2 %, respectively, in control population. Hence, genetic counseling should be provided to all KFS couples planning fertility, and preimplantation genetic diagnosis may be considered.

77. *What is gynecomastia?*

Gynecomastia is defined as enlargement of glandular breast tissue in males. Gynecomastia can be physiological during three phases of life: neonatal period, puberty, and old age. Neonatal gynecomastia is seen in 60–90 % of infants and commonly regresses within first year of life. It occurs as a result of transplacental transfer of maternal estrogens. During puberty, 48–64 % of boys can have gynecomastia, which is commonly bilateral but asymmetrical and painful. Approximately 50–70 % of elderly men (50–80 years) have gynecomastia, which is due to age-related decrease in testosterone (“andropause”)

and increase in adipose tissue mass with consequent augmentation in peripheral aromatization.

78. *How to differentiate between gynecomastia and lipomastia?*

The presence of subareolar adipose tissue without glandular tissue is termed as lipomastia or pseudogynecomastia. Lipomastia can be clinically differentiated from gynecomastia by palpation of subareolar tissue or comparison of subareolar tissue with subcutaneous fat in anterior axillary fold. Ultrasonography and FNAC, if required, can be performed to confirm the presence or absence of glandular tissue. The differentiation between gynecomastia and lipomastia is important to avoid anxiety and unnecessary evaluation.

79. *What is pubertal gynecomastia?*

Gynecomastia is common during early to midpuberty and usually occurs at the age of 13–14 years or during pubic hair stage P_3 to P_4. It is usually bilateral; however, 25% of adolescents may have unilateral gynecomastia. Pubertal gynecomastia is painful in majority (70%) because of rapid enlargement of the breast. It usually regresses within 2–3 years, but 10% of patients may have persistent gynecomastia. Pubertal gynecomastia is a result of inappropriate increase in serum estradiol levels in comparison to testosterone, thereby leading to altered testosterone/estradiol ratio (Fig. 7.16).

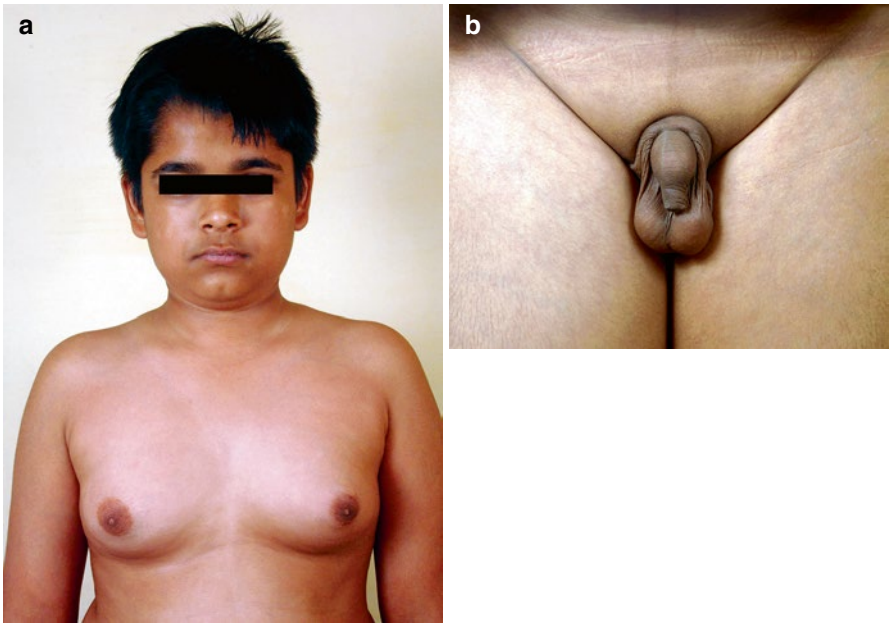

Fig. 7.16 (**a**) A 13-year-old boy with bilateral gynecomastia, (**b**) a testicular volume of 6 ml suggests that the boy has entered into puberty

80. *What is the cause of pubertal gynecomastia?*

During puberty, the level of serum estradiol increases by threefold, whereas serum testosterone increases by more than 30-fold. Therefore, the adult levels of serum estradiol are achieved earlier as compared to adult levels of serum testosterone, thereby leading to altered testosterone/estradiol ratio and consequent gynecomastia. The early rise in serum estradiol is due to LH-mediated increase in testicular aromatase activity as well as increased aromatase expression in adipose tissue during peripubertal period. Other possible mechanisms for pubertal gynecomastia include increased sensitivity to estradiol, peripubertal spurt in IGF-1 secretion, leptin and estrogen receptor polymorphisms, and increased CAG repeats in androgen receptor.

81. *When to evaluate a patient with gynecomastia?*

Gynecomastia (subareolar disk diameter) >5 cm in obese and >2 cm in lean subjects merits evaluation. However, all patients with recent onset, rapid or painful gynecomastia should also be evaluated, irrespective of size. In addition, patients with unilateral gynecomastia or suspicion of malignancy should also undergo further workup.

82. *What are the biochemical investigations required in a patient with gynecomastia?*

Once a decision to evaluate the patient has been made, renal and liver function tests should be obtained. If these are normal, then estimation of LH, FSH, testosterone, prolactin, and thyroid function test should be carried out. In those with rapidly progressive gynecomastia, estimation of serum β-hCG and estradiol level should be done. Further, those patients with suppressed LH and increased testosterone also merit evaluation for hCG-secreting tumors. Elevated levels of serum β-hCG point to a diagnosis of hCG-secreting germ cell tumors, whereas markedly increased serum estradiol levels suggest the possibility of Leydig/Sertoli cell tumor and rarely, adrenocortical carcinoma.

83. *A 16-year-old boy presented with gynecomastia and was diagnosed to have Klinefelter's syndrome. He presented after 3 years with history of rapid enlargement of breast. He was not on testosterone replacement. What is the likely cause?*

Gynecomastia is common in patients with Klinefelter's syndrome (38–75 %), and initiation of testosterone therapy can result in appearance/worsening of gynecomastia in these patients. However, the index patient had rapid progression of gynecomastia without testosterone replacement therapy. This should raise a suspicion of hCG secreting germ cell tumor, which is 500-fold more common in patients with KFS, as compared to the general population. The most common site of hCG secreting germ cell tumor in patients with KFS is mediastinum, followed by pineal gland and testes. Hence, serum hCG level should be estimated in this patient.

84. *What are the causes of gynecomastia?*

Gynecomastia occurs as a result of altered testosterone/estradiol ratio in favor of estradiol, which can be due to estrogen excess, androgen deficiency, or impaired testosterone action. In addition, various drugs are incriminated as the cause of gynecomastia. The various causes of gynecomastia are summarized in the figure given below (Figs. 7.17 and 7.18).

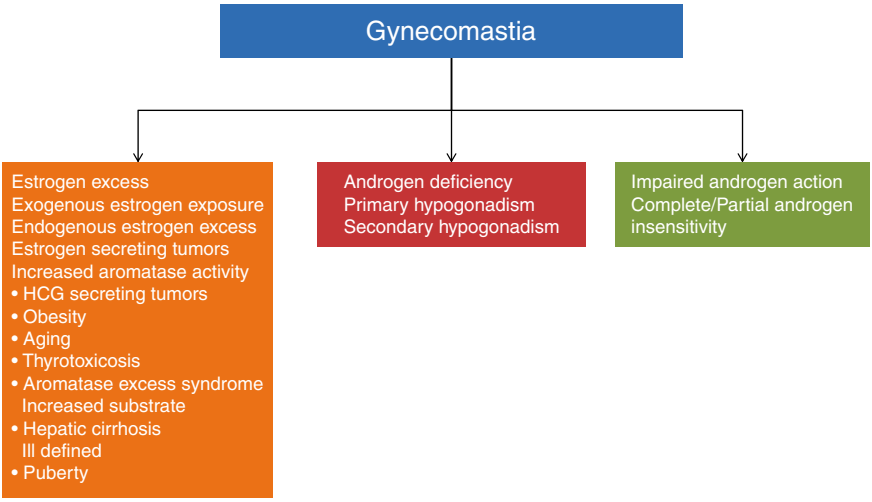

Fig. 7.17 Etiology of gynecomastia

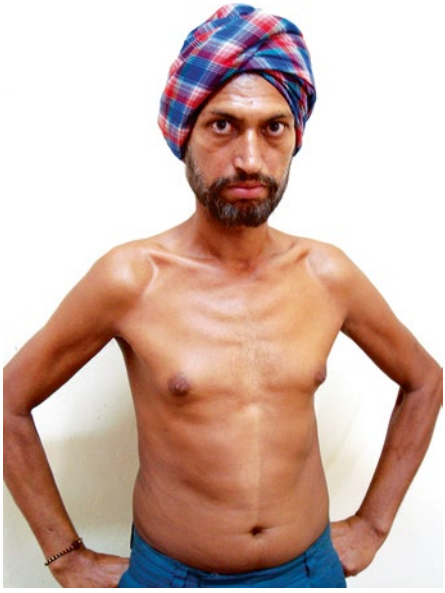

Fig. 7.18 A 35-year-old man with diffuse goiter and thyrotoxicosis. He had recent-onset gynecomastia

85. *What are the common drugs associated with gynecomastia?*

The drugs which commonly cause gynecomastia and the implicated mechanisms are summarized in the table given below.

Mechanism	Drugs
Estrogen-like activity	Digoxin, isoniazid
Increased substrate for aromatization	Androgens, hCG
Decreased estradiol metabolism	Cimetidine
Increased free estradiol (decreased binding of estradiol with SHBG)	Spironolactone, ketoconazole
Decreased androgen production	Spironolactone, ketoconazole, antiretroviral therapy
Androgen receptor blocker	Flutamide, cyproterone, cimetidine
Induction of aromatase activity	GH, thyroxine, hCG

86. *What are the available treatment options for the management of gynecomastia?*

All patients with painful or rapidly progressive gynecomastia and those having cosmetic concerns should be treated. The treatment of gynecomastia depends on the underlying etiology. Discontinuation of the offending drug, if possible, usually results in regression of gynecomastia within a few weeks to months. Patients with gynecomastia due to hyperthyroidism respond to antithyroid drugs, and those with gynecomastia due to hypogonadism respond to androgen therapy; however, testosterone therapy itself can induce/worsen gynecomastia. Patients with estrogen-/hCG-secreting tumors should be managed surgically. Obese subjects should be encouraged for weight loss. If there is no reversible cause for gynecomastia or if gynecomastia has not responded to these measures, medical/surgical therapy should be considered.

87. *What are the drugs available to treat gynecomastia?*

Patients with recent-onset gynecomastia respond better to medical therapy than those with long-standing gynecomastia (>1–2 years), as long-standing gynecomastia is associated with stromal fibrosis. Selective estrogen receptor modulators (SERMs) like tamoxifen and raloxifene have been shown to be effective in approximately 90 % of patients, irrespective of etiology of gynecomastia. Tamoxifen is commonly used at doses of 10–20 mg daily for a period of 3–9 months. Aromatase inhibitors like anastrozole and testolactone are less effective than SERMs; however, they are likely to be more effective in testosterone-induced gynecomastia and in those with aromatase excess states. Non-aromatizable androgen like dihydrotestosterone (e.g., DHT topical gel) has also been tried in the management of gynecomastia. Surgical therapy should be considered in those with long-standing gynecomastia,

those who do not respond to medical therapy, or those who want immediate results. Liposuction and reduction mammoplasty are the available surgical options.

88. *What are the causes of testicular enlargement in a boy with delayed puberty who is on testosterone replacement therapy?*

Normally with testosterone replacement therapy in a boy with delayed puberty, there is no alteration in testicular size. However, increase in testicular volume on testosterone replacement therapy in a boy with delayed puberty suggests the diagnosis of CDGP or reversible idiopathic hypogonadotropic hypogonadism (IHH). *FGFR1*, *GNRHR*, and *CHD7* mutations are associated with reversible IHH. Mechanisms implicated in reversal of IHH include modulation of GnRH neuron plasticity or differentiation of progenitor cells present in subcortical white matter into neuronal lineage in response to testosterone therapy. In addition, the neuroregenerative process in olfactory placode continues throughout the life which may be further stimulated in response to gonadal steroid therapy.

89. *What are the causes to be considered in a well-virilized male with bilateral small testes?*

Approximately 90 % of testicular volume is contributed by seminiferous tubule and the rest by Leydig cells. During pubertal development, progressive virilization corresponds with increase in testicular volume. However, patients with Klinefelter's syndrome may have virilization without corresponding increase in testicular volume. This is because Leydig cell functions are usually normal during peripubertal period under intense LH drive, whereas seminiferous tubules progressively degenerate possibly due to an extra X chromosome. Recent-onset acquired postpubertal testicular failure (e.g., orchitis) may also manifest with similar presentation as regression of secondary sexual characteristics is tardy as compared to testicular atrophy. Further, testosterone therapy in patients with hypogonadism of any etiology results in virilization without corresponding increase in testicular volume (Figs. 7.19 and 7.20).

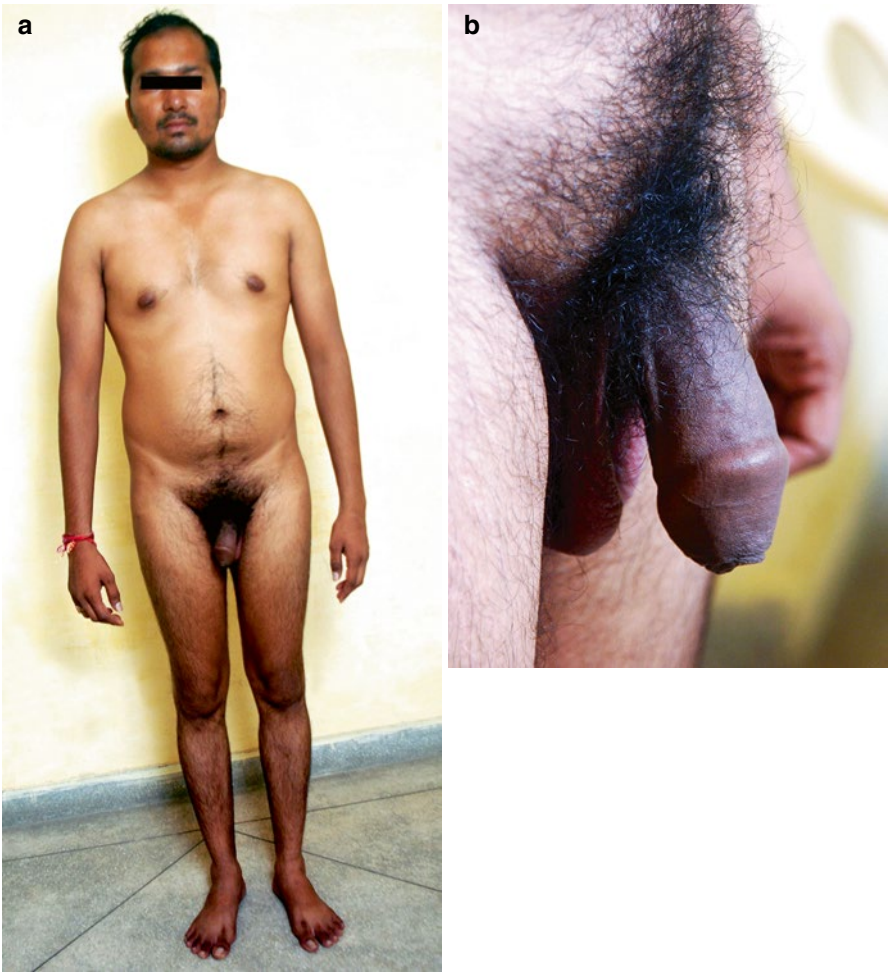

Fig. 7.19 (**a**) A 30-year-old well-virilized male. (**b**) Note small testes with normal penile length. High gonadotropins and 47 XXY karyotype confirmed the diagnosis of Klinefelter's syndrome

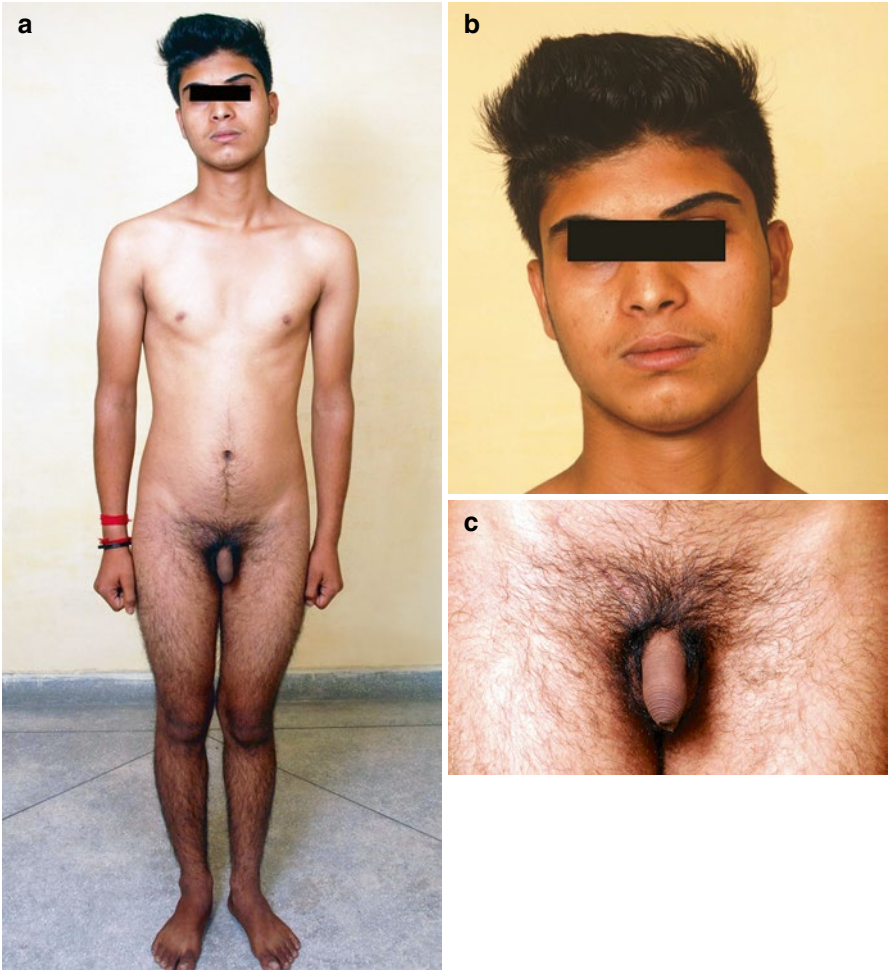

Fig. 7.20 An 18-year-old boy with acquired bilateral testicular failure. Note the normal penile length and single atrophic testes. He had mumps orchitis (left testis) at 10 years of age with small palpable left residual testis. Thereafter, he had normal pubertal events. At age 17 years, he had right testicular torsion and underwent orchidectomy

90. *What are the causes of poor virilization with near normal-sized testes?*

Poor virilization despite near normal-sized testes occurs due to decreased androgen production/action with normal seminiferous tubule growth and development during peripubertal period. The disorders include partial IHH,

fertile eunuch syndrome, minimal androgen insensitivity syndrome (MAIS), and juvenile hypothyroidism. In addition, acquired hypogonadotropic hypogonadism may also result in undervirilization with normal-sized but soft testes (e.g., prolactinoma, nonfunctioning pituitary macroadenoma) (Figs. 7.21 and 7.22).

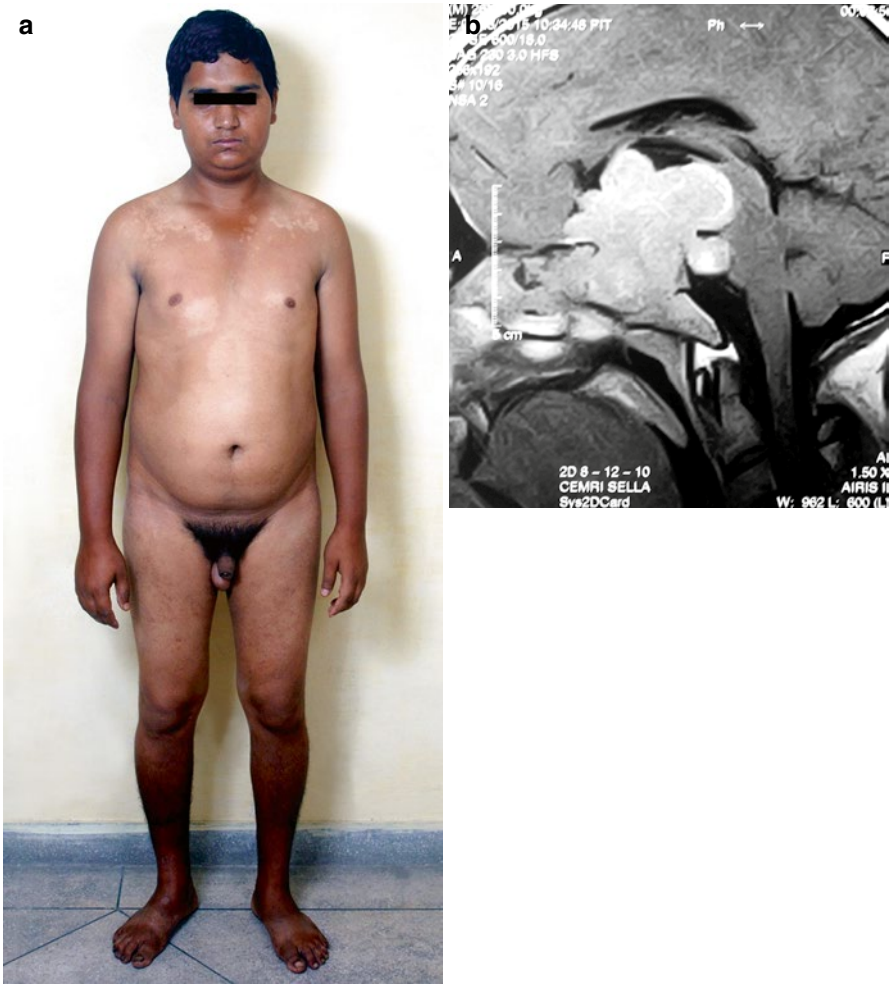

Fig 7.21 (**a**) A 16-year-old boy had testicular volume of 15 ml with poor virilization. (**b**) CEMRI sella showing giant pituitary macroadenoma. Serum prolactin was 4,794 ng/ml, confirming the diagnosis of prolactinoma

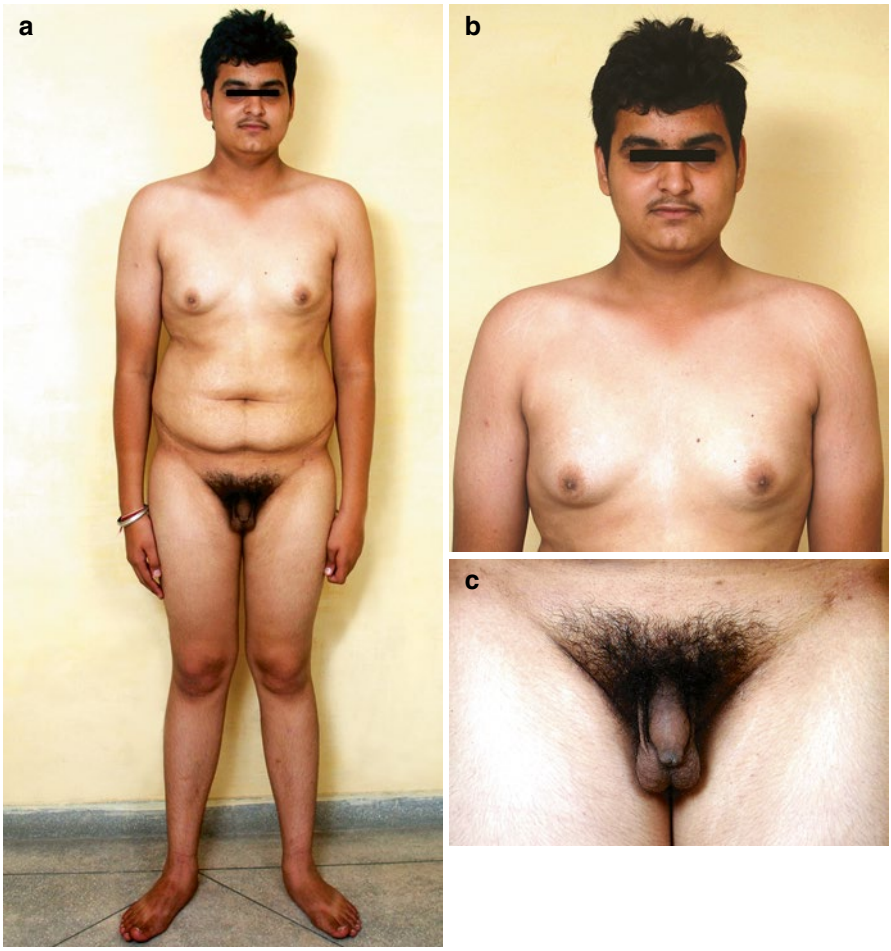

Fig. 7.22 (**a**) An 18-year-old boy presented with poor virilization and (**b**) gynecomastia; (**c**) his testicular volume was 12 ml with serum testosterone of 4 nmol/L suggestive of partial hypogonadotropic hypogonadism

91. *What are the disorders to be considered in a well-virilized male with bilateral nonpalpable testes?*

A well-virilized male with bilateral nonpalpable testes who is not on androgen replacement therapy suggests the diagnosis of idiopathic bilateral cryptorchidism. Testosterone production by the Leydig cells remains uninterrupted as Leydig cells are heat-resistant, whereas germ cells get atrophied as they are heat-sensitive. In addition 46,XX CAH with severe virilization (Prader 5) manifests apparently as virilized male with bilateral nonpalpable testes. Further, patients with hypogonadism of any etiology with bilateral nonpalpable testes

(e.g., hypogonadotropic hypogonadism and Klinefelter syndrome with cryptorchidism, vanishing testes syndrome and post-orchidopexy bilateral testicular atrophy) virilize only with androgen replacement therapy. Therefore, careful treatment history is warranted to narrow the differential diagnosis in such a scenario (Fig. 7.23).

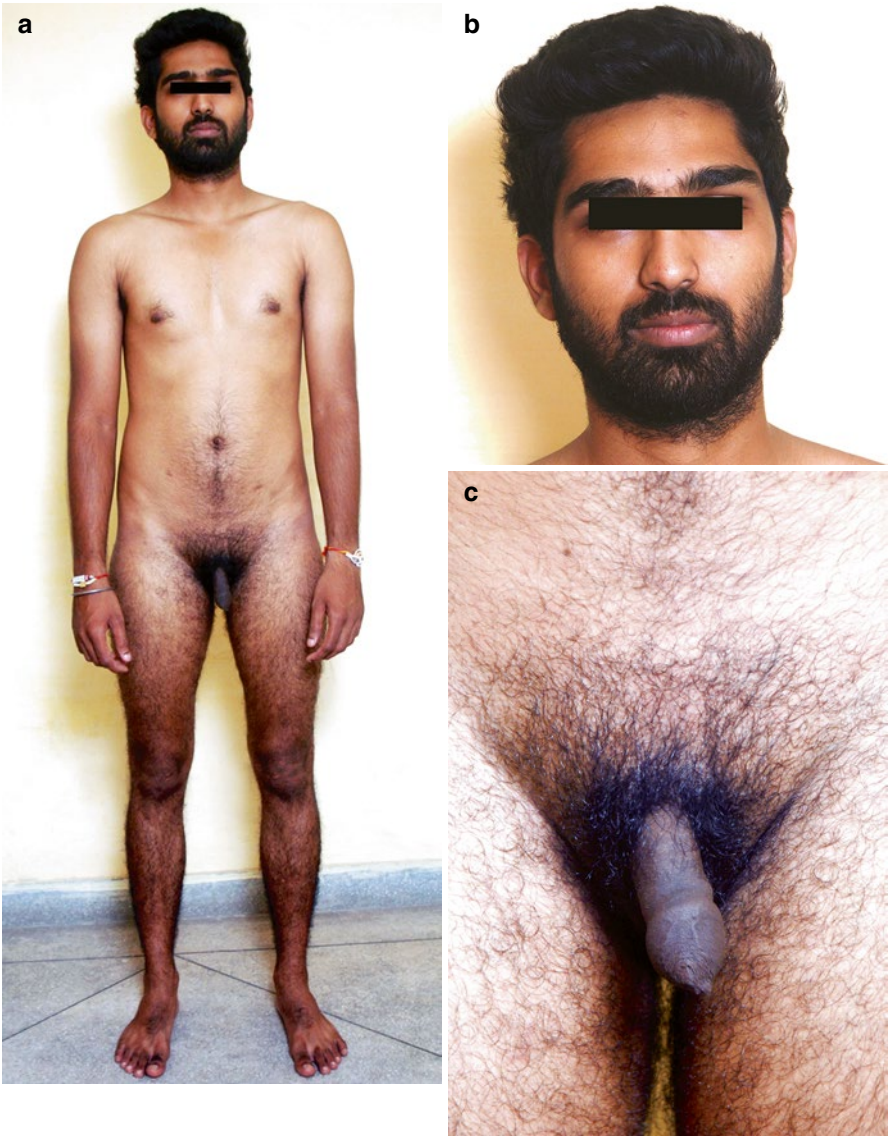

Fig. 7.23 A 20-year-old male presented with bilateral nonpalpable testes. He was well virilized without any androgen replacement. Ultrasonography localized both the testes in the inguinal canal

92. *A 25-year-old male presented with primary infertility. On evaluation, he had eunuchoidal habitus, sparse facial hair with Tanner staging A_+, P_2 and bilateral 4 ml, firm testes and stretched penile length of 8 cm. Hormonal profile showed serum LH 15.2 mIU/ml, FSH 40.2 mIU/ml, and testosterone 15 nmol/L. What to do next?*

The differential diagnoses in this young man, who presented with primary infertility and small testes and had normal testosterone with high gonadotropins, include androgen insensitivity syndrome, Sertoli-cell-only syndrome, and Klinefelter's syndrome. The possibility of androgen insensitivity syndrome (AIS) is considered in view of poor virilization and inappropriately elevated gonadotropin in relation to testosterone. However, the presence of eunuchoidal habitus, very small testes, and rise in FSH greater than LH excludes the diagnosis of AIS. In patients with AIS, very high LH and normal to high FSH with elevated testosterone are characteristic biochemical abnormalities. High LH is due to loss of negative feedback as a consequence of impaired testosterone action; whereas, FSH is primarily regulated by inhibin B which is normally produced by Sertoli cells in these patients. The possibility of Sertoli-cell-only syndrome is also unlikely because of eunuchoidal proportions, poor virilization, and very small testes. However, the biochemical profile in patients with Sertoli-cell-only syndrome may be similar as shown in the index patient. The possibility of Klinefelter's syndrome is high in the index patient, as he had eunuchoidal proportions, small-sized firm testes, and elevated gonadotropin with FSH greater than LH. The normal levels (low-normal to mid-normal) of serum testosterone in patients with Klinefelter syndrome are observed in 50 % of the patients as seen in the index case. It occurs as a result of preserved Leydig cell function during early pubertal period and higher SHBG levels due to relatively increased estradiol. His karyotype was 47,XXY, thus confirming the diagnosis of Klinefelter's syndrome.

Further Readings

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Turner Syndrome

8

8.1 Case Vignette

A 15-year-old female presented with growth failure and poor development of secondary sexual characteristics. She was born of a non-consanguineous marriage by normal vaginal delivery at term with birth weight of 2.7 Kg. Her neonatal period was uneventful and she did not have history of prolonged physiological jaundice or hypoglycemia. There was no history of swelling over hands and feet. She had normal developmental milestones. She was noted to have growth failure since the age of 6 years onward; however, no treatment was sought for the same. There was no history of chronic systemic illness or gastrointestinal symptoms and her appetite was normal. Her scholastic performance was average and she was studying in eighth standard. She did not have history of headache, visual field defect, or previous history of head injury, meningitis, or encephalitis. There was no history of cold intolerance, easy fatiguability, constipation, decreased appetite, or hypotensive episodes. She did not have any development of secondary sexual characteristics till the age of presentation. Her family history was noncontributory. There was no history of any treatment received so far. On examination, her height was 126 cm (−5.5 SDS in CDC growth chart and −2.2 SDS in Turner growth chart, height age 8.5 years, target height 156 cm), weight was 23 Kg (weight age 7 years), upper: lower segment ratio 1, with arm span of 131 cm. Her blood pressure was 100/70 mmHg and pulse rate 92/min regular with no radiofemoral delay. She had multiple pigmented nevi over face, low hairline, nystagmus in primary gaze, and cubitus valgus. She had a small diffuse goiter and deep tendon reflexes were normal. Her sexual maturation score was $A_1P_1B_1$. She did not have any skeletal deformities and hearing was apparently normal. Cardiovascular system examination did not reveal any abnormality and other systemic examination was noncontributory. On investigations, hemoglobin was 13.4 g/dl and liver and renal function tests, blood glucose, and calcium profile were normal. Hormonal profile revealed T_3 1.49 ng/ml (N 0.8–2.0), T_4 8.21 μg/dl (N 4.8–12.7), TSH 3.05 μIU/ml (N 0.27–4.2), TPO antibody <5.0 IU/ml (N <34), 0800h cortisol 559 nmol/L (N 171–536), LH 32.8 mIU/ml (N 1.7–8.6), FSH 117 mIU/ml (N 1.5–12.4), E_2 5 pg/ml (N 12.5–166, follicular phase) and IGF1

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281 ng/ml (N 237–996). Bone age was 12 years. Her 30 cell karyotype was 46Xi(X)(q10) (isochromosome). Serum IgA tTG was negative and serum total IgA was low normal; therefore, the patient was subjected for duodenal biopsy, which was reported to be normal. She underwent growth hormone dynamic tests after appropriate priming and peak response after insulin–hypoglycemia and glucagon stimulation were 9 ng/ml and 19 ng/ml, respectively. Ultrasonography of the abdomen revealed horseshoe kidney and hypoplastic uterus, and ovaries could not be visualized. Two-dimensional echocardiography did not display any abnormality. With this profile, she was diagnosed to have Turner syndrome and was initiated on estradiol valerate at a dose of 0.25 mg/day and was planned to increase gradually every 3–6 months (Fig. 8.1).

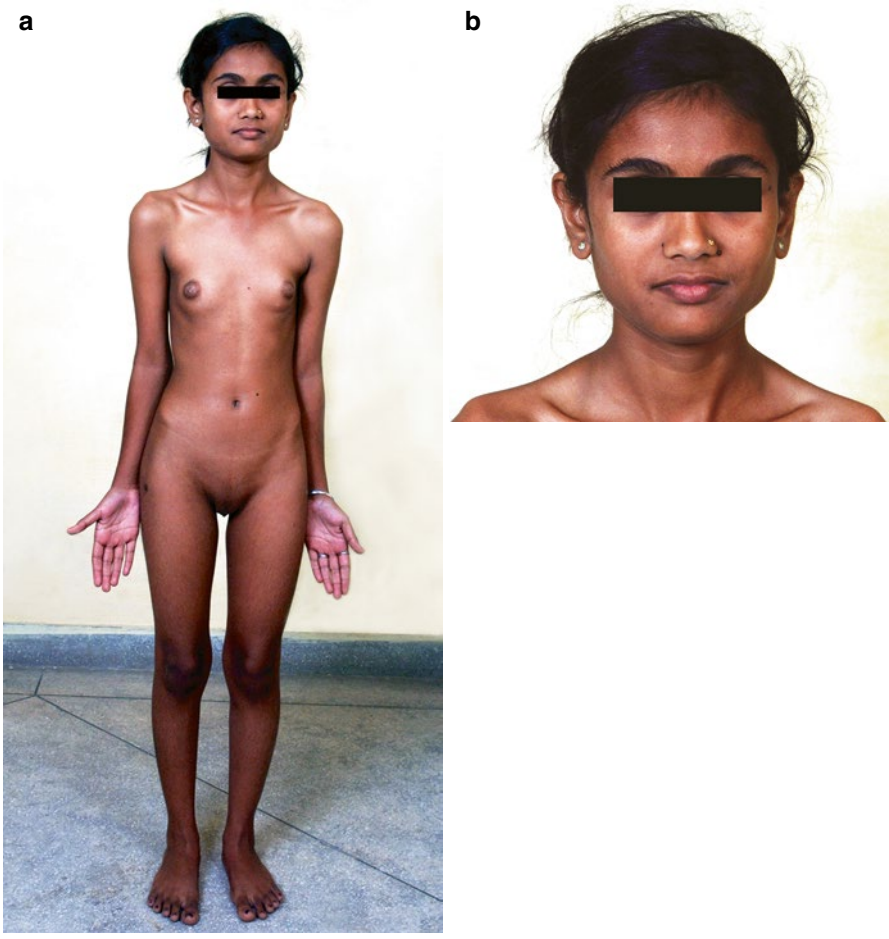

Fig. 8.1 (**a**) A 15-year-old girl presented with short stature and delayed puberty. (**b**) Note multiple facial nevi and webbing of neck. (**c**) Karyotype shows 46Xi(X)(q10), isochromosome (*red arrow*). Patient is presently on estrogen replacement therapy

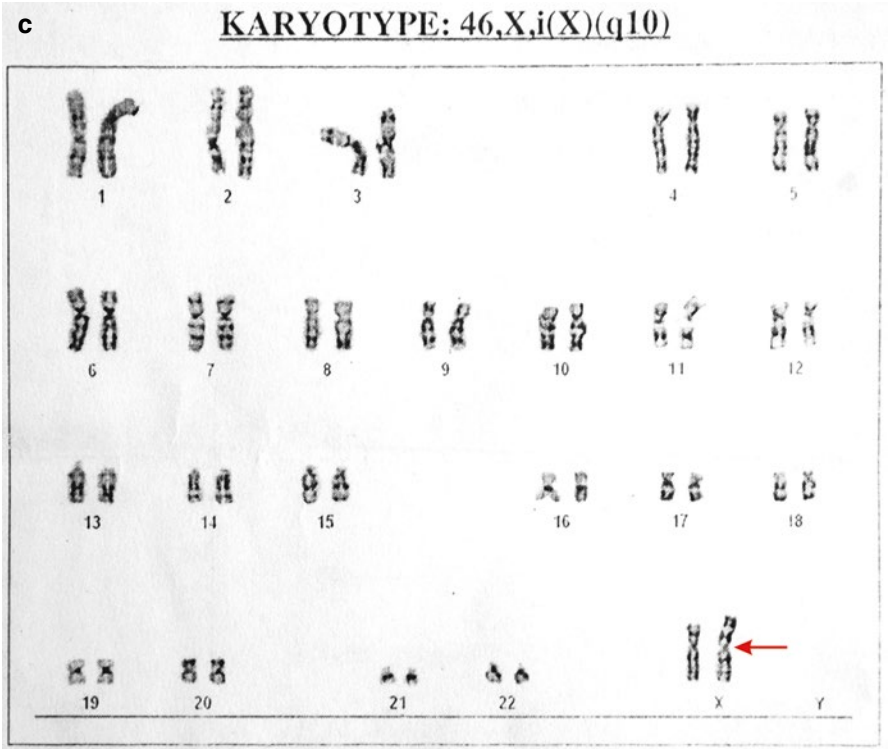

Fig. 8.1 (continued)

8.2 Stepwise Analysis

Linear growth failure and poor development of secondary sexual characteristics in an adolescent raise the possibilities of chronic systemic illness, uncontrolled type 1 diabetes, multiple pituitary hormone deficiency, idiopathic growth hormone deficiency, juvenile primary hypothyroidism, and childhood Cushing's syndrome. Further, a possibility of Turner syndrome should always be considered in a female child who presents with short stature and delayed puberty. Though she did not have any clinical evidence of chronic systemic disease, her weight was more compromised than height (height age > weight age). However, relevant investigations to exclude the systemic diseases were noncontributory. She had phenotypic abnormalities like multiple pigmented nevi over face, low hairline, and cubitus valgus suggestive of Turner syndrome which was further substantiated by high level of FSH and karyotype [46Xi(X)(q10)]. Patients of Turner syndrome born-at-term usually (90 %) have mean birth weight deficit of 600 g, and approximately 20 % of neonates have low birth weight as well as low birth length(≤2SDS). The index patient had a birth weight of 2.7 Kg which is within the normal limits. Mean height deficit in an adult with Turner syndrome is approximately 20 cm, and this is contributed by progressive

decline in growth during intrauterine period, childhood phase, and peripubertal period. Seventy percent of height deficit occurs during prepubertal period and the rest during peripubertal period due to absence of estrogen-mediated growth spurt. Therefore, patients with Turner syndrome have maximum height deficit at the age of 14 years followed by modest increase in height due to delayed epiphyseal fusion. The growth failure is an essential feature of Turner syndrome and occurs due to *SHOX* haploinsufficiency and estrogen deficiency. *SHOX* haploinsufficiency is associated with chondrocyte resistance to GH-IGF1 at epiphyseal growth plate and presence of estrogen is required not only for growth in peripubertal period (by promoting GH-IGF1 surge) but also during prepubertal period, as prepubertal ovary also produces a small amount of estrogen and promotes IGF 1 generation. In addition, 10 % of children with Turner syndrome have concurrent GH deficiency. The index patient had a height SDS of −5.5 on CDC growth chart and −2.2 SDS on Turner-specific growth chart. Estimation of height-deficit on CDC growth chart helps to detect the growth faltering at an early age and allows timely initiation of rhGH therapy. However, growth faltering on Turner-specific growth chart suggests the concurrent presence of other growth-related disorders like celiac disease, primary hypothyroidism, and GH deficiency. Patient was subjected to additional workup for abovementioned disorders and they were essentially normal. The classical phenotypic features of Turner during infancy are lymphedema, cystic hygroma, webbed neck, coarctation of aorta, and partial anomalous pulmonary venous connections. During childhood and adolescence growth failure, hearing defect, delayed puberty and skeletal anomalies are the presenting features. Lymphedema in infancy is due to maldevelopment of lymphatics as a result of X-chromosome haploinsufficiency. The cardiac manifestations (coarctation of aorta 11 % and bicuspid aortic valve 16 %) may be a result of compression of cardiovascular outflow tract by fetal cystic hygroma, which occurs due to jugular lymphatic obstruction or it may be a direct consequence of X-chromosome haploinsufficiency. Baseline 2D echocardiography should be performed in all patients with diagnosis of Turner syndrome, and cardiac MRI is recommended whenever the child is able to tolerate it without sedation. If the baseline cardiac evaluation is normal, then monitoring with echocardiography is recommended at five-year intervals. Further, cardiac evaluation is advised in patients with Turner syndrome during peripubertal period, before contemplating pregnancy, and when they develop hypertension. Resolution of cystic hygroma during late gestation due to opening up of jugular lymphatics manifest later as webbed neck. Skeletal anomalies associated with Turner syndrome include short neck, shield chest, cubitus valgus, short fourth and fifth metacarpal and metatarsals, Madelung deformity, and kyphoscoliosis. These abnormalities are not present at birth, but evolve during childhood and are the result of *SHOX* haploinsufficiency. Ocular manifestations include strabismus, hypermetropia, epicanthal folds, pseudo-ptosis, telecanthus, upward slanting of palpebral fissures, red-green color blindness, and rarely nystagmus. Our patient had nystagmus in primary gaze; however, the cause of nystagmus is not known in patients with Turner syndrome. Renal abnormalities in patients with Turner syndrome include horseshoe kidney (20–50 %), duplication of renal pelvis, renal dysplasia, and ectopic kidney. Karyotype of the index patient confirmed the diagnosis of Turner syndrome 46Xi(X)(q10) (isochromosome). Fifty percent of patients

with Turner syndrome have 45XO karyotype, whereas mosaic forms (45XO/46XX) contribute approximately to 25 % of cases, and the rest have structural abnormalities of X chromosome (e.g., long-arm or short-arm deletion, isochromosome, or ring chromosome). Patients with deletion of short arm of X chromosome (Xp-) predominantly present with growth failure, while those with long-arm involvement (Xq-) have ovarian failure as the presenting manifestation. Further, patients with ring chromosome usually have mental retardation and those with isochromosome have associated autoimmune disorders. Although our patient had isochromosome, she did not have autoimmune thyroid disease or celiac disease. The aims of treatment in a patient with Turner syndrome are to improve the adult height potential, timely induction and maintenance of secondary sexual characteristics, and appropriate management of comorbidities like cardiovascular, renal, and otological disorders. The index patient was initiated with low-dose estradiol valerate. Delaying the induction of puberty beyond 12 years of age is not recommended as this may adversely affect psychosocial development and bone health of the child. Further, there is evidence to support that estradiol when initiated at low doses at 12 years of age and slowly built-up thereafter allows normal pubertal development without influencing the adult height potential of the child. Progesterone should be added cyclically, either after the onset of breakthrough bleed or 2 years after the initiation of estrogen therapy. The rhGH therapy in children with Turner syndrome is rewarding if initiated at the onset of growth faltering, and it can be started as early as 9 months of age. The index patient had bone age of 12 years and did not have epiphyseal fusion; therefore, rhGH therapy may still be effective in terms of final adult height gain.

8.3 Clinical Rounds

1. *What is Turner syndrome*?

   Turner syndrome (TS) is a chromosomal disorder characterized by short stature, gonadal failure, and typical physical features in a phenotypic female with loss of either entire X chromosome or complete/partial loss of short arm (p) of X chromosome.

2. *Who should not be considered to have Turner syndrome despite the deletion of X chromosome*?

   Deletion of a part of X chromosome distal to Xq24 is associated with gonadal failure; however, these individuals do not have characteristic phenotypic features of Turner syndrome and hence not considered to have Turner syndrome. Patients with small distal short-arm deletions (Xp-) with intact Xp22.3 have many characteristic skeletal abnormalities of Turner syndrome, but have a low risk of gonadal failure; hence, they are also not considered to have Turner syndrome. Individuals with a male phenotype are also not considered to have Turner syndrome, regardless of the karyotype.

3. *Do all women with a karyotype 45, XO qualify for a diagnosis of Turner syndrome*?

   The diagnosis of Turner syndrome requires the presence of characteristic features in a phenotypic female along with karyotypic abnormalities. Hence, presence of karyotypic abnormalities without phenotypic features does not qualify for a diagnosis of Turner syndrome.

4. *What is SHOX*?

   Homeobox-containing genes are involved in growth, differentiation, and organogenesis. *SHOX* (short-stature homeobox) is one of the homeobox-containing genes and is located on the pseudoautosomal region (PAR) of short arm of X and Y chromosomes. *SHOX* is expressed as early as 6 weeks of intrauterine life in various tissues including humerus, radius, ulna, wrist, and first and second pharyngeal arches and is responsible for chondrocyte growth and differentiation in these tissues.

5. *What are pseudoautosomal genes*?

   Pseudoautosomal genes are genes which are present on sex chromosomes but behave like autosomal genes. Normally, an autosomal gene works in tandem with its homologous pair on the corresponding chromosome for its expression, whereas genes in the sex chromosomes need only one copy for their expression (either in X or Y chromosome). Genes in the pseudoautosomal region (e.g., *SHOX*) require both the copies for their optimal expression and do not undergo lyonization. These genes are present in the distal ends of X and Y chromosomes. The pseudoautosomal region in sex chromosomes is depicted in the figure given below (Fig. 8.2).

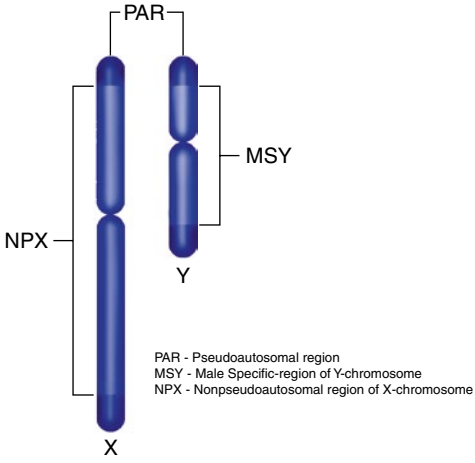

Fig. 8.2 Pseudoautosomal region in sex chromosomes

6. *What are the manifestations of SHOX insufficiency*?

Two copies of *SHOX* gene are normally expressed in males as well as in females. As *SHOX* regulates skeletal growth and development, its haploinsufficiency results in short stature and overdose results in tall stature (e.g., Klinefelter's syndrome 47, XXY). The skeletal manifestations of *SHOX* insufficiency include short stature, short neck, short metacarpals/metatarsals, cubitus valgus, mesomelia, Madelung wrist deformity, high-arched palate, micrognathia, and abnormal auricular development (Figs. 8.3 and 8.4).

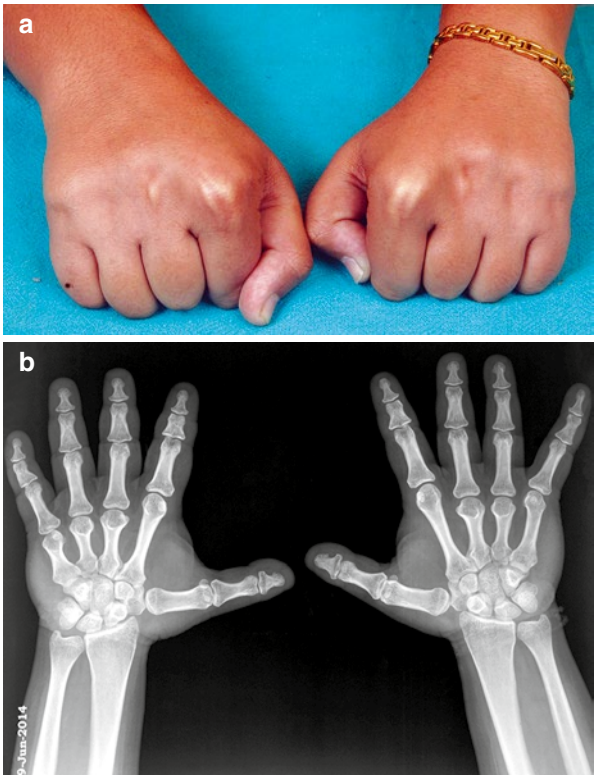

Fig. 8.3 (**a**) "Clinched fist" sign to demonstrate short third, fourth, and fifth metacarpals. (**b**) X-ray of hand showing short metacarpals in the same patient

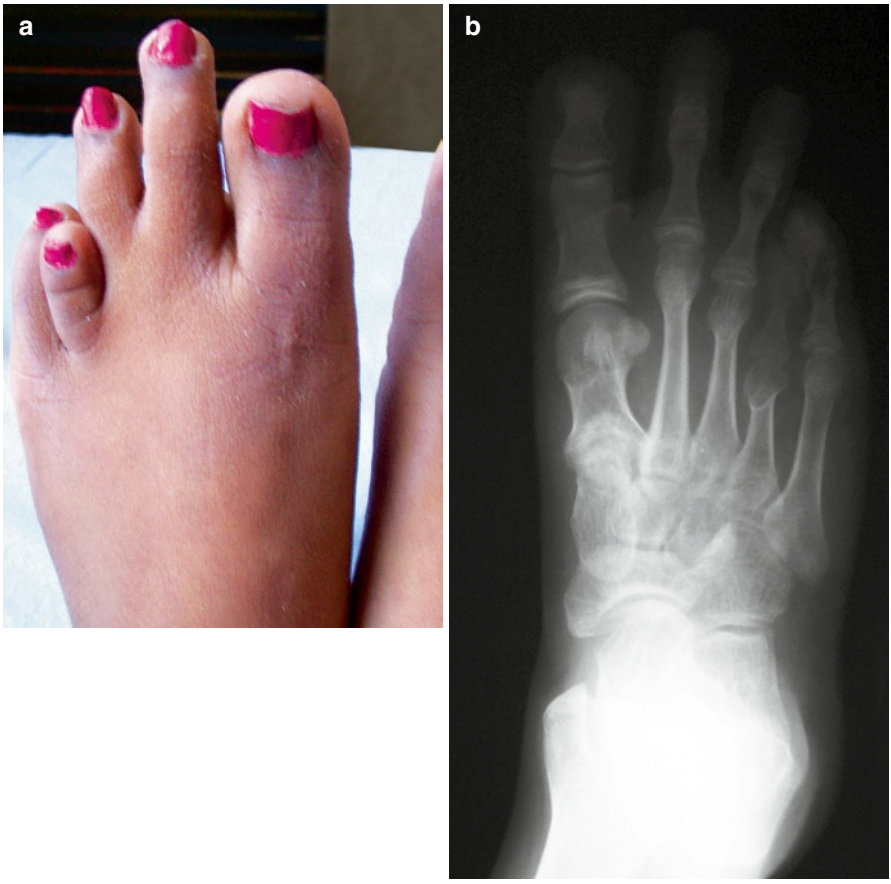

Fig. 8.4 (**a**) Short fourth metatarsal in the same patient. (**b**) X-ray of foot confirms the same finding

7. *What is Madelung deformity*?

   Madelung deformity is characterized by shortening and bowing of the radius with dorsal subluxation of distal ulna. The deformity usually becomes clinically evident by adolescence. The causes include *SHOX*-insufficiency-related disorders, mucopolysaccharoidosis, achondroplasia, trauma, or idiopathic in origin.

8. *What are SHOX insufficiency-related disorders*?

SHOX insufficiency-related disorders include Turner syndrome, Leri–Weil dyschondrosteosis, and Langer mesomelic dysplasia. The differences among these disorders are summarized in the table given below.

Characteristics	Turner syndrome	Leri–Weil dyschondrosteosis	Langer mesomelic dysplasia
SHOX gene defect	Haploinsufficiency	Haploinsufficiency	Homozygous deficiency
Gender	Only females	Both sexes	Both sexes
Mean height SDS	−3.2 SDS	−2.4 SDS	−6.2 SDS
Skeletal defects	Mesomelia ±	Mesomelia +	Mesomelia +++
	Cubitus valgus	Cubitus valgus	Hypoplasia/aplasia of ulna, fibula, and mandible
	Short 4th and 5th metacarpals	Short 4th and 5th metacarpals	
	Madelung deformity (8 %)	Madelung deformity (75 %)	
	High arched palate	High arched palate	
Gonadal failure	Yes	No	No
Cardiac and renal anomalies	Yes	No	No

In addition, *SHOX* haploinsufficiency has also been described in individuals with idiopathic short stature.

9. *What is Lyon's hypothesis*?

Lyon's hypothesis states that one of the X chromosomes in somatic cells of a normal female embryo undergoes inactivation during 16–64 cell stage (second week of life). This inactivation occurs in a random fashion and may involve X chromosome derived from either parent. This silencing of X chromosome in a female is a compensatory response to achieve equality in genetic dose between a male and female, as X chromosome consists of >1,000 genes as compared to Y chromosome with barely 200 genes. This silencing of second X chromosome is mediated by the gene *XIST*.

10. *Why do patients with TS have phenotypic abnormalities despite the fact that only one X chromosome is active in a normal female*?

    In a normal female, one of the X chromosomes undergoes inactivation (lyonization); however, this inactivation is not complete and some genes escape lyonization, which are essential for survival and development of a normal female. These include genes present in the pseudoautosomal region (e.g., *SHOX*) and some genes in other parts of the X chromosome (10–15 %). Patients with TS (45,XO) have only one X chromosome, and the absence of those genes which escape lyonization in the second X chromosome results in phenotypic abnormalities of TS.

11. *Is 45,XO compatible with life*?

    It is known that 3 % of all conceptuses are 45,XO and 99 % of these are aborted. This shows that the presence of both copies of X chromosomes is required for survival. However, 1 % of fetuses with a karyotype of 45,XO are still able to survive, and this can be explained by the presence of occult 45,XO/46XX mosaicism.

12. *How common is 45,XO karyotype in Turner syndrome*?

    Approximately 45–60 % of individuals with Turner syndrome have 45,XO karyotype. 45,XO/46,XX mosaicism is the next common karyotypic abnormality, which is present in 20–30 % of patients. Other abnormalities like deletion of short arm (p-), isochromosome q, ring chromosome, and marker chromosome contribute to the rest.

13. *What is ring chromosome*?

    Ring chromosome is a structurally abnormal chromosome resulting from fusion of both arms of a chromosome, after the breakage of genetic material from both the distal ends. Ring chromosome [46,Xr(X)] is the cause of Turner syndrome in approximately 10 % of patients. The presence of ring chromosome in Turner syndrome (TS) is associated with spontaneous menarche in one-third, higher incidence of mental retardation, and lower incidence of congenital malformations. The figure illustrated below shows formation of ring chromosome (Fig. 8.5).

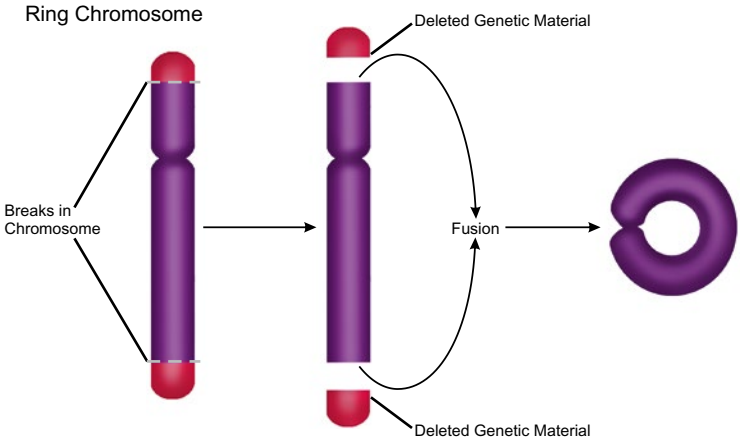

Fig. 8.5 Evolution of the ring chromosome

14. *What is isochromosome*?

Isochromosome is a structurally abnormal chromosome resulting from loss of one arm of a chromosome with duplication of other arm. Isochromosome q[46,Xi(Xq)] contributes to approximately 8 % of patients with TS. Presence of isochromosome q is associated with increased incidence of autoimmune disorders, deafness, and lower incidence of structural abnormalities. The figure given below illustrates the development of isochromosome (Fig. 8.6).

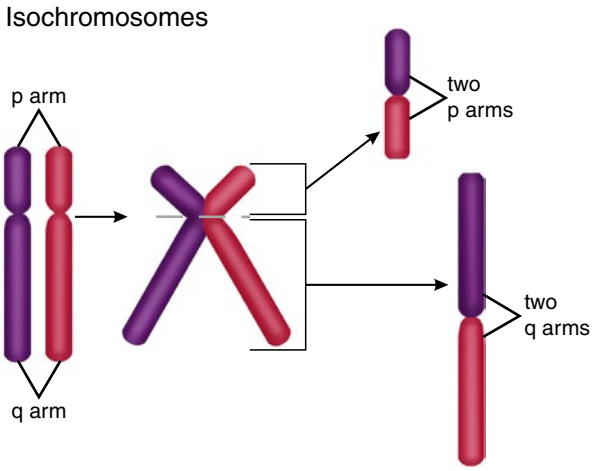

Fig. 8.6 The development of isochromosome (Xpi or Xqi)

15. *What is marker chromosome*?

Marker chromosome is a small segment of supernumerary chromosome, whose origin cannot be determined by conventional cytogenetic methods. These fragments of chromosomes may arise from autosome or sex chromosomes (X or Y) and require molecular approaches for definitive characterization. The presence of marker chromosome in a patient with TS should prompt active search for Y chromosome material. In addition, presence of marker chromosomes is also associated with increased risk of developing intellectual disability in these patients.

16. *How to suspect TS during intrauterine period*?

TS should be suspected in a fetus with abnormal features on ultrasonography including increased nuchal translucency, cystic hygroma, left-sided cardiac defects, renal anomalies, and growth retardation. In addition, a positive triple or quadruple test should also raise a suspicion of TS. However, these ultrasonographic features or biochemical tests are not specific for TS, and a karyotype should be carried out for confirmation of diagnosis.

17. *How to suspect TS at birth or during infancy*?

Turner syndrome can be suspected at birth/infancy by the presence of lymphedema, webbed neck, low posterior hairline, abnormal auricles, left-sided cardiovascular anomalies (bicuspid aortic valve and coarctation of aorta), and renal anomalies (horseshoe-shaped kidney). The skeletal abnormalities associated with TS are not present at birth; they progressively develop with growth of the child.

18. *How to confirm the prenatal diagnosis of TS*?

Karyotype is mandatory for the diagnosis of TS. For cytogenetic analysis, chorionic villous sampling can be performed between 10 and 12 weeks of gestation, whereas amniocentesis can be performed between 15 and 17 weeks. In the presence of ultrasonographic abnormalities and 45,XO or mosaicism, the likelihood of having clinical TS is very high. However, with 45,XO/46,XX mosaicism without ultrasonographic abnormalities, probability of TS is low. Hence, karyotype should be repeated after birth in all babies with a prenatal diagnosis of TS.

19. *What is the cause of webbed neck in Turner syndrome*?

Lymphatic aplasia or hypoplasia during intrauterine period results in accumulation of lymph in the jugular lymph sac and consequently, formation of a large swelling in the neck, i.e., cystic hygroma. Progressive resolution of lymph in cystic hygroma results in redundant and loose skin around the neck, leading to

webbed neck. Other manifestations of lymphatic aplasia/hypoplasia include lymphedema, low posterior hairline, low-set ears, and nail dysplasia. Lymphatic abnormalities are thought to be due to haploinsufficiency of an unidentified gene present on short arm of X chromosome (Fig. 8.7).

Fig. 8.7 (**a**) An 18-year-old female with Turner syndrome having low-set ears, webbed neck, and multiple nevi. (**b**) Low hairline in another patient of Turner syndrome

20. *What is the clinical course of lymphedema in patients with TS*?

Lymphedema is present in 25–50 % of patients with TS and usually manifests at birth in majority (76 %) of these patients. Lymphedema commonly resolves spontaneously by 2 years of age in most of the patients. However, it can recur at any age thereafter, especially after initiation of rhGH or estrogen therapy. Supportive therapy including manual massage for lymphatic drainage and compression stockings is helpful in alleviating lymphedema; surgery may be required in severe and resistant cases.

21. *What are the clinical implications of presence of webbed neck in patients with TS*?

Webbed neck is one of the few manifestations of TS, which is present even at birth and hence helps in the early diagnosis. In addition, patients with webbed neck have three times increased risk (36 % vs. 12 %) of having cardiac anomalies as compared to those who do not have webbed neck.

22. *Why are cardiac anomalies more common in patients of TS with webbed neck*?

The cause for association between cardiovascular malformations and webbed neck is not well known. However, the proposed mechanisms include compressive effect of enlarged lymph sac on the developing cardiac outflow tract during intrauterine period and dysfunction of a common gene required for lymphatic as well as cardiovascular development.

23. *What are the cardiovascular anomalies in Turner syndrome*?

Cardiovascular anomalies occur in 25–45 % patients with TS. Bicuspid aortic valve (16 %) and coarctation of aorta (11 %) are the most common cardiovascular defects. Other cardiovascular anomalies include aortic dilatation, aortic aneurysm, elongated transverse aortic arch, partial anomalous pulmonary venous connection (PAPVC), and left-sided superior vena cava (Fig. 8.8).

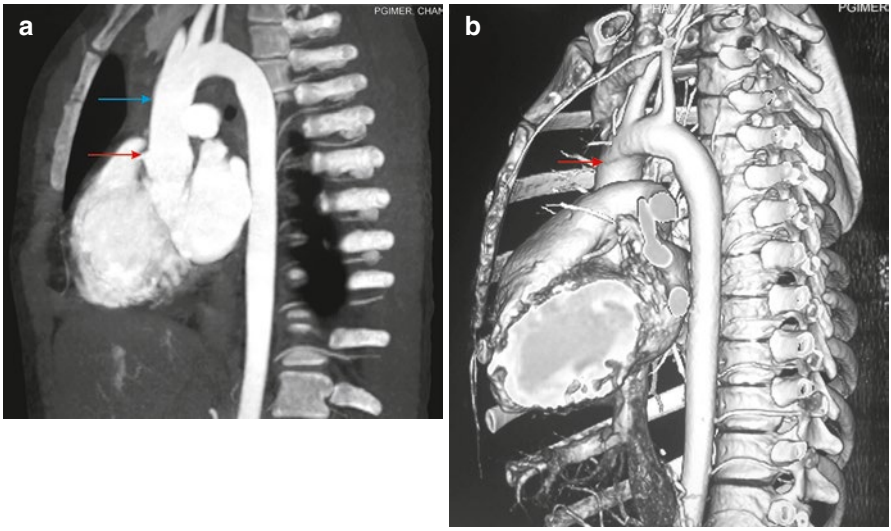

Fig. 8.8 (**a**) Sagittal reconstructed MIP and (**b**) VRT images of thoracic CT angiography showing mild dilatation of aortic root (*red arrow*) and ascending thoracic aorta near the aortic arch (*blue arrow*) in a patient with Turner syndrome

24. *What are the ECG abnormalities present in patients with TS*?

The most common ECG abnormality present in patients with TS is sinus tachycardia. Other abnormalities include right-axis deviation, QT prolongation, short PR interval, and nonspecific T wave abnormalities.

25. *What is the importance of pulse and blood pressure examination in patients with TS*?

Sinus tachycardia is common in patients with TS, which is due to dysautonomia and can be evident even during intrauterine period. Patients with TS having sinus tachycardia are at increased risk of developing aortic dissection in the presence of aortic dilatation. Radio-femoral delay is a clinical clue for the presence of coarctation of aorta. Blood pressure examination should be carried out in all four limbs to detect the presence of coarctation of aorta.

26. *What are the risk factors for aortic dissection in patients with TS*?

Bicuspid aortic valve, coarctation of aorta, elongated transverse arch, aortic dilatation, systemic hypertension, and sinus tachycardia are the risk factors for aortic dissection in patients with TS. The predisposition for the development of aortic dilatation/dissection is possibly due to abnormality in vascular collagen.

27. *What is elongated transverse aortic arch*?

Elongated transverse aortic arch (ETA) is one of the common cardiovascular abnormalities present in patients with TS. ETA refers to the flattening of the aortic arch with kinking along the lesser curvature, which is seen as increased distance between the origins of left common carotid artery and left subclavian artery. ETA can be well visualized on cardiac MRI and is present in 49 % of patients with TS.

28. *How to define aortic dilatation*?

The normal diameter of ascending aorta is between 20 and 37 mm. An aortic diameter >4 cm is considered as aortic dilatation and surgical intervention are indicated if diameter exceeds 5 cm. Patients with TS have lower body surface area and smaller vasculature, hence, the aortic size is corrected for body surface area, and aortic size index (ASI) is used for defining aortic dilatation. ASI is calculated as diameter of ascending aorta (calculated at the level of right pulmonary artery) divided by body surface area. An ASI >2 cm/m^2 warrants close surveillance, while an ASI of >2.5 cm/m^2 warrants urgent referral for further evaluation.

29. *When to perform cardiac imaging in patients with TS*?

Baseline electrocardiogram, echocardiography, and cardiac MRI are recommended in all patients with TS at diagnosis. In children, cardiac MRI can be delayed till the age when the procedure can be performed without sedation. However, if echocardiography fails to adequately visualize aortic valve, aortic arch, or pulmonary veins, cardiac MRI can be done with sedation. Cardiac echocardiography and MRI should be repeated every 5–10 years thereafter, if the baseline evaluation was normal. More frequent monitoring is required in patients who have hypertension, ETA, aortic dilatation, or those planning pregnancy.

30. *Why to perform cardiac MRI in patients with TS*?

Cardiac MRI is more sensitive than echocardiography for the detection of coarctation of aorta and can detect partial anomalous pulmonary venous connection (PAPVC), persistent left superior vena cava, and elongated transverse aortic arch which are not easily detected on echocardiography.

31. *Does karyotype help in predicting the risk of cardiovascular abnormalities in TS*?

Yes. Cardiovascular defects are present in 30–39 % of patients with a karyotype 45,XO, while they are present in 24 % of patients with mosaicism and 11–12 % in those with structural abnormalities of X chromosome.

32. *What is the growth pattern in patients with TS*?

Short stature is the most consistent phenotypic abnormality in patients with TS and is virtually present in all. Growth failure in TS begins in utero and these newborns are born small for their gestational age with a median height SDS of −1.17. They continue to grow slow during infancy and childhood, reaching a height SDS of −3 by the age of 3 years. In addition, they lack pubertal growth spurt, and therefore, the average adult height of an untreated individual with TS is 20 cm shorter (−3.2 SDS) than normal women of the same population (Fig. 8.9).

Fig. 8.9 A 16-year-old girl with classical Turner syndrome. Note the webbing of the neck and cubitus valgus

33. *What are the causes of short stature in patients with Turner syndrome*?

Approximately two-third of the height deficit in patients with TS is contributed by *SHOX* haploinsufficiency, as two copies of SHOX gene are required for chondro-osteogenesis and normal linear growth. Other putative genes in the X chromosome are implicated for rest of the height deficit. In addition, lack of estrogen during prepubertal period also contributes to height deficit, as prepubertal ovary too secretes a small amount of estrogen which has growth-promoting effect. Further, coexisting disorders like celiac disease and autoimmune thyroid disease can also contribute to short stature and should be suspected when girls with TS falter on TS-specific growth charts.

34. *Which growth chart should be used for monitoring linear growth in children with TS*?

The height of a girl with TS should be plotted on TS-specific growth charts as well as standard growth charts. Monitoring of growth on standard growth charts is important as it allows early detection of growth abnormalities and timely initiation of rhGH therapy. In addition, a girl with TS should also be monitored on TS-specific growth chart, and if the height of the child falters on TS-specific growth chart (either crosses centile curve downward or height ≤ –2SDS), secondary causes of growth retardation like hypothyroidism, celiac disease, malnutrition, or growth hormone deficiency should be actively sought.

35. *What are the alterations in growth hormone dynamics in TS*?

The data regarding GH-IGF1 axis in patients with TS is conflicting. Most of the studies show that integrated GH secretion is normal during prepubertal years, while it is low during peripubertal period. This is because of lack of pubertal GH surge in these patients due to estrogen deficiency and when treated with estrogen, GH secretion is normalized. GH response to provocative stimuli is also normal in majority of patients with TS, while 10–20 % of these patients may have coexisting GH deficiency. Serum IGF1 levels have been reported to be normal or low in various studies. Low IGF1 has been attributed to GH resistance at hepatocytes, decreased acid-labile subunit, and increased IGFBP3 proteolysis. Some studies have also reported IGF1 resistance in girls with TS.

36. *What are the otological disorders in children with TS*?

Abnormalities of external, middle, and inner ear are common in patients with TS. The external ear abnormalities include low-set ears, abnormal downward slopping of the helix, and upward slanting of external auditory canals. Abnormalities of middle ear predispose children with TS to recurrent and bilateral suppurative otitis media (SOM). This is due to malalignment of eustachian tube to middle ear as a result of abnormal skull base anatomy, persistent lymphatic effusion due to lymphatic hypoplasia, and hypotonia of tensor palati muscles. Recurrent episodes of otitis media result in scarring of tympanic membrane and subsequent conductive hearing loss (CHL). This is usually nonprogressive and resolves with advancing age due to growth of facial structures. The inner ear abnormality manifests as sensorineural hearing loss (SNHL), classically at a frequency of 1.5–2 KHz or above 8 KHz. This is due to reduced sensory cells in cochlea. SNHL typically manifests during adulthood and is progressive, unlike CHL, which presents in childhood and resolves with time. Otological disorders are attributed to *SHOX* haploinsufficiency, as *SHOX* gene is expressed in first and second pharyngeal arches which develop into maxilla, mandible, ear ossicles, and muscles of soft palate.

37. *What are the learning disabilities which are present in patients with Turner syndrome*?

Patients with TS have preserved intellectual function and verbal abilities with mild impairment in visuospatial, executive, and social cognitive domains. This is reflected as poor sense of direction, difficulty in learning to drive, ability to plan and execute tasks, arithmetic skills, and subtle defects in social behavior. In addition, patients with ring chromosome or marker chromosome have an increased risk of mental retardation. Hence, neuropsychological testing should be routinely performed.

38. *What are the common autoimmune disorders in patients with TS*?

The incidence of autoimmune disorders is twofold higher in patients with TS and those with an isochromosome Xq have higher incidence of autoimmune disorders as compared to patients with other chromosomal abnormalities. The most common autoimmune disease is Hashimoto's thyroiditis (30–50 %). Despite the high prevalence of thyroid autoantibody positivity, overt hypothyroidism is uncommon. Therefore, it is recommended that patients with TS should be annually screened for Hashimoto's thyroiditis. Other autoimmune disorders associated with TS include celiac disease (4–6 %), juvenile rheumatoid arthritis, Graves' disease (2.5 %), vitiligo, and Crohn's disease.

39. *Why are autoimmune disorders common in patients with TS*?

Autoimmune disorders are common in patients with TS; however, the exact mechanism remains elusive. X chromosome contains majority of genes involved in immune regulation. Therefore, haploinsufficiency of these genes has been proposed as one of the potential mechanisms for increased risk of autoimmune disorders in TS. Turner syndrome is a meiotic nondisjunctional chromosomal disorder and patients with other nondisjunctional chromosomal abnormities (Down's syndrome and Klinefelter's syndrome) are also predisposed to autoimmune diseases, thereby suggesting a role of nondisjunctional chromosomal abnormalities in the pathogenesis of autoimmune disorders.

40. *Why do children with TS lack pubarche despite adrenarche*?

Despite timely occurrence of adrenarche, children with TS do not develop pubarche or develop it later as compared to normal girls. It is thought that presence of functioning ovaries is required to convert weaker adrenal androgens to more potent androgens, thereby resulting in pubarche. This explains the absence

of pubarche in children with TS who have ovarian failure. In addition, estrogen acts in concert with androgens at pilosebaceous unit and induces pubarche. This also explicits the appearance of pubic hair after initiation of estrogen supplementation in children with TS. The reason for early adrenarche in children with TS is not known; however, it is postulated that an unidentified factor produced by ovaries inhibits adrenarche and this is absent in patients with TS due to gonadal failure (Fig. 8.10).

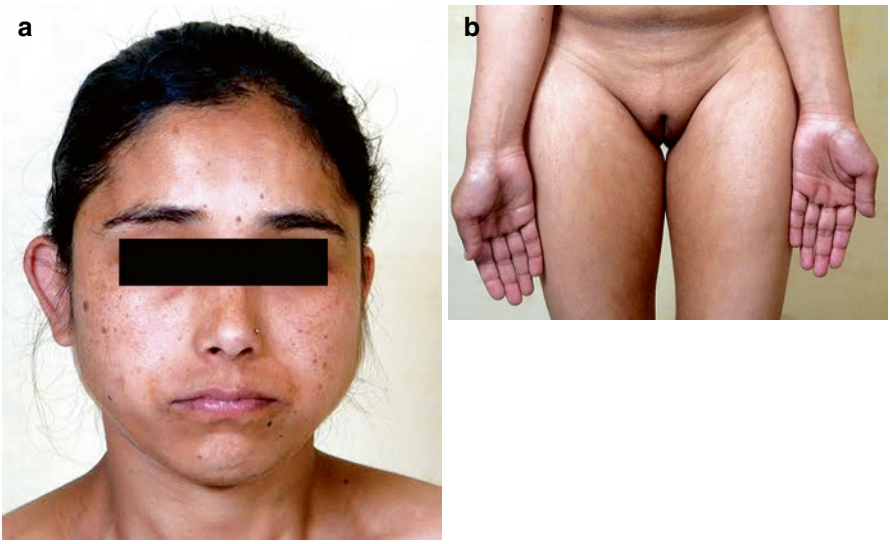

Fig. 8.10 (**a**) A 20-year-old woman with Turner syndrome. (**b**) Absence of pubarche in the same patient

41. *What is the cause of gonadal failure in TS*?

Gonadal failure is present in nearly 95 % of patients with Turner syndrome. It can present as delayed/arrested puberty, primary amenorrhea, infertility, or premature ovarian failure. Ovarian development requires two copies of multiple genes present on both short arm (e.g., Xp11) and long arm (e.g., Xq24) of X chromosome. These genes do not undergo lyonization and hence loss of even a single copy of these genes will result in streak gonads. In addition, reactivation of lyonized X chromosome (which occurs at 8 weeks of intrauterine life) is essential for germ cells (oogonia) to enter into meiotic division. Therefore, absence of one X chromosome in patients with TS results in poor development of ovaries and accelerated follicular atresia even during intrauterine period.

42. *What are the differences in patients with classical and mosaic Turner syndrome*?

The differences in patients with classical and mosaic Turner syndrome are summarized in the table given below.

Parameters	Classical Turner	Mosaic Turner
Karyotype	45,XO	45,XO/46,XX (most common)
Incidence	50–60 %	20–30 %
Height	Severe short stature	Mildly affected
Phenotypic abnormalities	Severe	Mild
Cardiac abnormalities	30–39 %	24 %
Renal abnormalities	46 %	39 %
Gonadal function	Invariably affected	Spontaneous puberty 30 %
		Menarche 10 %
		Fertility 2–3 %
Mortality	High (SMR 4.4)	Low (SMR 2.2)

43. *What are the disorders which share phenotypic features of Turner syndrome*?

Individuals with Noonan's syndrome and mixed gonadal dysgenesis share several phenotypic features of Turner syndrome like short stature, webbing of neck, low posterior hairline, and cardiac and renal anomalies. In addition, patients with Leri–Weil dyschondrosteosis also share many characteristic skeletal manifestations of Turner syndrome including short stature, cubitus valgus, short fourth metacarpal and high-arched palate.

44. *What are the differences between Turner syndrome and Noonan's syndrome*?

Although both Turner syndrome and Noonan's syndrome share several phenotypic features like short stature, cubitus valgus, low-set ears, low posterior hairline, and malformed ears and ptosis, there are several differences between these disorders, which are summarized in the table given below.

Characteristics	Turner syndrome	Noonan's syndrome
Gender	Only females	Both sexes
Inheritance	Sporadic	Autosomal dominant/sporadic
Karyotype	45,XO	Normal
	45,XO/46,XX	
Cardiac defects	Left sided	Right sided
	Bicuspid aortic valve	Pulmonary stenosis
	Coarctation of aorta	Hypertrophic cardiomyopathy
Gonadal function	Ovarian failure invariable	Females: normal
		Males: cryptorchidism, delayed puberty, infertility
Mental retardation	Absent	25 %
	Mild learning disabilities +	

45. *Whom to screen for TS*?

Turner syndrome should be suspected in any girl with short stature or delayed puberty or having characteristic phenotypic abnormalities of TS, and a 30-cell karyotyping should be performed in all these patients.

46. *Why is 30-cell karyotype recommended for the diagnosis of TS*?

Examination of at least 30 cells is required to detect 10 % mosaicism with 95 % confidence interval. The rationale for selecting 10 % mosaicism is probably based on the fact that approximately 10 % of aneuploidy (45, XO) is required to express the phenotypic abnormalities of Turner syndrome in women with 45,XO/46,XX karyotype.

47. *A 16-year-old girl presented with short stature and delayed puberty. On evaluation she had the characteristic phenotype of Turner syndrome. Her karyotype was 46,XX. How to proceed further*?

The index patient has short stature, delayed puberty, and characteristic phenotypic features of Turner syndrome, with normal karyotype. Since the clinical suspicion of TS is high, additional investigations are required. However, before further investigations, it should be confirmed that 30 cells have been examined on karyotyping to allow detection of mosaicism. Once it is ensured, tissue karyotyping should be carried out from skin fibroblasts, hair follicles, or gonadal tissue.

48. *When to screen for Y chromosome material in patients with TS*?

Patients with TS who have features of virilization or found to have marker chromosomes on karyotyping should be screened for the presence of Y chromosome material. DNA studies like polymerase chain reaction or FISH using a Y centromeric probe are the recommended techniques for the detection of Y chromosome material.

49. *What are the implications of presence of Y chromosome fragments in patients with TS*?

Y chromosome or its fragments are present in approximately 5 % of patients with TS, and its presence is associated with 12 % increased risk of developing gonadoblastoma. Although gonadoblastoma is a benign tumor, it may transform into malignant germ cell tumors including dysgerminoma in approximately 60 % of patients. Therefore, prophylactic laparoscopic gonadectomy is recommended in patients with TS who have Y chromosome or its fragments. Patients of TS with Y chromosome material may rarely have posterior labial fusion or clitoromegaly at birth.

50. *A patient having TS presented with clitoromegaly. How to proceed further*?

The presence of virilization in a patient with TS should raise the suspicion of gonadoblastoma, as these tumors often present with virilization. In addition, presence of Y chromosome material can cause virilization, even without the development of gonadoblastoma. CECT abdomen to localize the tumor and DNA studies/FISH to detect Y chromosome material are recommended in this scenario. Absence of gonadoblastoma/Y-cell material merits evaluation for other causes of virilization including adrenal or midline tumors. Administration of oxandrolone as a growth-promoting therapy in patients with TS can also lead to development of virilization.

51. *How does estimation of serum FSH help in the diagnosis of TS*?

Ovarian failure is one of the characteristic manifestations of TS, and accelerated follicular atresia begins as early as eighteenth week of intrauterine period. Biochemically, ovarian failure is characterized by raised FSH levels, with a classical biphasic pattern. Serum FSH is elevated from day 3 of life till the age of 5 years, followed by a decline to baseline and starts rising again after 10 years of age. Therefore, estimation of serum FSH is complementary in the diagnosis of TS from birth to 5 years and after 10 years of age. However, during the window period (5–10 years of age), estimation of serum FSH does not distinguish between children with TS from healthy girls.

52. *How to evaluate a patient with Turner syndrome*?

All patients with TS require cardiac, auditory, ophthalmic, orthodontic, and psychosocial evaluation. In addition, renal ultrasonography, thyroid function tests and anti-tissue transglutaminase antibody are also recommended in these patients. Fasting plasma glucose, lipid profile, liver function tests, and bone mineral density should also be monitored in adult women with TS. These parameters should be periodically reassessed, thereafter.

53. *When to initiate recombinant human growth hormone* (*rhGH*) *in patients with Turner syndrome*?

Short stature is virtually present in all patients with TS, and rhGH therapy has been shown to be safe and beneficial in these individuals. GH dynamic tests are not required before initiation of rhGH therapy, as most of these patients have normal response to GH dynamic tests and rhGH is indicated in these children for improving their height potential, irrespective of GH status. However, higher doses of rhGH are required for optimal height gain to overcome the chondrocyte resistance in patients with TS as compared to individuals with isolated GH deficiency. In addition, rhGH therapy in children with TS also improves the facial features as well as the peak bone mass. Therapy with rhGH should be

started once the child starts faltering on standard growth chart. Recombinant hGH therapy can be safely initiated in these patients as early as 9 months of age, as shown in the Turner–Toddler study. The initiation of rhGH therapy at an early age helps in the attainment of near-normal target height and induction of puberty at an appropriate age.

54. *What is the evidence for the use of rhGH in children with TS*?

Therapy with rhGH has been shown to be effective in promoting linear growth in children with TS. The landmark clinical trials using rhGH in children with TS are shown in the table given below. The Canadian study was the first randomized–controlled trial in children with TS which clearly demonstrated the benefit of rhGH therapy in children with TS. The Turner–Toddler study showed that rhGH can be safely initiated as early as 9 months of age, while the Dutch study showed the optimal dose of rhGH for growth promotion in children with TS. The Dutch study has shown that although a higher dose of rhGH (4 IU/m^2/day × 1 year followed by 6 IU/m^2/day × 6 years) was effective than low dose (4 IU/m^2/day × 7 years), increasing the dose further (4 IU/m^2/day × 1 year, 6 IU/m^2/day × 1 year, 8 IU/m^2/day × 5 years) did not result in significant improvement in height.

Studies	**Canadian study (2005)**	**Toddler–Turner study (2007)**	**Dutch study (1999)**
No. of patients recruited	154	89	68
No. of patients completed study	104	79	65
Age (years)	7–13	0.75–4	2–11
Inclusion criteria based on height	<10^{th} centile on WHO chart and height velocity <6 cm/year	–	<50^{th} percentile for healthy Dutch girls
Baseline Height SDS (GH vs. placebo)	−0.2 vs. −0.1 (TS chart)	−1.4 vs. −1.8 (CDC)	–
No. of patients treated with rhGH	61	45	68
Controls	43	43	–
Duration of rhGH therapy (years)	5.7	2	7
Dose of rhGH used	0.30 mg/Kg/week (6 days a week)	50 µg/Kg/day (0.3 mg/Kg/week)	*Group A* 4 IU/m^2/day × 7 years
			Group B 4 IU/m^2/day × 1 year, 6 IU/m^2/day × 6 years
			Group C 4 IU/m^2/day × 1 year, 6 IU/m^2/day × 1 year, 8 IU/m^2/day × 5 years

Studies	Canadian study (2005)	Toddler–Turner study (2007)	Dutch study (1999)
Height SDS after rhGH therapy	−0.2 to +1.4 SDS (TS chart)	−1.4 to −0.3	–
Height SDS in controls	−0.1 to +0.2 SDS (TS chart)	−1.8 to −2.2	–
Height gain	+ 7.2 cm	–	*Group A*: +12.5 cm
			Group B: + 14.6 cm
			Group C: +16 cm

55. *Why are the doses of rhGH higher in children with Turner syndrome*?

The doses of rhGH are relatively higher in children with TS as compared to children with growth hormone deficiency. The recommended dose is 0.36–0.46 mg/Kg/week, administered daily at bedtime. This is because TS is GH-resistant state due to intrinsic defect in chondrocytes as a result of *SHOX* haploinsufficiency.

56. *How to monitor a child with Turner syndrome receiving recombinant human growth hormone therapy*?

Patients with TS on rhGH therapy should be monitored at an interval of 3–6 months. Height should be monitored on standard growth chart. Doses of rhGH can be adjusted according to growth response and serum IGF-1 levels, which should be maintained in the mid-normal reference range. Kyphoscoliosis, slipped capital femoral epiphysis, and benign intracranial hypertension are more common in patients of TS treated with rhGH, and hence close surveillance for these side effects is mandatory. Lack of optimal growth response to rhGH therapy mandates evaluation for hypothyroidism and celiac disease, after compliance to therapy has been ensured. Recombinant hGH therapy can be discontinued after attainment of a near target height or a height velocity reduced to <2 cm per year or bone age >14 years.

57. *What are the predictors of response to rhGH therapy in patients with TS*?

Younger age at initiation of therapy, higher doses of rhGH (0.375 mg/Kg/week), prolonged duration of treatment, timely induction of puberty (approximately at 10–12 years of age), and greater genetic potential (target height) predict good response to rhGH therapy in patients with TS. However, karyotype of the patient does not influence the response to rhGH therapy.

58. *What are the concerns regarding rhGH therapy in children with TS*?

Recombinant hGH therapy in children with TS is associated with an increased risk of kyphoscoliosis, slipped capital femoral epiphysis, and benign intracranial hypertension, as compared to children with isolated GHD or idiopathic short stature. Lymphedema may reappear in some children after initiation of rhGH therapy. There was concern regarding increased incidence of aortic dilatation and dissection with rhGH therapy; however, it has been refuted in long-term studies. Patients with TS are predisposed for the development of diabetes mellitus; however, therapy with rhGH does not increase this risk, despite worsening of insulin resistance. Children with TS who have Y-cell line are at an increased risk for the development of gonadoblastoma/malignant germ cell tumors and theoretically, rhGH therapy may further increase the risk. However, there is limited data to support or refute this concern, as most of the studies of rhGH therapy in children with TS either have not included patients with Y-cell line or have included them after gonadectomy.

59. *What are the benefits of rhGH therapy other than improvement in linear growth in children with TS*?

Apart from increased linear growth, rhGH therapy is associated with improvement in facial features, body proportions, and body composition. Decreased diastolic blood pressure and favorable lipid profile are other benefits of rhGH therapy. The data regarding the beneficial effects of rhGH on bone mineral density are conflicting; however, it seems that GH is important for the maintenance of BMD in prepubertal children with TS, while estrogen plays a key role in accrual of peak bone mass during peripubertal period.

60. *What are the strategies to improve final adult height in children with TS, in addition to rhGH therapy*?

The strategies to improve final adult height, in addition to rhGH therapy include oxandrolone, delayed induction of puberty, and use of low-dose estrogen therapy during childhood. In a recent study, it was shown that oxandrolone therapy initiated at the age of 9 years resulted in a height gain of 4.6 cm, despite the induction of puberty at 12 years of age. In the same study, it was also shown that delaying the pubertal induction till the age of 14 years resulted in a height gain of 3.8 cm, without oxandrolone. However, combining these two strategies (oxandrolone and delayed induction of puberty) did not result in any additional gain in height. Because of adverse effects on psychosocial development and bone health, delay in induction of puberty is not recommended as a measure to improve height in patients with TS. In another study, it was shown that low-dose estrogen therapy initiated during childhood (as early as 5 years), along

with rhGH resulted in an additional height gain of 2.1 cm as compared to rhGH therapy alone.

61. *How is oxandrolone helpful in promoting linear growth in children with TS*?

Oxandrolone is a non-aromatizable androgen which is administered orally. At a dose of 0.05 mg/Kg/day (maximum dose 2.5 mg/day), it has been shown to be effective in promoting linear growth in children with TS. The mechanism of growth-promoting action of oxandrolone is not well understood. The proposed mechanism includes increase in GH secretion and/or IGF1 generation. The side effects of oxandrolone include hypertension, hepatotoxicity, virilization, and regression of breast development.

62. *When to induce puberty in girls with Turner syndrome*?

In normal girls, puberty starts by the age of 8–13 years. In children with TS, the recommended age for the induction of puberty is 12 years. This age is chosen as it has been shown that induction of puberty with low-dose estrogen at this age does not interfere with growth-promoting effects of GH (as it promotes IGF1 generation). Further, low-dose estrogen therapy at this age does not influence bone maturation. In addition, waiting till 12 years of age also gives an opportunity to watch for spontaneous puberty which occurs in 10–20 % of children with TS. In the past, delaying the induction of puberty till 14–15 years was thought to improve the final height; however, it is no longer recommended as this results in adverse effects on psychosocial development and bone mineral density.

63. *How to induce puberty in girls with TS*?

Before initiating estrogen for induction of puberty, serum FSH levels should be determined to confirm gonadal failure. Puberty can be induced by estrogen, which can be administered either through oral, transdermal, or intramuscular (depot) route; however, transdermal route is preferred. Oral estrogen therapy is initiated with estradiol valerate or micronized estradiol at dose of 0.25 mg/day or ethinyl estradiol 5 μg/day or conjugated equine estrogen 0.162 mg/day. The initiating dose for transdermal estradiol is 6.25 μg/day. The dose should be gradually titrated up at every 3–6 months interval, so that the adult dose (2 mg estradiol valerate or equivalent) is reached over a period of 2–4 years. Progesterone should be added once breakthrough bleed occurs or after at least 2 years of estrogen therapy. Later, the treatment should be maintained with estrogen and progesterone administered cyclically. The dose of estrogen should be reduced to 1 mg of estradiol valerate or its equivalent beyond the age of 30 years. Hormone replacement therapy should be continued up to the age of menopause for that ethnic group or till 50 years.

64. *Why is transdermal route preferred over oral route for estrogen therapy*?

Estrogen can be administered either through oral or transdermal route. However, transdermal route is preferred as it has favorable effect on body composition, IGF1 generation, lipid profile, proinflammatory markers, and procoagulant activity and is associated with lesser risk of venous thromboembolism, as compared to oral route. After oral administration of estrogen, hepatocytes are exposed to very high concentration of estrogen, due to hepatic first-pass effect, which results in increased secretion of procoagulant factors, proinflammatory markers, and very low-density lipoprotein. In addition, exposure of hepatocytes to high concentration of estrogen also leads to inhibition of GH-mediated IGF1 generation via upregulation of suppressors of cytokine signaling (SOCS)-2 and 3. Transdermal therapy employs estrogen at very low doses as compared to oral therapy, because estrogen administered transdermally is directly absorbed to systemic circulation, without any first-pass effect on the liver. Further, transdermal estrogen promotes IGF1 generation as opposed to oral estrogen therapy.

65. *Why are oral contraceptive pills not preferred for the induction of puberty*?

Pubertal development is a slow and progressive process along with a gradual rise in estradiol concentration, which results in optimal development of breast and uterus. Use of oral contraceptive pills for the induction of puberty should be discouraged, because they contain higher dose of estrogen than that recommended for induction of puberty. Therapy with high doses results in rapid but suboptimal breast and uterine development, as it does not mimic the normal physiology of gradual rise in estrogen over 3–4 years. The use of high-dose estrogen for induction of puberty results in development of tubular breasts (as a result of predominant glandular tissue development in subareolar region, which is cosmetically unacceptable) as opposed to normal breast development (globular breast). In addition, progesterone in oral contraceptive pill also interferes with the action of estrogen at breast and uterus.

66. *Which is the preferred estrogen for induction of puberty*?

Many preparations of estrogen are available commercially; however, preparations containing 17β-estradiol are preferred, because it is the predominant circulating estrogen in a premenopausal female. The available preparations of estrogen are given in the table below. Estradiol valerate, micronized estradiol, and estradiol patch are 17β-estradiol-containing formulations.

Compound	Trade name	Route of administration	Dose/day
Estradiol valerate	Progynova	PO	0.25–2 mg
Ethinyl estradiol	Lynoral	PO	5–50 μg
Micronized estradiol	Estrace	PO	0.25–2 mg
Conjugated equine estrogen	Premarin	PO	0.162–0.625 mg
Estradiol patch	Alora	Transdermal	6.25–100 μg

Although conjugated equine estrogen (CEE) is a natural estrogen, it is not preferred for the induction of puberty because it is a mixture of more than 100 types of estrogens, with estrone being the predominant form, rather than estradiol. Use of ethinyl estradiol is associated with higher incidence of cardiovascular events, hypertension, and thromboembolism and hence should be avoided for induction of puberty.

67. *What are the fertility prospects in patients with TS*?

Although pregnancy is rare in TS, fertility prospects in TS can be improved either by cryopreservation of ovarian tissue or donor oocyte followed by in vitro fertilization and embryo transfer. Cryopreservation of ovarian tissue should be performed during prepubertal period and is preferred in patients with mosaicism. Donor oocyte may be the only fertility option for majority of those with classical TS and pregnancy outcomes are similar to non-TS women who have undergone embryo transfer after oocyte donation.

68. *What are the contraindications to pregnancy in patients with TS*?

Presence of cardiovascular anomalies like aortic dilatation, bicuspid aortic valve and hypertension, or past history of surgery for cardiac defects increases the risk of aortic dilatation and dissection during pregnancy and should be considered as relative contraindications for pregnancy. However, ASI >2 cm/m^2 is an absolute contraindication to pregnancy. Therefore, thorough cardiac evaluation including cardiac MRI is mandatory in women with TS planning pregnancy.

69. *What are the pregnancy outcomes in women with TS*?

Although most women with TS are infertile, spontaneous pregnancy can occur in 2–3 % of these patients and most of these women have mosaicism. Pregnancy in women with TS is associated with increased incidence of miscarriages, stillbirths, and congenital malformations. High incidence of adverse pregnancy outcomes in TS is attributed to defective oocyte, increased probability of chromosomal abnormalities in the fetus, and decreased endometrial receptivity. In addition, due to their small pelvic size, most women with TS require cesarean section.

70. *What are the causes of mortality in Turner syndrome*?

Mortality in women with Turner syndrome is threefold higher than in the general population. Cardiovascular disorders, including coronary artery disease, cerebrovascular disease, aortic aneurysm, and congenital heart disease are the most common causes of mortality and contribute to 41 % of excess mortality in TS. Pneumonia, recurrent urinary tract infection with renal dysfunction, and diabetes also lead to excess mortality in women with TS. Mortality is higher in those with classical TS (SMR 4.4) as compared to those with mosaic TS (SMR 2.2), and this is due to aortic aneurysm and cardiovascular congenital anomalies.

Further Readings

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Disorders of Sex Development

9

9.1 Case Vignette

A 20-year-old individual presented with poor development of secondary sexual characteristics and primary amenorrhea. She was born of a non-consanguineous marriage at term by vaginal delivery at home without any perinatal complications and was assigned female gender. There was no history of maternal virilization or any drug intake by the mother during pregnancy. The child did not have history of salt crisis or failure to thrive. The index patient identified self as a female, used to play with girls, and had preference for girl's toys. The developmental milestones and growth were normal and the child had an average scholastic performance. During adolescence, patient had development of axillary and pubic hair and mild phallic enlargement. In addition, patient could also feel the presence of some globular structure in both labioscrotal folds. Patient continued to identify self as a female and had a preference for male partner. She had eight siblings and one elder sibling also had genital ambiguity and was reared as a male. There was no family history of primary infertility, gynecomastia, salt crisis, precocious puberty, and neonatal deaths. The patient did not receive any medical treatment or surgical intervention till presentation. On examination, she had height of 161 cm (upper segment 77 cm, lower segment 84 cm, and arm span 167 cm), weight 55 Kg, and blood pressure 100/60 mmHg. Tanner staging was A_+, P_4, B_2. External genitalia revealed phallus of 4.5 cm with presence of chordee and ventral–urethral groove. There were two

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distinct openings with presence of posterior labial fusion. Both the gonads were palpable in the inguinal region (measuring 10 ml and 8 ml on the left and right side, respectively) which could be brought down to labioscrotal folds. Labioscrotal folds were partially rugosed and pigmented. The external masculinization score was 2. The patient did not have temporal recession, facial hair, acne, male torso, body hairs, and deepening of voice. Systemic examination was unremarkable. On investigation, hormonal profile showed LH 32.5 mIU/ml (N 1.7–8.6), FSH 48.5 mIU/ml (N 1.5–12.4), T 6.4 nmol/L (N 9.9– 27.8), DHT 281 pg/ml (N 240–650), E_2 39.7 pg/ml (N 7.63–42.6), 0800h cortisol 371.5 nmol/L (N 171–536), prolactin 11.4 ng/ml (N 4–15), T_4 8.7 µg/dl (N 4.8–12.7), and TSH 1.53 µIU/ml (N 0.27–4.2). Ultrasound and MRI abdomen confirmed the absence of Mullerian structure and showed the presence of homogeneous solid structure in both inguinal canal with right one measuring 3.2 × 2.7 × 1 cm and left measuring 3.8 × 2.2 × 1 cm. Karyotype from mononuclear cell was 46,XY. Patient underwent hCG stimulation test and the results are summarized in the table given below.

	Baseline (nmol/L)	**Post-hCG stimulated (nmol/L)**
Androstenedione (A)	20.6	25.7
Testosterone (T)	6.4	8.8
T/A ratio	0.31	0.34

Genitoscopy showed separate urethral opening and a blind vaginal pouch. A diagnosis of 46,XY DSD due to 17β-hydroxysteroid dehydrogenase type 3 deficiency (androgen biosynthetic defect) was established. Considering her gender identity and role, she underwent bilateral gonadectomy and phallic recession surgery. Gonadal tissue histology showed presence of Leydig cells and seminiferous tubules consistent with testes. She was treated with estradiol valerate for the development of secondary sexual characteristics at a dose of 0.5 mg once a day and progressively increased to 2 mg per day (Fig. 9.1).

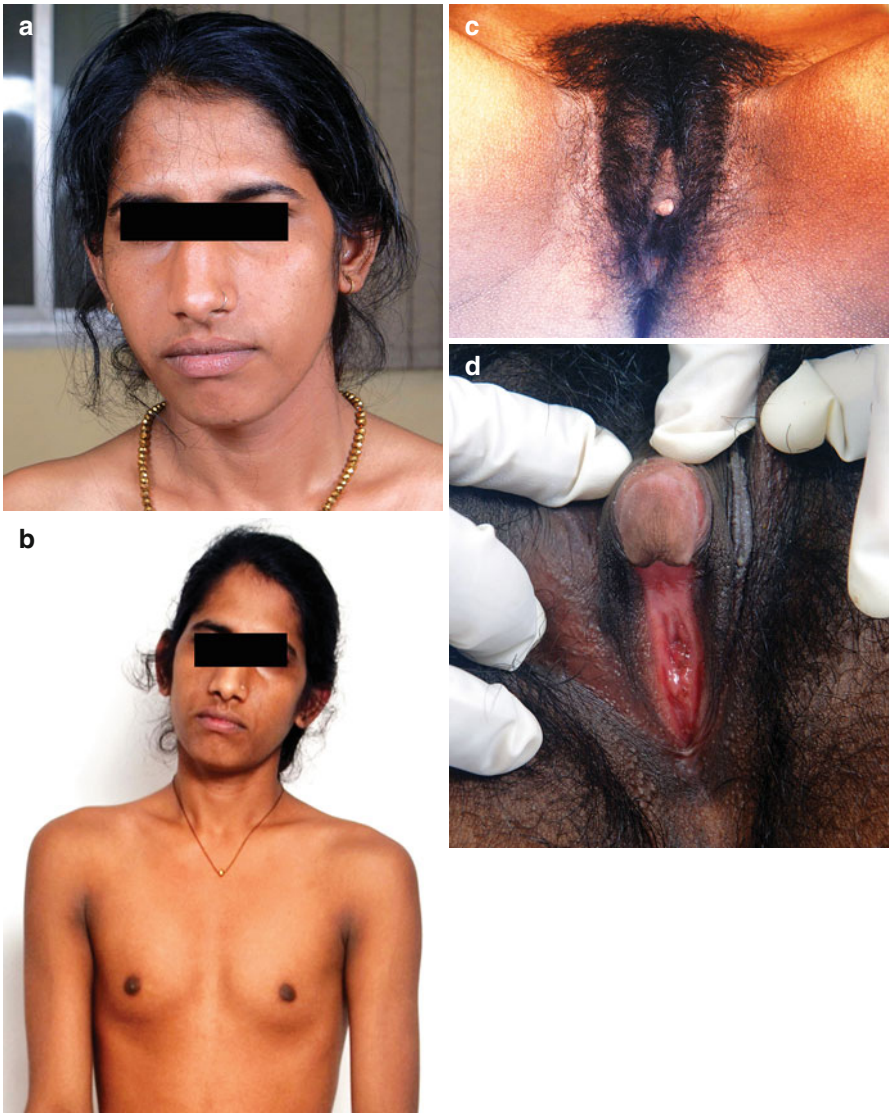

Fig. 9.1 (**a**) A 20-year-old patient, reared as female presented with genital ambiguity (**b**) bilateral gynecomastia. (**c**) External genitalia showing phallus with glans. (**d**) Posterior labial fusion with two distinct openings at the perineum

9.2 Stepwise Analysis

The index patient presented with primary amenorrhea, poor development of secondary sexual characteristics, genital ambiguity, and bilateral palpable gonads. The differential diagnosis to be considered with this presentation includes androgen biosynthetic defect, ovotesticular DSD, partial 46,XY gonadal dysgenesis, and partial androgen insensitivity syndrome. The possibility of androgen biosynthetic defect is considered in view of peripubertal onset of virilization and feminization, absence of uterus, and bilateral palpable gonads with 46,XY karyotype. The diagnosis of ovotesticular DSD should also be considered as she experienced some degree of virilization as well as feminization during peripubertal period. Bilateral palpable gonads are usually not a feature of ovotesticular DSD, unless there are bilateral ovotestes or testis and ovotestis on either side. This was further confirmed by the presence of bilateral testicular tissue on histology. 46,XY partial gonadal dysgenesis is usually associated with varying degree of genital ambiguity and lack of uterus as seen in the index patient; however, the palpable gonads in the labioscrotal folds do not favor this diagnosis, as dysgenetic testes usually do not descend to the scrotum. This diagnosis was further denied by high serum androstenedione level. The diagnosis of partial androgen insensitivity (PAIS) is less likely in view of poor breast development with markedly undervirilized genitalia. Further, low serum testosterone excluded this possibility as well. The androgen biosynthetic defects due to 17α-hydroxylase, 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), and 5α-reductase deficiency are the contenders for the differential diagnosis in the index patient. 17α-hydroxylase deficiency in 46,XY DSD is usually associated with tall stature with eunuchoidal proportions, hypertension and female external genitalia, and undetectable or low serum androstenedione (A) and testosterone (T) level. Patients with 5α-reductase deficiency are usually associated with normal body proportions, varying degree of genital ambiguity with palpable gonads, absence of gynecomastia, virilization during peripubertal period, and high serum testosterone and low dihydrotestosterone (DHT) levels. Therefore, the possibilities of these two biosynthetic defects are excluded as she had high androstenedione and low testosterone levels. Patients with 17β-HSD3 deficiency usually have female external genitalia with palpable gonads, eunuchoidal proportions, peripubertal virilization, and varying degree of gynecomastia. Low testosterone, high androstenedione, and elevated gonadotropins and hCG stimulated low T/A ratio (<0.8) confirmed the diagnosis of 17β-HSD3 deficiency in the index patient. The T/A ratio is confirmatory for the diagnosis of 17β-HSD3 deficiency only in the presence of high levels of androstenedione. Further, hCG stimulation test is only required in prepubertal children, as during peripubertal period endogenous LH drive is sufficient to stimulate the Leydig cell for unmasking the respective enzyme deficiency as was observed in the index patient who had modest Δ rise in T/A ratio after hCG stimulation. High gonadotropins, both LH and FSH, are also a feature of 17 β-HSD3 deficiency as was seen in the index patient. High LH is attributed to decreased negative feedback by low testosterone, and elevated FSH is the result of decreased inhibin B which occurs due to low intratesticular testosterone-mediated inhibition of spermatogenesis and

possibly abnormally positioned testes. Patients with 46,XY DSD due to 17 β-HSD3 deficiency usually do not have virilization of external genitalia during embryogenesis; however, these patients exhibit some degree of virilization during peripubertal period as a result of extra-glandular conversion of androstenedione to testosterone by 17β-HSD5, an isoenzyme, which is possibly induced by some environmental factor during adolescence. The index patient was assigned female gender as probably mild genital ambiguity would have been overlooked at birth; however, during adolescence she had appearance of pubic hair, phallic enlargement, and some degree of breast development. Sometimes patients with 17β-HSD3 and 5α-reductase deficiency may exhibit reversal of gender identity and role during adolescence. Nevertheless, she did not have any change in gender role and orientation. Absence of uterus is characteristic of androgen biosynthetic defects associated with 46,XY DSD which was also observed in the index patient. Corrective surgery is indicated in concordance with the gender role and behaviour of an individual as well as the extent of virilization. The index patient had gender identity, role, and orientation as a female and the degree of virilization of external genitalia was mild (EMS 2); therefore, a decision to feminize her was propounded and corrective surgery was done accordingly.

9.3 Clinical Rounds

1. *What are the "disorders of sex development*?"

   The term "disorders of sex development" (DSD) refers to congenital disorders associated with discordance in chromosomal, gonadal, or anatomical (phenotypic) sex of an individual. Previously, the terms intersex, pseudohermaphrodite, and hermaphrodite were used to describe individuals with genital ambiguity. However, these terms were derogatory and lacked scientific basis. The new classification is based on karyotype and includes disorders associated with defects in chromosomal, gonadal, or anatomical sex, even in the absence of genital ambiguity.

2. *What is the basis of origin of chromosomal DSD*?

   Chromosomal DSD occurs as a result of nondisjunction of homologous chromosomes (sister chromatids) either during meiosis/mitosis or due to chimerism. Normally, during the period of anaphase, there is separation of sister chromatids with equal distribution of genetic material into two chromatids. The failure to separate or unequal distribution of genetic material into two chromatids results in aneuploidy. Meiotic nondisjunction results in disorders like classical Klinefelter syndrome (47,XXY) and Turner syndrome (45,XO), whereas mitotic nondisjunction results in mosaic variants of Turner syndrome (45,XO/46,XX) and Klinefelter syndrome (46,XY/47,XXY). Ovotesticular DSD (46,XX/46,XY) occurs due to sex chromosome chimerism.

3. *What is the difference between mosaicism and chimerism*?

Mosaicism is characterized by coexistence of two or more cell lines (e.g., 45,X/46,XY or 46,XY/47,XXY) originating from a single zygote as a result of mitotic nondisjunction, while chimerism is characterized by coexistence of two or more cell lines having different genetic origin in a same individual. Chimerism occurs as a result of double fertilization (dispermy) of a binucleate ovum or fusion of two zygotes before implantation. The ovotesticular disorders are the example of chimerism (e.g., 46,XX/46,XY).

4. *What is the difference between sex determination and sex differentiation*?

The development of bipotential gonad into either ovary or testis is called sex determination and is genetically determined. Sex differentiation is the process of development of internal and external genitalia of a male or female, as a result of appropriate function of the respective gonad. Both these events occur during the critical period of embryogenesis during seventh to twelfth week of intrauterine life. The sequence of events that constitute sex determination and differentiation are shown in the figure given below (Fig. 9.2).

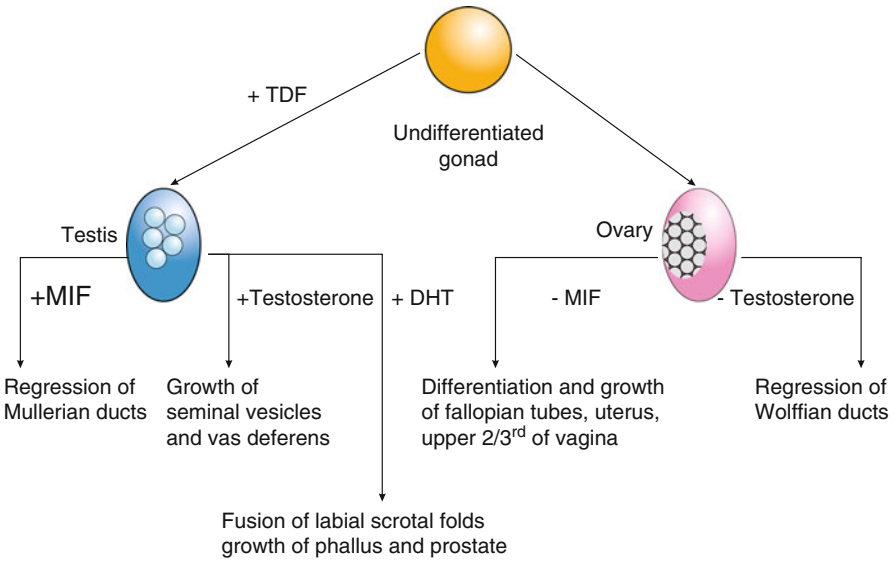

Fig. 9.2 Sex determination and differentiation in primitive gonad

5. *What are the factors responsible for sex determination*?

Sex determination is an active process wherein a bipotential gonad develops into testis or ovary. The development of bipotential gonad into testis initiates at 6–7 weeks of intrauterine life and is determined by genes like *SRY*, *SOX9*, *SF1*, *WT1*, and *DHH*. Expression of *SRY* gene (Yp11.3) initiates the development of testis by regulating multiple downstream sex-determining factors including *SOX9*. The development of ovary from bipotential gonad is not a passive process, as believed previously and the genes determining ovarian development include *WNT4, FOXL2* and *RSPO1*.

6. *What is the importance of DAX1 in sex determination*?

DAX1 (dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome, gene 1) located on Xp20.3 is necessary for the development of testes/ovaries, hypothalamus, and pituitary gland (GnRH–gonadotropin–gonadal axis) and adrenal cortex. As the name suggest, *DAX1* is a dose-dependent gene and plays an important role in sex determination. Expression of one copy of *DAX1* facilitates the development of bipotential gonad into testis/ovary as determined by the chromosomal sex. Deletion or inactivating mutation of *DAX1* is associated with hypogonadotropic hypogonadism and congenital adrenal hypoplasia, whereas duplication of *DAX1* results in gonadal dysgenesis.

7. *What are the derivatives of Wolffian duct and Mullerian duct in males and females*?

The derivatives of Wolffian duct and Mullerian duct in male and female are summarized in the table given below.

	Male	**Female**
Wolffian duct	Epididymis Vas deferens Seminal vesicle Ejaculatory duct Ureter Renal pelvis and calyces Collecting ducts	Ureter Renal pelvis and calyces Collecting ducts Gartner's duct
Mullerian duct	Appendix testis Utricle of prostate	Fallopian tubes Uterus and cervix Upper two-thirds of vagina

8. *How does external genitalia develop*?

   The development of external genitalia in both gender occurs from the common genital primordia. Exposure to androgens determines the differentiation toward a male phenotype, whereas absence of androgens leads to a female phenotype. The development of external genitalia in both gender is summarized in the table given below.

Structure	Male	Female
Genital tubercle	Glans penis	Clitoris
Urethral (urogenital) folds	Shaft of the penis	Labia minora
Labioscrotal (urogenital) swelling	Scrotum	Labia majora
Urogenital sinus	Prostate	Urethra
	Prostatic urethra	Lower one-third of vagina

9. *What is the difference between hormonal regulation of development of internal and external genitalia in a male*?

   Once bipotential gonad develops into testes, the process of sexual differentiation is initiated by secretion of testosterone and anti-Mullerian hormone (AMH) by testes. AMH is produced by Sertoli cells by seventh week of gestation and acts in a paracrine manner to cause regression of Mullerian ducts. Testosterone is secreted by Leydig cells from eighth week of gestation and causes stabilization and development of Wolffian structures by its paracrine action. Differentiation of external genitalia starts by ninth week of gestation and is accomplished by the endocrine action of testosterone. Virilization of external genitalia requires high concentration of androgens; however, concentration of circulating testosterone is too low to virilize the external genitalia during embryogenesis. This is overcome by conversion of testosterone to dihydrotestosterone which has ten times more affinity for the androgen receptor. Other DHT-mediated features of virilization include temporal recession, dense facial and body hair, male torso and hair in the upper pubic triangle.

10. *How to grade the degree of genital ambiguity in a child with DSD*?

    The commonly used scores to assess the degree of genital ambiguity in a child with DSD are Prader staging system and external masculinization score (EMS). Prader staging was developed to grade the degree of virilization of external genitalia in a 46,XX individual and comprises of five stages. External masculinization score (EMS) was developed to grade the degree of under-virilization of external genitalia in an individual with 46,XY DSD. Although both scores can be used to grade the genital ambiguity, EMS takes into account all physical

features associated with genital ambiguity independently and is more objective. EMS score for a normal male external genitalia is 12, whereas for a normal female external genitalia is 0. Both these scores are shown in the figure given below (Fig. 9.3).

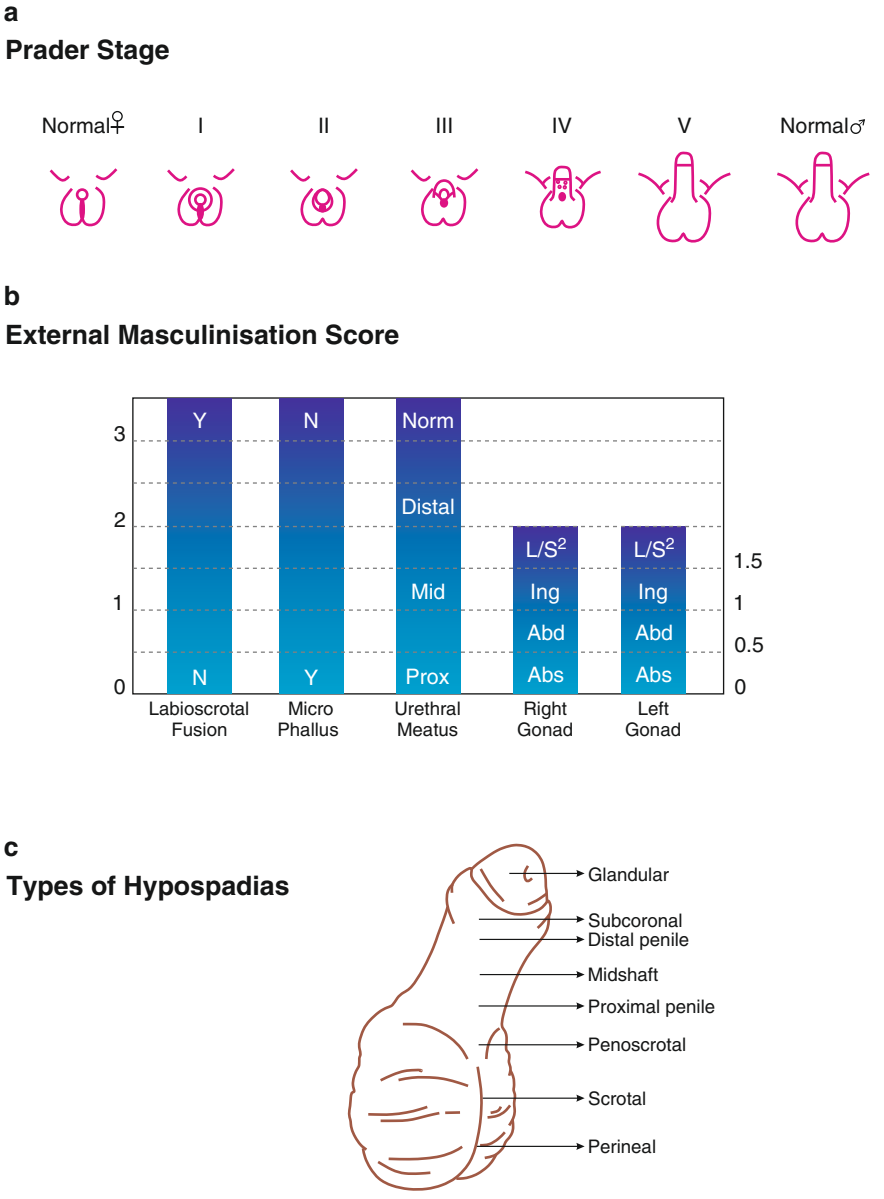

Fig. 9.3 (**a**) Staging to grade the degree of virilization of external genitalia in a 46,XX individual. (**b**) External masculinization score to grade the degree of undervirilization of external genitalia in a 46,XY individual. (**c**) Grading of hypospadias

11. *What are the features of prenatal virilization in a 46,XX newborn*?

The features that suggest prenatal virilization in a 46,XX newborn include clitoromegaly (clitoral length ≥9 mm), posterior labioscrotal fusion (anogenital ratio >0.5), and single urogenital opening. Androgen exposure prior to 12 weeks of intrauterine life results in clitoromegaly, single urogenital opening, and posterior labioscrotal fusion, whereas exposure to androgens after 12 weeks of intrauterine life results in isolated clitoromegaly (Fig. 9.4).

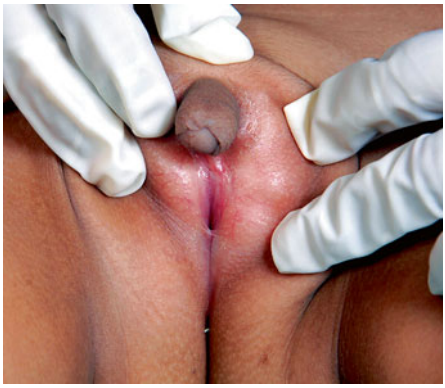

Fig. 9.4 Features of prenatal virilization in a 46,XX child. Note clitoromegaly, posterior labial fusion, and single urogenital opening

12. *What are the causes of blind vaginal pouch*?

Upper two-thirds of the vagina develops from Mullerian duct, whereas the lower one-third from urogenital sinus. Blind vaginal pouch refers to the presence of lower one-third of vagina with hypoplastic/absent Mullerian derivatives (uterus, fallopian tubes, and upper two-thirds of vagina). The causes of blind vaginal pouch in an individual with 46,XY DSD include complete androgen insensitivity syndrome, androgen biosynthetic defects (e.g., 17α-hydroxylase deficiency, 17β-hydroxysteroid dehydrogenase type 3 deficiency), and 5α-reductase type 2 deficiency. Presence of blind vaginal pouch in an individual with 46,XY DSD is due to defective secretion/action of testosterone and preserved secretion of AMH from the gonad. Preserved secretion of AMH results in regression of Mullerian derivatives whereas impaired secretion/action of testosterone results in differentiation of urogenital sinus into urethra and lower one-third of vagina (blind vaginal pouch). In addition, blind vaginal pouch can also be present in individuals with Mayer–Rokitansky–Kuster–Hauser syndrome and ovotesticular DSD (Fig. 9.5).

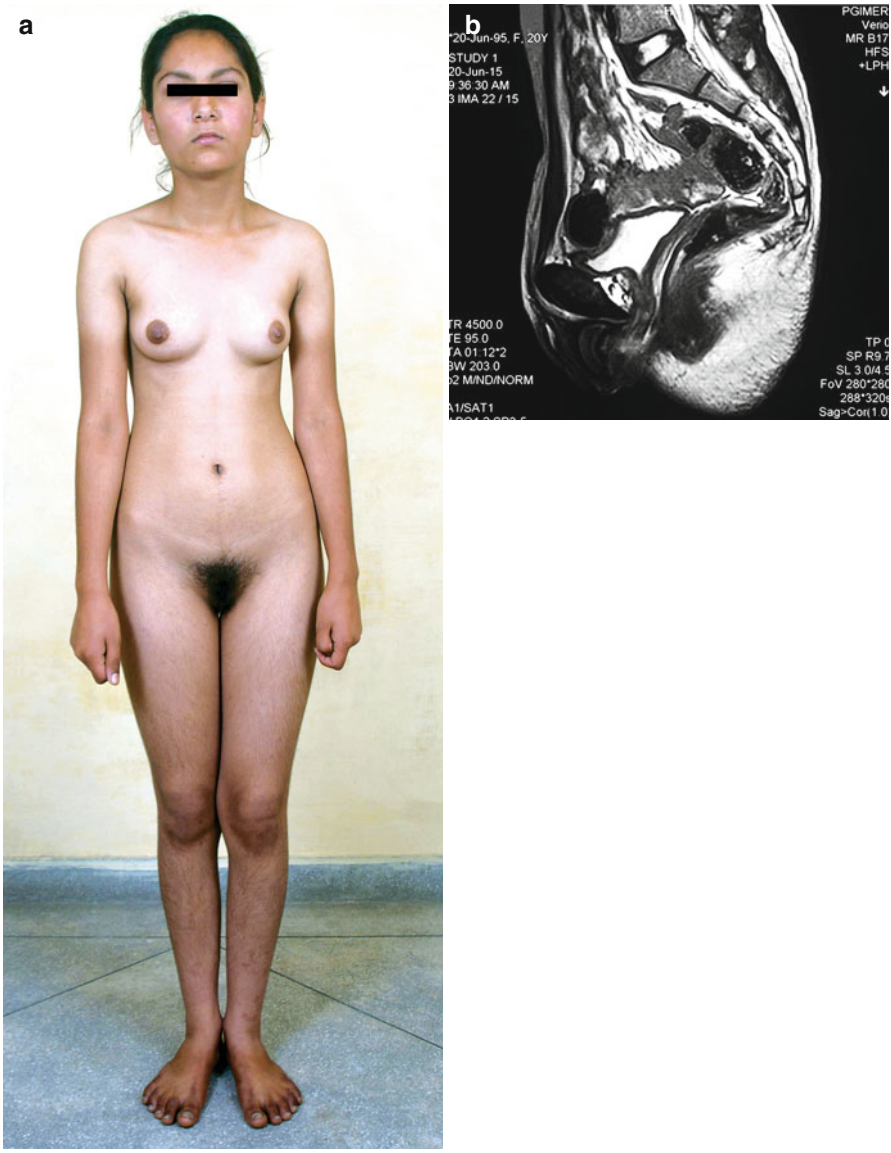

Fig. 9.5 (**a**) An 18-year-old female presented as primary amenorrhea with normal secondary sexual characteristics (**b**) MRI pelvis showing absence of uterus suggestive of Mayer–Rokitansky–Kuster–Hauser syndrome

13. *What are the disorders associated with the simultaneous presence of both Wolffian and Mullerian duct derivates*?

    The causes of presence of both Wolffian and Mullerian duct derivatives include persistent Mullerian duct syndrome (PMDS), ovotesticular DSD (OT-DSD), and mixed gonadal dysgenesis (MGD). Patients with PMDS usually present with inguinal hernia but do not have genital ambiguity. PMDS occurs either due to impaired secretion or action of AMH during early intrauterine life. Patients with OT-DSD and MGD usually have genital ambiguity and have Wolffian duct derivatives on the side of testis/ovotestis and Mullerian duct derivatives on the side of ovary/streak gonads.

14. *Why boys with hypogonadotropic hypogonadism do not have genital ambiguity*?

    The virilization of external genitalia during sexual differentiation is accomplished by the action of testosterone (after conversion to dihydrotestosterone) secreted from the Leydig cells during 9–12 weeks of gestation. However, the fetal hypothalamo–pituitary–testicular axis starts functioning only after 12 weeks of gestation. The prime drive for Leydig cell stimulation during this critical period (9–12 weeks of gestation) is maternal human chorionic gonadotropin (hCG); hence, patients with isolated hypogonadotropic hypogonadism (IHH) do not have genital ambiguity due to hCG-mediated testosterone secretion from fetal Leydig cells. The activation of hypothalamo–pituitary–testicular axis after 12 weeks is required for the continued production of testosterone, which is responsible for penile growth and descent of testes. Hence, patients with hypogonadotropic hypogonadism with decreased testosterone levels manifests with micropenis and cryptorchidism. Rarely, patients with DAX1 gene mutation may have genital ambiguity with IHH as DAX1 gene mutation is also associated with impaired Leydig cell function.

15. *Can patient with Klinefelter's syndrome present with ambiguous genitalia*?

    Patients with Klinefelter's syndrome do not have genital ambiguity as the presence of Y chromosome directs bipotential gonad to differentiate into testis and, consequently, virilization of external genitalia. However, there are few reports of genital ambiguity in patients with 47,XXY karyotype. This may be due to higher number of CAG repeats in the androgen receptor (i.e., androgen insensitivity) or double dose of DAX1 gene (as a result of failure of inactivation of DAX1).

16. *What are the causes of 46,XY DSD with ambiguous genitalia*?

The causes of ambiguous genitalia in a 46,XY individual are enlisted in the table given below.

Pathogenesis	**Etiology**
Disorders of testicular development	Partial gonadal dysgenesis
	Ovotesticular DSD
Disorders of androgen biosynthesis	CAH
	StAR inactivating mutation
	CYP11A1 deficiency
	3 β-HSD2 deficiency
	P450 oxidoreductase deficiency
	17β-HSD3 deficiency
	5α-reductase deficiency
	LH receptor inactivating mutation
Disorders of androgen action	Partial androgen insensitivity

17. *What are the causes of 46,XY DSD with female external genitalia*?

The disorders commonly associated with female external genitalia in a 46,XY individual include complete gonadal dysgenesis, complete androgen insensitivity syndrome (CAIS), and androgen biosynthetic defect due to 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase type 3 deficiency. All these disorders present during adolescence with primary amenorrhea. Tall stature, absent secondary sexual characteristics, and presence of Mullerian derivatives suggest a diagnosis of 46,XY complete gonadal dysgenesis (Swyer syndrome). Patients with CAIS are characterized by normal breast development, absent pubic and axillary hair, and lack of Mullerian derivatives. Presence of hypertension, absent secondary sexual characteristics, and lack of Mullerian derivatives suggest a diagnosis of 17 α-hydroxylase deficiency. Patients with 17β-hydroxysteroid dehydrogenase type 3 deficiency have female external genitalia at birth; however, they virilize during peripubertal period. In addition, other disorders associated with defective androgen biosynthesis like inactivating mutation of LH receptor, StAR protein, and side chain cleavage enzyme (CYP11A1) can also result in female external genitalia in a 46,XY individual (Fig. 9.6).

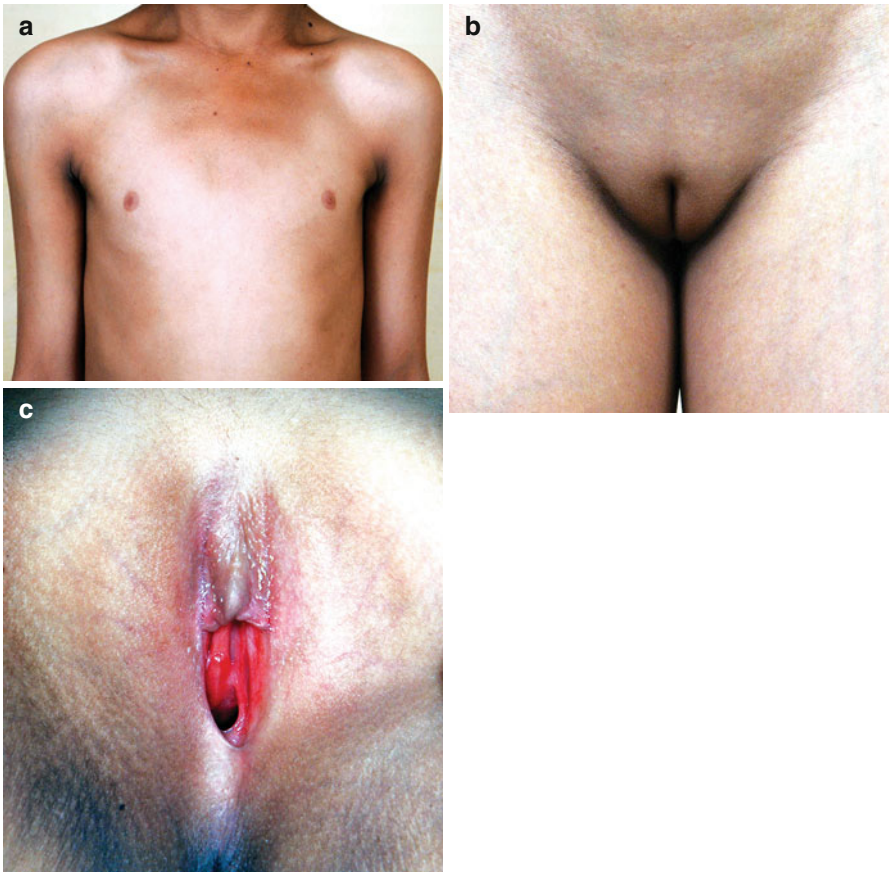

Fig. 9.6 (**a**) A 14-year-old individual reared as female who presented with primary amenorrhea, (**b**) absent secondary sexual characteristics and (**c**) unambiguous female external genitalia. Ultrasonography revealed bilateral inguinal testis. A diagnosis of 46,XY DSD due to androgen biosynthetic defect was considered

18. *What are the causes of 46,XX DSD with ambiguous genitalia*?

The causes of ambiguous genitalia in a 46,XX individual are enlisted in the table given below.

Pathogenesis	**Etiology**
Disorders of gonadal development	Ovotesticular DSD
	Testicular DSD
	SRY gene translocation
	SOX9 duplication
	Inactivating mutation of RSPO1 and WNT4
Disorders of androgen excess	Congenital adrenal hyperplasia
	21α-hydroxylase deficiency
	11β-hydroxylase deficiency
	P450 oxidoreductase deficiency
	3 β-HSD2 deficiency
	Placental aromatase deficiency
	Luteoma of pregnancy
	Exogenous androgens exposure during intrauterine period

19. *What are the causes of 46,XX DSD with apparently male external genitalia*?

The prerequisite for having apparently male external genitalia (Prader stage 5) in a 46,XX individual is exposure to androgens during the critical period of embryogenesis, i.e., between 8 and 12 weeks of gestation. Exposure to androgens after this period results only in isolated clitoromegaly. The causes of 46,XX DSD with male external genitalia (but with anorchidism) include CAH due to 21α-hydroxylase and 11β-hydroxylase deficiency, SRY gene translocation, SOX9 duplications, and RSPO1 inactivating mutations. In addition, maternal exposure to androgens/androgenic progestins during first trimester may also result in 46,XX DSD with male external genitalia.

20. *What are the causes of isolated micropenis*?

Micropenis is defined as stretched penile length <−2.5SD for that particular age. In a term newborn, stretched penile length <2.5 cm is used to define micropenis. Isolated micropenis (without any genital ambiguity) suggests impaired androgen synthesis/action or GH deficiency. The causes include hypogonadotropic hypogonadism, hypergonadotropic hypogonadism, androgen biosynthetic defect, androgen insensitivity syndrome, and neonatal GH deficiency. The normal values of stretched penile length at various stages of life are summarized in the table given below.

Stretched penile length in normal males (*cm*)

Age	**Mean ± SD (cm)**	**Micropenis (mean-2.5 SD, cm)**
Newborn: 30 weeks	2.5 ± 0.4	1.5
Newborn: 34 weeks	3.0 ± 0.4	2.0
Newborn: term	3.5 ± 0.4	2.5
0–5 months	3.9 ± 0.8	1.9
6–12 months	4.3 ± 0.8	2.3
1–2 years	4.7 ± 0.8	2.6
2–3 years	5.1 ± 0.9	2.9
3–4 years	5.5 ± 0.9	3.3
4–5 years	5.7 ± 0.9	3.5
5–6 years	6.0 ± 0.9	3.8
6–7 years	6.1 ± 0.9	3.9
7–8 years	6.2 ± 1.0	3.7
8–9 years	6.3 ± 1.0	3.8
9–10 years	6.3 ± 1.0	3.8
10–11 years	6.4 ± 1.1	3.7
Adult	13.3 ± 1.6	9.3

Adapted from *Indian Journal of Pediatrics*. 2000;67:455–60

21. *What is the difference between the terms "microphallus" and "micropenis*?"

The term "micropenis" should be preferred when stretched penile length is <−2.5SD for that particular age in the presence of palpable testes and normal location of urethral meatus without any genital ambiguity. Patients with isolated micropenis should be evaluated for hypogonadism, growth hormone deficiency, and DSD. The term (micro)phallus should be preferred in patients who have genital ambiguity and/or absence of palpable testes and these patients require evaluation for DSD (Fig. 9.7).

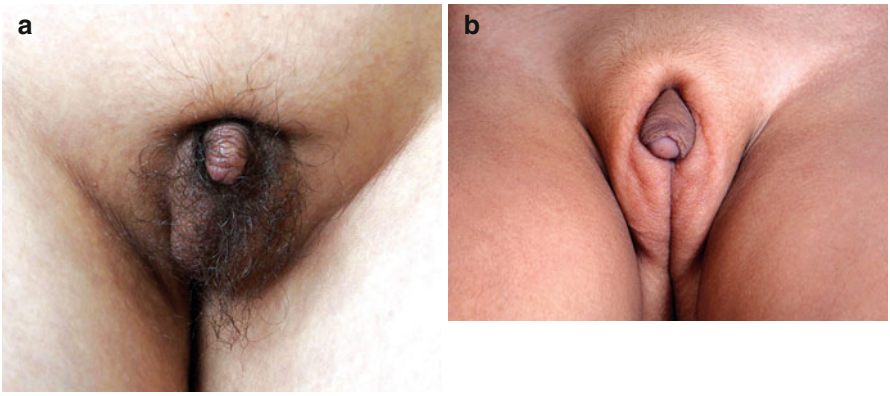

Fig. 9.7 (**a**) Micropenis in a patient with hypogonadotropic hypogonadism. Note the presence of testes in the same patient. (**b**) Phallus in a 3-year-old child with CAH

22. *What is hypospadias*?

Hypospadias is a congenital anomaly characterized by an abnormal site of urethral meatus. It is a common congenital abnormality in males and occurs in approximately 1 in 300, whereas it is rare in females with an incidence of 1 in 500,000. In males with hypospadias, the urethral opening may be located on the undersurface of penis between the tip of glans and perineum. Hypospadias can be classified as distal (glandular and sub-coronal), mid (distal penile, mid-shaft, and proximal penile), or proximal (penoscrotal, scrotal, and perineal). Distal hypospadias is the most common (70 %), followed by mid-hypospadias (20 %) and proximal hypospadias (10 %). Proximal hypospadias is usually associated with DSD, whereas distal hypospadias is commonly idiopathic. Hypospadias is commonly associated with chordee (ventral curvature of penis) and/or deficient foreskin with a dorsal hood.

23. *What is chordee*?

Chordee is defined as fixed ventral curvature of penis. Penis develops from all three germ layers; ectoderm develops into penile skin and prepuce, mesoderm into corpora cavernosa and glans penis, and endoderm into corpora spongiosa and penile urethra. Development of penis is androgen-dependant and possibly higher levels of DHT are required for the formation of penile urethra, as compared to the levels required for the development of corpora cavernosa, glans penis, and prepuce. Therefore, during the development of external genitalia in

an undervirilized 46,XY DSD, dorsal part of penis (corpora cavernosa) outgrows the ventral part (corpora spongiosa and penile urethra), thereby resulting in chordee.

24. *What is cryptorchidism*?

Cryptorchidism is defined as failure of one or both testis to descend into the scrotum. The undescended testes may lie anywhere along its route of descent from abdomen to scrotum or it may be ectopically placed. The prevalence of cryptorchidism in term newborn boys weighing >2.5 Kg varies between 1.8 % and 3.8 %. Spontaneous descent of testis occurs in majority of these infants (50–70 %) by 1–3 months of age, whereas spontaneous descent beyond 6–9 months is rare. The prevalence of cryptorchidism is only approximately 0.8 % at 1 year of age. Cryptorchidism is unilateral in approximately 80 %, whereas the rest have bilateral undescended testes. The most common site of undescended testes is high scrotal/pre-scrotal (48 %), followed by superficial inguinal ring (29 %), inguinal canal (22 %), and abdomen (<1 %). Ectopic testes contribute to <1 % of all cases of cryptorchidism and the sites of ectopic testes include just above penis (pre-penile), perineum, femoral canal, and intra-abdominal wall (Fig. 9.8).

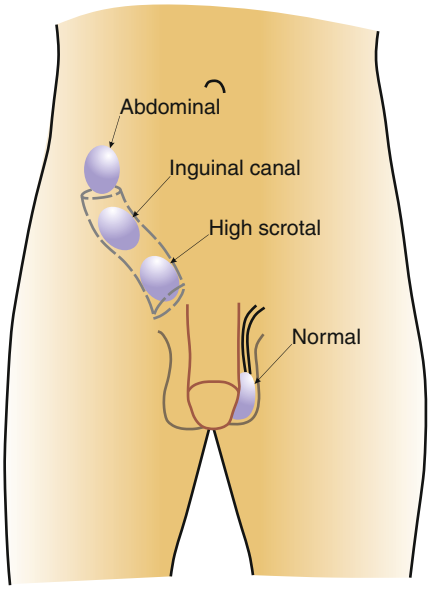

Fig. 9.8 Common sites of undescended testes

25. *What is retractile testis*?

Retractile testis refers to testis which has completed its descent to scrotum but which ascends to groin due to exaggerated cremasteric reflex. Retractile testis can be brought down to scrotum by manipulation and may remain in scrotum at least temporarily. Retractile testis is usually bilateral and is commonly observed in children between 2 and 6 years of age.

26. *What are the causes of cryptorchidism*?

The most common cause of isolated cryptorchidism is idiopathic, and the other common causes include hypogonadotropic hypogonadism, Klinefelter's syndrome, Noonan syndrome, and obesity–hypogonadism syndromes. DSD is an uncommon cause of cryptorchidism and accounts for only 3 % of all cases. Partial androgen insensitivity syndrome and androgen biosynthetic defects are the common DSDs that can also present with isolated cryptorchidism. However, if cryptorchidism is associated with hypospadias, the likelihood of having a DSD increases to 13 %, and the causes include partial androgen insensitivity syndrome, androgen biosynthetic defects, ovotesticular DSD, mixed gonadal dysgenesis, and 46,XY partial gonadal dysgenesis. In addition, bilateral non-palpable testis due to anorchidism with apparent male genitalia (Prader 5) is seen in congenital adrenal hyperplasia due to 21α-hydroxylase deficiency and vanishing testes syndrome (congenital anorchia).

27. *How to differentiate bilateral undescended testes from anorchidism*?

All children with bilateral cryptorchidism should be evaluated for the presence of testes by imaging. Ultrasonography is useful, especially for detection of testes located in the inguinal region, whereas MRI is required to localize the testes at deep inguinal ring and abdomen. During infancy and peripubertal period, estimation of gonadotropins and testosterone helps in determination of presence or absence of testis, because of reactivation of HPG-axis during these periods of life. High gonadotropin level with low testosterone is highly suggestive of anorchia or dysgenetic testes; whereas, low gonadotropin level with low testosterone suggest cryptorchidism due to hypogonadotropic hypogonadism. However, in prepubertal period, estimation of gonadotropins and testosterone cannot reliably distinguish anorchia from cryptorchidism; hence, hCG stimulation test should be performed in this scenario. Estimation of inhibin B and AMH can be used to differentiate anorchia from cryptorchidism at any age. An undetectable serum inhibin B or anti-Mullerian hormone is highly suggestive of anorchia. Finally, diagnostic laparoscopy is the gold standard method to localize the testis (Fig. 9.9).

Fig. 9.9 (**a**–**c**) A 20-year-old individual presented with bilateral non-palpable testes and no genital ambiguity. CEMRI could not localize any gonadal tissue and testosterone response to hCG was undetectable. He was diagnosed to have hypergonadotropic hypogonadism due to vanishing testes syndrome. The patient is on testosterone replacement therapy

28. *What is the role of different imaging modalities in the evaluation of cryptorchidism*?

    Imaging modalities that can be used to localize the testes include ultrasonography, CT, and MRI; CT scan should be avoided to prevent radiation exposure to the gonads. The sensitivity and specificity of ultrasonography and MRI in localizing non-palpable testes are summarized in the table given below.

Imaging modality	Location	Sensitivity (%)	Specificity (%)
Ultrasonography	Inguinoscrotal	52	88
	Intra-abdominal	44	93
MRI	Inguinoscrotal	85	100
	Intra-abdominal	55	100

29. *How to differentiate between bilateral undescended testes and anorchidism by hCG stimulation test*?

    The dose of hCG for the assessment of testosterone response depends on the age of the child: 500 IU for infants, 1,000 IU for children aged 1–10 years, and 1,500 IU for >10 years. hCG is administered intramuscularly for 3 days and serum testosterone is estimated at baseline and after 24h following the last dose. A positive response is defined as a twofold rise in serum testosterone from baseline or a peak testosterone level >5 nmol/L (radioimmunoassay) after hCG stimulation test and denotes the presence of functioning Leydig cells. A negative response suggests anorchia.

30. *When to evaluate a newborn for DSD*?

    All newborns with overt genital ambiguity should be evaluated for DSD. Newborns with an apparent female external genitalia having isolated clitoromegaly, posterior labial fusion, or inguinal/labial mass require evaluation for DSD. Newborns with apparent male genitalia and having external masculinization score <11 also merit evaluation for DSD (e.g., those with isolated micropenis will have EMS 9 and those with isolated proximal or mid-hypospadias will have EMS 10 or less). In addition, those with familial hypospadias or having discordance between prenatal karyotype and genital appearance require evaluation for DSD.

31. *How to approach a neonate with genital ambiguity*?

The first step in the evaluation of a neonate with genital ambiguity is a meticulous clinical examination for the presence or absence of gonad/s. Palpable gonad/s in a child with ambiguous genitalia suggests the presence of testes or ovotestis; as testes descend, ovaries do not and ovotestes/dysgenetic testes partially descend. Absence of palpable gonad/s in a child with ambiguous genitalia suggests either undervirilized 46,XY infant or virilized 46,XX infant. The external genitalia in undervirilized 46,XY infant and virilized 46,XX infant is usually symmetrical; however, asymmetrical external genitalia suggests the diagnosis of mixed gonadal dysgenesis (MGD) or ovotesticular DSD (OT-DSD). An approach to genital ambiguity in a newborn based on clinical examination and estimation of serum 17(OH)P is given in the figure below (Fig. 9.10).

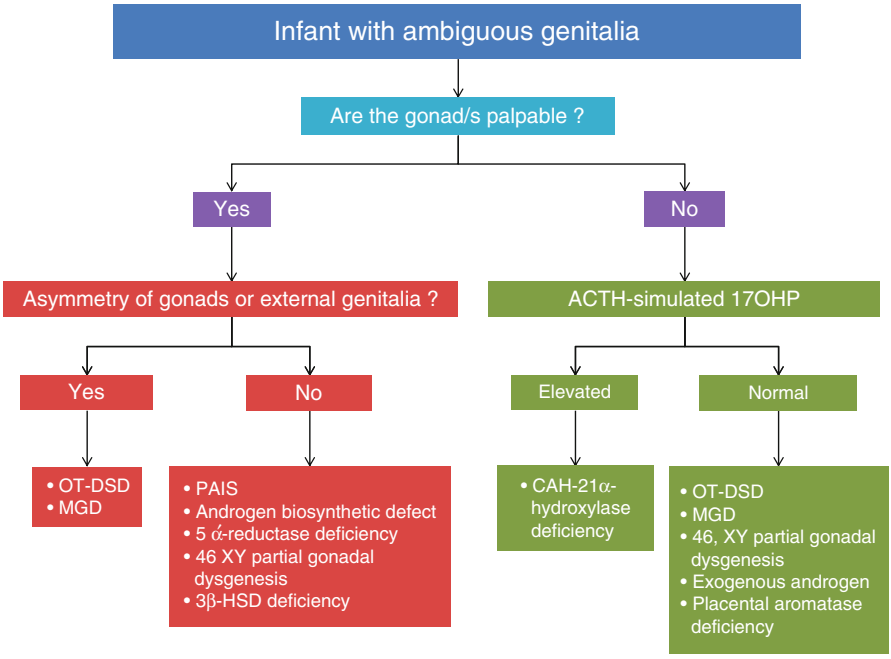

Fig. 9.10 Approach to a newborn with ambiguous genitalia

32. *How to evaluate a child with genital ambiguity and palpable gonads*?

In a child with genital ambiguity and palpable gonads, karyotyping, serum testosterone, anti-Mullerian hormone (AMH), and hCG stimulation test help to

establish the etiological diagnosis. Age-specific cutoffs for serum testosterone and AMH are to be used for interpretation of the results. Estimation of serum LH is helpful during minipuberty and after the onset of puberty; however, it has limited utility during "window period." The diagnostic workup of a child with ambiguous genitalia and palpable gonads is shown in the figure given below (Fig. 9.11).

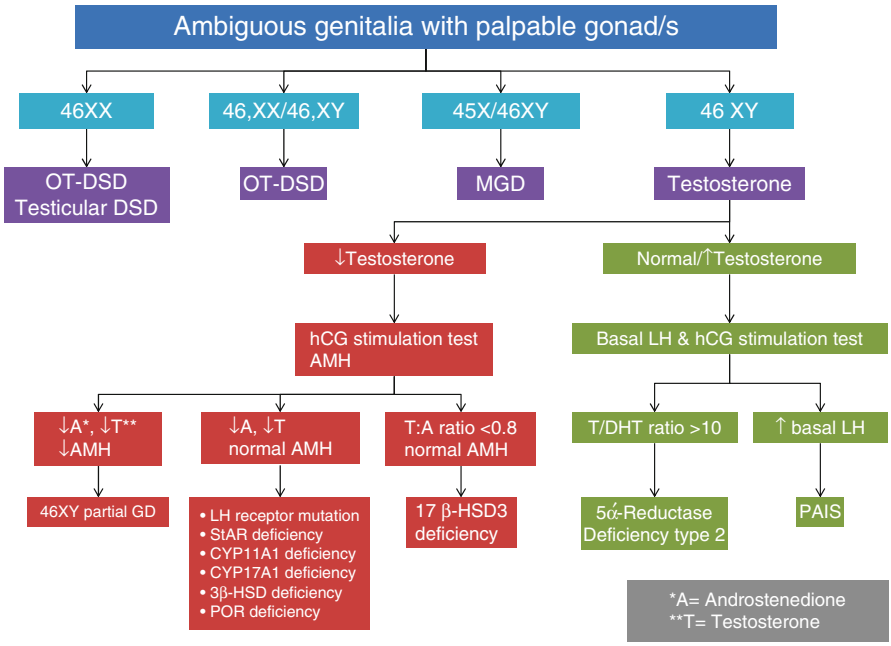

Fig. 9.11 Approach to a child with ambiguous genitalia and palpable gonads

33. *A 2-year-old child was brought with history of genital ambiguity since birth. The clinical profile of the patient is depicted below. What are the differential diagnoses in the index case*?

This child was reared as a boy and was brought for evaluation of genital ambiguity at the age of 2 years. Examination of genitalia revealed bifid scrotum with rugosity, bilateral scrotal testes (size 2 ml each), phallus with chordee, ventral urethral groove, and perineoscrotal hypospadias. The external masculinization score was 6. Since both the gonads are palpable, a clinical diagnosis of 46,XY DSD was considered. The differential diagnosis in this child includes androgen biosynthetic defects (ABSD), partial androgen insensitivity (PAIS), and 5α-reductase type 2 deficiency. Rarely, CAH due to 3β-HSD2 and POR deficiency can be associated with 46,XY DSD (Fig. 9.12).

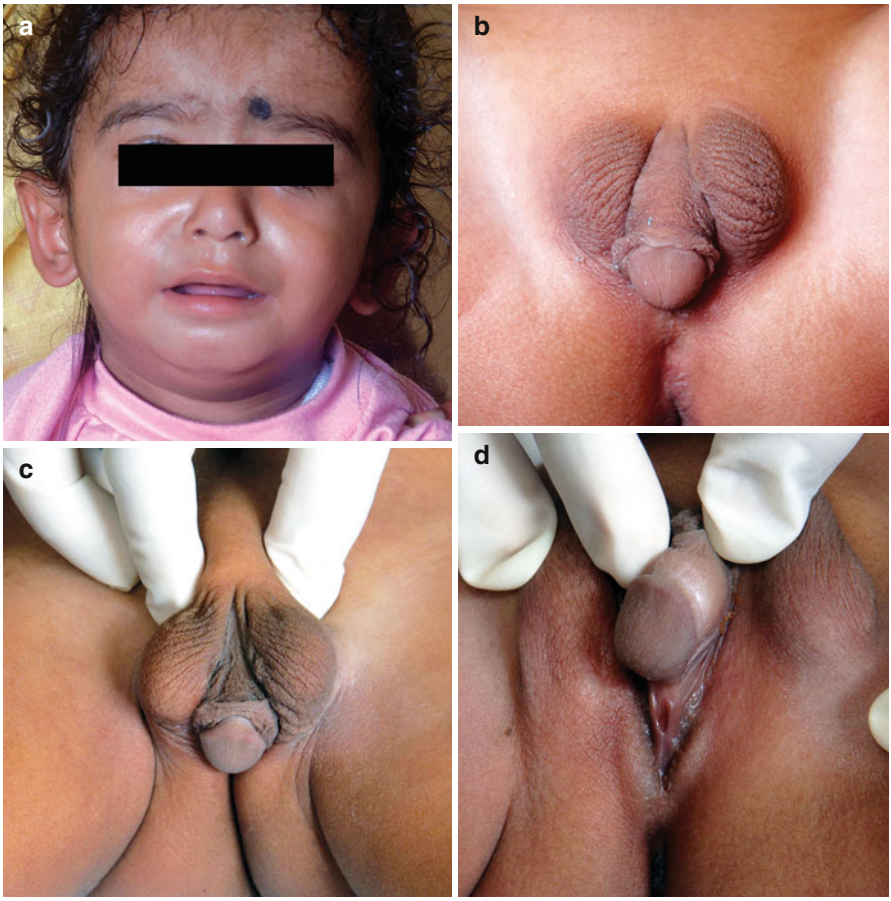

Fig. 9.12 (**a**) A 2-year-old child with (**b**, **c**) bifid scrotum and palpable testes in the labioscrotal fold and (**d**) perineoscrotal hypospadias with chordee. The karyotype of the child was 46,XY and a diagnosis of androgen biosynthetic defect (17β-HSD 3 deficiency) was established

34. *What is the utility of hCG stimulation test in patients with DSD*?

hCG stimulation test aids in the differential diagnosis in patients with 46,XY DSD suspected to have androgen biosynthetic defects, 5α-reductase type 2 deficiency, and androgen resistance syndrome. hCG is administered intramuscularly for 3 days at a dose of 500–1,500 IU/day, based on the age of the patient, and serum testosterone, androstenedione, and dihydrotestosterone are estimated at baseline and 24h after the last dose of hCG. The interpretation of the results of hCG stimulation test is summarized in the table given below. In addition,

hCG stimulation test is also useful to differentiate anorchidism from bilateral cryptorchidism.

Diagnosis	Androstenedione	Testosterone	Dihydrotestosterone	Remarks
Androgen resistance syndrome	Elevated	Elevated	Elevated	–
5α-reductase type 2 deficiency	Normal to elevated	Elevated	Low	T/DHT ratio >10
Androgen biosynthetic defects (17β-HSD3)	Elevated	Low	Low	T: Δ^4 ratio <0.8
Dysgenetic testes	Low	Low	Low	No response to even prolonged hCG stimulation test

35. *What are the causes of presence of Mullerian derivatives in a child with genital ambiguity and palpable gonads*?

The presence of Mullerian structures in a child with ambiguous genitalia and palpable gonads suggests dysgenetic testes or ovotestes. Inability of dysgenetic testes/ovotestes to produce sufficient quantity of AMH and testosterone results in persistence of Mullerian derivates and genital ambiguity, respectively. The differential diagnosis includes ovotesticular DSD, mixed gonadal dysgenesis, and 46,XY partial gonadal dysgenesis.

36. *How to evaluate a child with genital ambiguity and non-palpable gonads*?

Absence of palpable gonads in a child with ambiguous genitalia suggests either undervirilized 46,XY infant or virilized 46,XX infant. The most common cause of virilization in 46,XX infant is CAH due to 21α-hydroxylase deficiency, and it is important to recognize this disorder as it can be life threatening due to salt-wasting crisis. Undervirilization in 46,XY infant with non-palpable gonads suggest 46,XY partial gonadal dysgenesis or 17α-hydroxylase deficiency. In addition, infants with mixed gonadal dysgenesis and ovotesticular DSD can also present with genital ambiguity and non-palpable gonads. Karyotype and stimulated 17(OH)P can establish the diagnosis in a child with ambiguous genitalia and non-palpable gonads. After establishing the diagnosis, gonads should be localized in infants with 46,XY DSD, OT-DSD, and MGD (Fig. 9.13).

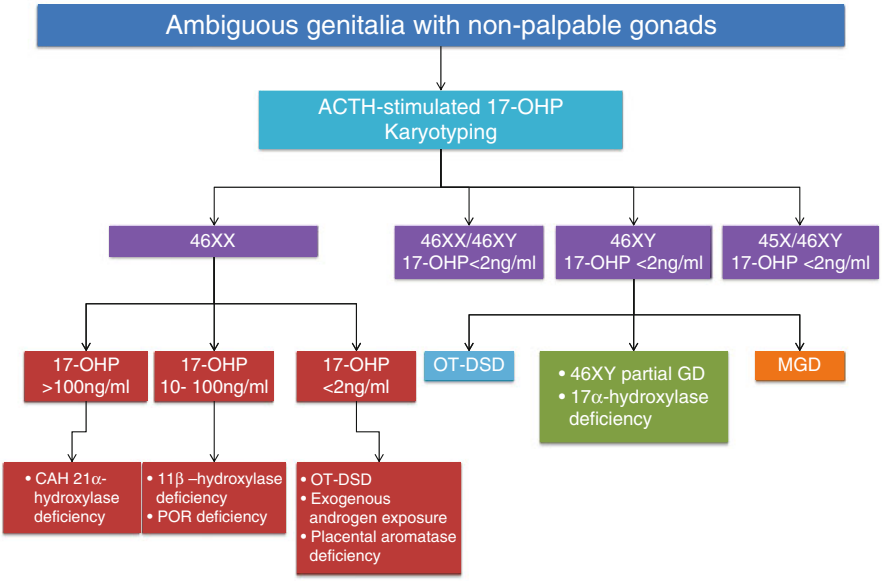

Fig. 9.13 Approach to a child with genital ambiguity and non-palpable gonads

37. *A 6-day-old newborn presented with genital ambiguity. On examination, external genitalia was Prader stage 3 and gonads were non-palpable. Karyotyping is awaited. How to proceed*?

ACTH-stimulated serum 17(OH)P and karyotyping are the first-line investigations in the evaluation of a child with genital ambiguity and non-palpable gonads. CAH due to 21α-hydroxylase deficiency is the most common differential diagnosis in this scenario and early diagnosis and timely initiation of therapy may prevent salt-wasting crisis. As the results of karyotyping and serum 17(OH)P are not immediately available, ultrasonography can be used to evaluate the presence/absence of Mullerian derivatives (uterus, fallopian tubes, and upper two-thirds of vagina). Presence of Mullerian derivatives in a child with genital ambiguity and non-palpable gonads increases the likelihood of CAH; therefore, blood pressure, serum sodium, potassium, and blood glucose should be closely monitored, and glucocorticoid therapy should be initiated, if required. The definitive diagnosis in the newborn can be established once results of karyotype and 17(OH)P are available.

38. *What is the importance of family history in the differential diagnosis of DSD*?

Family history provides important clues in the differential diagnosis of DSD as summarized in the table given below.

History	Etiology
Consanguinity	Congenital adrenal hyperplasia
	5α-reductase type 2 deficiency
	Androgen biosynthetic defects
	Gonadal dysgenesis
Maternal virilization during pregnancy	Placental aromatase deficiency
	P450 oxidoreductase deficiency
Neonatal deaths	CAH with salt crises
Precocious puberty (heterosexual or isosexual)	CAH
Primary amenorrhea	Androgen insensitivity syndrome
	Gonadal dysgenesis
	CAH due to 17α-hydroxylase deficiency
Gynecomastia	Androgen insensitivity syndrome
	Androgen biosynthetic defects
Advanced maternal age	Turner syndrome
	Klinefelter syndrome

39. *What are the DSDs which present without genital ambiguity*?

46,XY DSDs which do not manifest with genital ambiguity include CAIS, Swyer syndrome (46,XY complete gonadal dysgenesis), and, rarely, severe 17α-hydroxylase deficiency. Patients with these disorders have female external genitalia. In addition, patients with congenital anorchia (vanishing testes syndrome) present with bilateral non-palpable gonads, with unambiguous male external genitalia. 46,XX DSDs which can present with apparently male genitalia without any genital ambiguity include CAH due to 21α-hydroxylase and 11β-hydroxylase deficiency, SRY gene translocation, SOX9 duplications, and RSPO1-inactivating mutations. Patients with CAH can present with apparently normal male external genitalia without palpable gonads, whereas the latter disorders are associated with normal male external genitalia and palpable gonads. In addition, Turner syndrome and Klinefelter are the sex chromosomal DSDs that present without genital ambiguity.

40. *Whom to evaluate for DSD during adolescence*?

A possibility of DSD should be considered in any phenotypic female who present during adolescence with primary amenorrhea, significant virilization, inguinal/labioscrotal mass, or cyclical hematuria. The various presenting manifestations of DSD during adolescence in a phenotypic female are summarized in the table given below.

Presentation	**Etiology**
Primary amenorrhea	Turner syndrome
	Mullerian agenesis
	Complete androgen insensitivity syndrome (CAIS)
	46,XY complete gonadal dysgenesis
	46,XX complete gonadal dysgenesis
	17α-hydroxylase deficiency
Peripubertal virilization	5α-reductase deficiency type 2
	17β-HSD3 deficiency
	Partial androgen insensitivity syndrome (PAIS)
	Ovotesticular DSD
	Mixed gonadal dysgenesis (MGD)
	Aromatase deficiency
Inguinal/labioscrotal mass	Complete androgen insensitivity syndrome
	Partial androgen insensitivity syndrome (PAIS)
	17β-HSD3 deficiency
	Ovotesticular DSD
Cyclical hematuria	Ovotesticular DSD
	Congenital adrenal hyperplasia

In addition, DSD should also be considered in boys with delayed puberty and the causes include Klinefelter's syndrome, PAIS, and 46,XY gonadal dysgenesis (mild variants).

41. *What are the DSDs associated with genital ambiguity and peripubertal gynecomastia*?

The causes of genital ambiguity with peripubertal gynecomastia are partial androgen insensitivity syndrome (PAIS), androgen biosynthetic defects (ABSD), ovotesticular disorders (OT-DSD), and rarely Klinefelter's syndrome (Fig. 9.14).

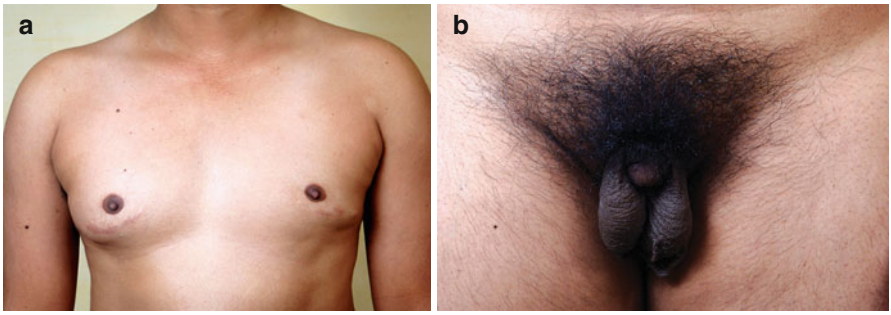

Fig. 9.14 (**a**) A 20-year-old patient presented with genital ambiguity and peripubertal gynecomastia. Note the surgical scar of reduction mammoplasty. (**b**) External genitalia showing Tanner pubic hair stage P_4, small phallus, penoscrotal hypospadias, and bilateral testicular volume 10–12 ml. Hormonal profile was suggestive of PAIS

42. *What are the DSDs associated with inguinal hernia*?

The descent of testes from abdominal cavity to scrotum is accompanied with a peritoneal lining called processus vaginalis, which is gradually obliterated after the completion of testicular descent. Persistence of processus vaginalis predisposes for the development of inguinal hernia and hydrocele. Inguinal hernia is more common in boys as compared to girls (10:1) and majority (80–85 %) of inguinal hernia is unilateral. Bilateral inguinal hernias are uncommon and its presence in a girl child should raise a suspicion of complete androgen insensitivity syndrome (CAIS). The other DSDs that are associated with inguinal hernia are 17-β hydroxysteroid dehydrogenase type 3 deficiency (17-βHSD3), ovotesticular DSD (OT-DSD), and persistent Mullerian duct syndrome (PMDS). The contents of hernial sac differ in all these disorders; testis in CAIS and 17β-HSD3 deficiency, ovotestis in OT-DSD and Mullerian derivatives in PMDS (Fig. 9.15).

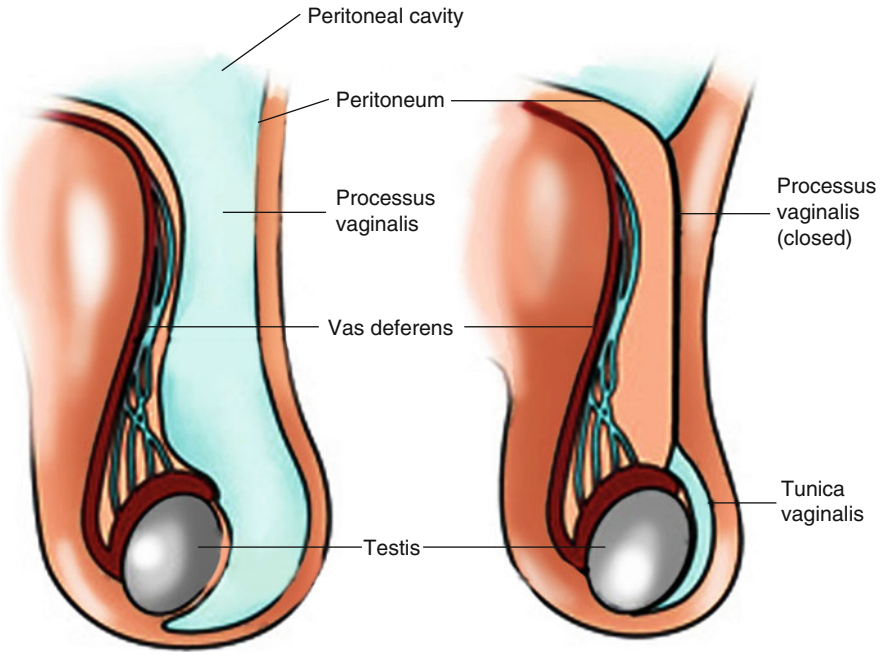

Fig. 9.15 Obliteration of processus vaginalis during embryogenesis

43. *What are the DSDs that present as primary amenorrhea with virilization during peripubertal period*?

The DSDs that present as primary amenorrhea with virilization during peripubertal period are partial androgen insensitivity, androgen biosynthetic defect, 5α-reductase deficiency, and aromatase deficiency.

44. *What are the DSDs associated with cyclical hematuria*?

Cyclical hematuria in patients with DSD represents menstrual bleed. Presence of functional hypothalamo–pituitary–ovarian axis, intact uterus, and outflow tract, and persistent urogenital sinus are the prerequisites for cyclical hematuria. The cause of DSD associated with cyclical hematuria is ovotesticular DSD. However, CAH due to 21-α hydroxylase deficiency and 11-β hydroxylase deficiency in a 46,XX female, who is on medical therapy and have not undergone urogenital reconstructive surgery may also have cyclical hematuria.

45. *What are the DSDs associated with eunuchoidal body proportions*?

The DSDs associated with eunuchoidal body proportions include 46,XY and 46,XX complete gonadal dysgenesis, androgen biosynthetic defects (17β-HSD3, 17α-hydroxylase deficiency), and placental aromatase deficiency.

46. *How to classify gonadal dysgenesis*?

Gonadal dysgenesis refers to defective development of gonads, which can vary from hypoplastic and dysfunctioning gonads mainly composed of fibrous tissue without any germ cells (ovarian-like stroma, i.e., streak gonads) to incomplete gonadal development (dysgenetic gonads). Dysgenetic gonads commonly contain hypoplastic seminiferous tubules and fibrous tissue (dysgenetic testis). The differences between various types of gonadal dysgenesis are summarized in the table given below.

Types	**Gonad**	**Genitalia**
Complete or pure gonadal dysgenesis (46,XX or 46,XY)	Bilateral streak gonads	External: female
		Internal: female
Partial gonadal dysgenesis (46,XX or 46,XY)	Bilateral dysgenetic gonads	External: female to ambiguous
		Internal: commonly female
Mixed gonadal dysgenesis (45,X/46,XY)	Dysgenetic testis on one side and streak on the other side	External: ambiguous
		Internal: female/male

47. *What are the somatic features associated with 46,XY gonadal dysgenesis*?

The presence of extragonadal manifestations provide clues to the diagnosis of 46,XY gonadal dysgenesis. These are summarized in the table given below.

Extragonadal manifestations	**Syndrome**	**Mutation**
Wilm's tumor, aniridia, genitourinary abnormalities	WAGR syndrome	WT1
Wilm's tumor and early-onset nephropathy	Denys–Drash syndrome	WT1
Late-onset nephropathy	Frasier syndrome	WT1
Skeletal anomalies	Camptomelic dysplasia	SOX9
Mental retardation	–	WNT4, DMRT1, ATRX
α-Thalassemia	–	ATRX

48. *When to suspect mixed gonadal dysgenesis in a patient with DSD*?

Asymmetry of external genitalia/gonads, short stature, and features of Turner syndrome in a child with DSD suggest the diagnosis of MGD. Asymmetry of external genitalia refers to well-formed scrotum on one side and hypoplastic labioscrotal sac on contralateral side, usually with a palpable gonad on the side of well-formed scrotum. Short stature is present in majority of patients, whereas Turner stigmata are found in one-third of patients with MGD.

49. *When to consider a diagnosis of ovotesticular DSD in a child with ambiguous genitalia*?

Ovotesticular DSD should be considered in all newborns with genital ambiguity and asymmetry of external genitalia/gonads or inguinal hernia. In addition, it should also be suspected in those children who present with genital ambiguity and bilateral non-palpable gonads, after excluding CAH due to 21α-hydroxylase deficiency. During peripubertal period, OT-DSD should be considered in individuals with genital ambiguity who are reared as boys and present with cyclical hematuria or gynecomastia. It should also be pondered in individuals with genital ambiguity who are reared as girls and present with primary amenorrhea and virilization (Fig. 9.16).

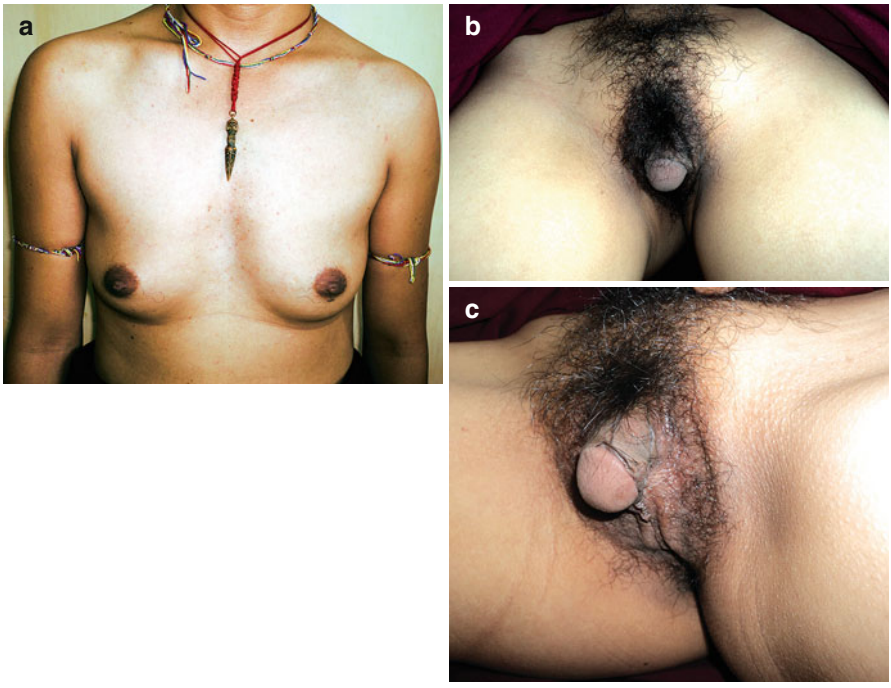

Fig. 9.16 (**a**) A 22-year-old individual with ovotesticular DSD who presented with cyclical hematuria. Note the presence of breast development. (**b**, **c**) Tanner pubic hair stage P_3, phallus with chordee, and bilateral non-palpable gonads

50. *How to establish the diagnosis of OT-DSD*?

The characteristic karyotype 46,XX/46,XY is present only in 20 % patients with OT-DSD, whereas 46,XX karyotype is present in 70 % and 46,XY karyotype in 10 %. Hence, the diagnosis of OT-DSD should be established histopathologically by demonstration of ovarian tissue (containing primordial or maturing follicles) and testicular tissue (containing seminiferous tubules with spermatogonia) either in the same gonad or in different gonads. Ovotestes are the most frequent gonad present in patients with OT-DSD, followed by ovary and testis. 50 % of patients with OT-DSD have unilateral ovotestes with either a testis or ovary on the other side, 30 % of patients have bilateral ovotestes, while the rest (20 %) have ovary on one side and testis on the other side. Testis is commonly located in scrotum, ovary in the abdomen, and ovotestis can be present anywhere along the route of testicular descent from abdomen to labioscrotal fold.

51. *What are the DSDs associated with ovotestis*?

The term ovotestis denotes the presence of ovarian follicles and seminiferous tubules within the same gonad. The classical disorder associated with ovotestis is 46,XX or 46,XX/46,XY or 46,XY ovotesticular DSD. Other rare disorders associated with ovotestis include SRY gene translocation and inactivating mutations of RSPO1, SOX9, and WNT4.

52. *A 20-year-old individual, who was reared as male, was referred for diagnostic evaluation for DSD. He had history of surgery for gynecomastia at the age of 15 years and multiple surgeries for hypospadias. The clinical profile of the patient is depicted below. What is the differential diagnosis in the index case*?

The index patient had immature facies, poor facial hair, and feminine voice suggestive of hypogonadism. Genital examination revealed Tanner pubic hair stage P_4, bilateral scrotal testes (size 15 ml bilateral), microphallus (stretched penile length 7 cm), penile hypospadias (despite multiple corrective surgeries), and redundant skin folds (after corrective surgery). The differential diagnosis in this individual with genital ambiguity, bilateral palpable gonads, and gynecomastia includes partial androgen insensitivity and androgen biosynthetic defects. The two disorders can be differentiated based on serum testosterone and LH levels. High normal to elevated serum testosterone with normal to raised LH suggest a diagnosis of partial androgen insensitivity, whereas low serum testosterone and elevated LH suggest androgen biosynthetic defects. The index patient had karyotype 46,XY, had serum testosterone 16 nmol/L and LH 12 μIU/ml, and was diagnosed to have PAIS (Fig. 9.17).

Fig. 9.17 (**a**) A 20-year-old individual with gynecomastia (**b**) bilateral scrotal testes (**c**) penile hypospadias with redundant skin folds (after corrective surgery for hypospadias) due to PAIS

53. *How to differentiate between PAIS, 5α-reductase type 2 deficiency, and 17β-HSD3 deficiency*?

In a patient with bilateral palpable gonads and genital ambiguity, the most common differential diagnoses are PAIS, 5α-reductase type 2 deficiency, and 17β-HSD3 deficiency. The differentiating features among these disorders are summarized in the table given below.

Parameters	**PAIS**	**5α-reductase type 2**	**17β-HSD3 deficiency**
Inheritance	X-linked recessive	Autosomal recessive	Autosomal recessive
Genital ambiguity	Less severe	Severe	Severe
Internal genitalia (Wolffian derivatives)	Often normal	Normal	Normal
Mullerian derivatives	Absent	Absent	Absent

Parameters	PAIS	5α-reductase type 2	17β-HSD3 deficiency
Prostate	Normal/hypoplastic	Hypoplastic	Normal/hypoplastic
Gynecomastia	Present	Absent	Present
Facial hair	Reduced	Reduced to absent	Reduced
Temporal recession	May be present	Absent	May be present
Gonads	Inguinal/scrotal	Inguinal/scrotal	Intra-abdominal/inguinal
Basal testosterone	Normal to elevated	Normal to elevated	Low
LH and FSH	Normal to elevated	Normal to elevated	Elevated
hCG stimulation test	–	T/DHT ratio >10	T/A ratio <0.8
Sex of rearing (commonly)	Male	Female	Female
Sex reversal (if reared as female)	Uncommon	Common	Common
Therapy	High-dose androgens	High-dose androgens Topical DHT gel	Gonadectomy and estrogen, if reared as female Androgen, if reared as male

54. *A 20-year-old individual who was reared as male presented with poor facial hair and genital ambiguity. The clinical profile of the patient is depicted below. What are the differential diagnoses in the index case*?

The index patient had immature facies and poor facial hair suggestive of hypogonadism. Genital examination revealed Tanner pubic hair stage P_4, bilateral scrotal testes (size 6 ml each), microphallus (stretched penile length 5 cm), ventral–urethral groove, and penile hypospadias. The external masculinization score was 7. He did not have gynecomastia. Since both the gonads were palpable, a clinical diagnosis of 46,XY DSD was considered. The differential diagnosis in this individual includes androgen biosynthetic defects (ABSD), partial androgen insensitivity (PAIS), and 5α-reductase type 2 deficiency. In the index patient, biochemical evaluation revealed serum testosterone 24 nmol/L, LH 7μIU/ml, and FSH 18.8 μIU/ml. As serum testosterone is in the upper normal range, a diagnosis of ABSD is unlikely. Hence to differentiate between partial androgen insensitivity (PAIS) and 5α-reductase type 2 deficiency, a hCG stimulation test was performed. The stimulated testosterone/dihydrotestosterone ratio was 4.9. Absence of gynecomastia in the index patient favors a diagnosis of 5α-reductase type 2 deficiency, whereas a T/DHT ratio of 4.9 makes this diagnosis unlikely. High-normal serum testosterone and LH and T/DHT ratio of 4.9 suggest a diagnosis of PAIS, although absence of gynecomastia is uncommon in PAIS. High FSH in the index patient represents either germ cell failure or it may be a manifestation of decreased testosterone

action on Sertoli cell, thereby resulting in decreased production of inhibin B. Therefore, genetic analysis is required for a definitive diagnosis in the index patient (Fig. 9.18).

Fig. 9.18 (**a**) A 20-year-old individual with poor facial hair growth. (**b**) Tanner pubic hair stage P_4, bilateral scrotal testes (**c**, **d**) microphallus, ventral–urethral groove, and penile hypospadias

55. *Does the presence of gynecomastia help in the differential diagnosis of 46,XY DSD with genital ambiguity*?

In patients with 46,XY DSD with genital ambiguity, the common differential diagnosis include partial androgen insensitivity syndrome (PAIS), androgen biosynthetic defects (ABSD), ovotesticular disorders (OT-DSD), 5α-reductase type 2 deficiency, and 46,XY partial gonadal dysgenesis. The presence of gynecomastia suggests PAIS, ABSD, and OT-DSD, whereas patients with 5α-reductase type 2 deficiency and 46,XY partial gonadal dysgenesis do not have gynecomastia. Presence of gynecomastia in PAIS and ABSD is due to deficient secretion/action of testosterone and induction of aromatase activity by elevated LH, whereas in OT-DSD, it is due to estrogen secretion from ovotestes/ovary. Patients with 5α-reductase type 2 deficiency do not have gynecomastia as they have normal to high serum testosterone with normal LH, whereas patients with 46,XY partial gonadal dysgenesis do not have gynecomastia as the dysgenetic testes is unable to produce sufficient quantity of testosterone to be aromatized, even in the presence of elevated LH.

56. *What are the variants of CAH associated with genital ambiguity in both genders*?

CAH due to 21α-hydroxylase deficiency and 11β-hydroxylase deficiency is associated with genital ambiguity only in 46,XX individuals, and CAH due to 3 β-HSD2 and 17α-hydroxylase deficiency is associated with genital ambiguity only in 46,XY individuals. The only variant of CAH that manifest genital ambiguity in both genders is CAH due to POR deficiency. In addition, CAH due to 3 β-HSD2 deficiency may also have genital ambiguity in both genders, although genital ambiguity is rare and less severe (isolated mild clitoromegaly) in females.

57. *What are the DSDs associated with maternal virilization during pregnancy*?

CytochromeP450 oxidoreductase deficiency (POR), placental aromatase deficiency, luteoma of pregnancy, and exposure to androgenic progestins/androgens are the causes of DSDs associated with maternal virilization during pregnancy.

58. *What are the characteristic features of aromatase deficiency*?

The acquisition of aromatase activity by placenta is important to prevent virilization of the female fetus by androgens secreted from fetal adrenal gland. DHEA (and 16α-hydroxy DHEA) synthesized from the fetal adrenal cortex is

converted to androstenedione (and 16α-hydroxy androstenedione) by the placental enzyme 3β-HSD1, which is aromatized to estrone (E1) by the enzyme placental aromatase. The placental 17 β-HSD1 converts estrone into active estrogens, estradiol (E2) and estriol (E3). In patients with placental aromatase deficiency, accumulation of fetal adrenal androgens results in virilization of 46,XX fetus and the mother. The severity of virilization of fetus varies from isolated clitoromegaly to Prader stage 4. During peripubertal period, affected females present with primary amenorrhea, poor breast development, hirsutism, virilization, hypergonadotropic hypogonadism, and tall stature. Affected males have persistent linear growth, eunuchoidal body proportions, genu valgum, and impaired fertility.

59. *A 32-year-old male presented with primary infertility and erectile dysfunction. On evaluation, he was well virilized and had normal proportions (upper segment to lower segment ratio 0.97) with sexual maturation score of A+,P_5 and testicular volume of 4–6 ml bilaterally. Hormonal profile showed LH 6.9 mIU/ml, FSH 14.1 mIU/ml, and testosterone 7.1 nmol/L. Semen analysis showed azoospermia and fine needle aspiration cytology was consistent with Sertoli cell-only syndrome. How to evaluate further*?

The index patient had primary gonadal failure, predominantly germ cell, as evidenced by high FSH, high normal LH, and low testosterone. The important causes of predominant germ cell failure include Klinefelter's syndrome, cryptorchidism, orchitis, postradiation/chemotherapy, Sertoli cell-only syndrome, Y chromosome microdeletion, and FSH receptor polymorphism. Therefore, any patient with predominant germ cell failure (elevated FSH) where cause is not evident should have a karyotype analysis to exclude the diagnosis of Klinefelter's syndrome. Hence, the index patient was subjected for karyotype analysis which revealed XX male syndrome (Fig. 9.19).

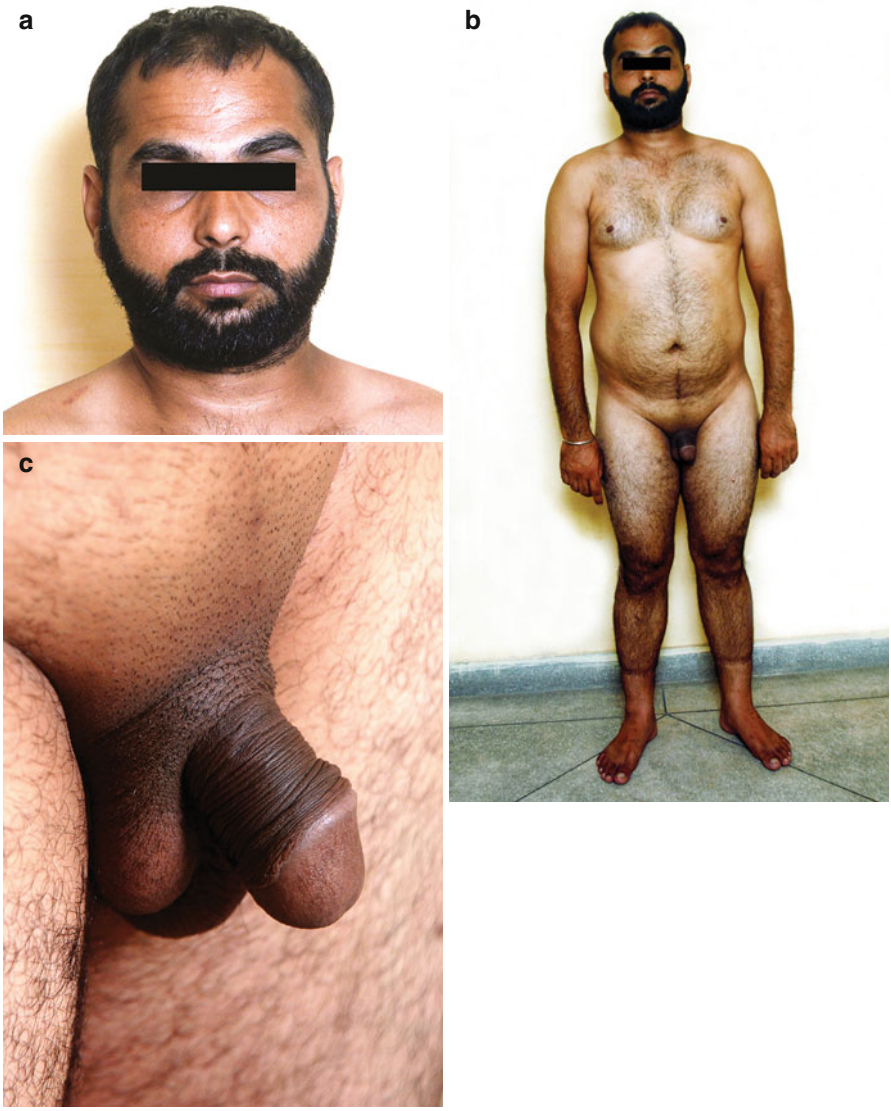

Fig. 9.19 (**a**–**d**) A 32-year-old well-virilized male presented with primary infertility and karyotype confirmed 46,XX male syndrome

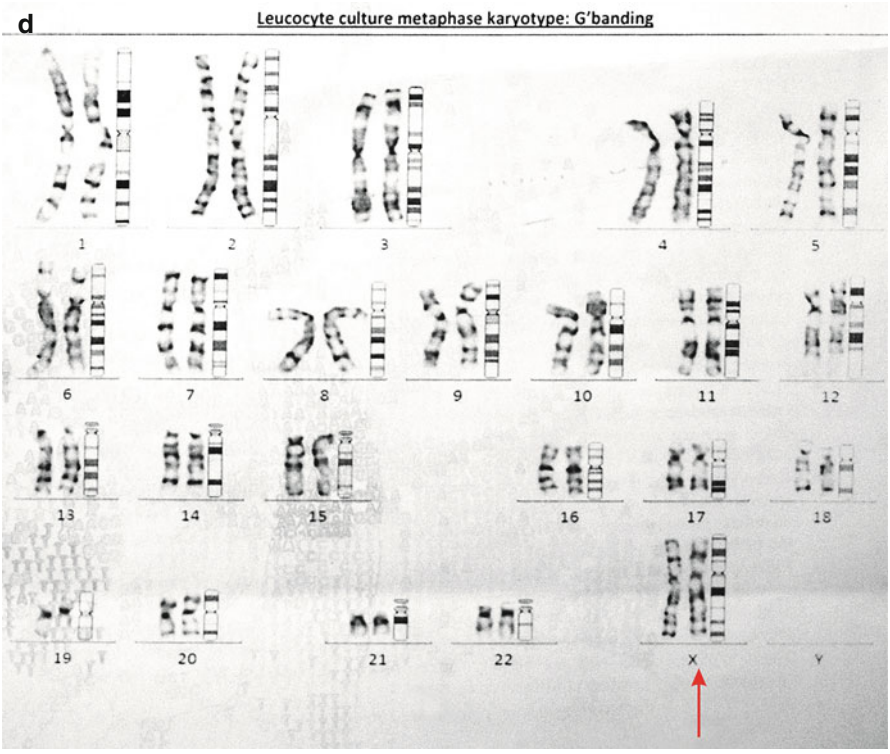

Fig. 9.19 (continued)

60. *What is XX male syndrome*?

46,XX male syndrome is a DSD characterized by unambiguous male phenotype with 46,XX karyotype, varying degree of virilization, short stature, small testes, primary infertility, and gynecomastia. The consistent biochemical abnormality is elevated FSH, whereas serum LH levels are variable depending on the degree of Leydig cell failure. *SRY* gene which is important for male sex determination is translocated on pseudoautosomal region of X chromosome and consequent male phenotype with 46,XX karyotype. The germ cell failure occurs as a result of absence of azoospermia factor (AZF) which is present on Y chromosome or due to presence of two X chromosomes.

61. *How to assess the psychosexual development of an individual*?

Psychosexual development ("behavioural sex") of an individual comprises of gender identity, gender role, and gender orientation. "Gender identity" refers to one's own perception of his/her gender, as male or female. "Gender role" refers to the behavior of an individual typical of a male or female. "Gender orientation" refers to the preference toward male or female as a sexual partner.

62. *What is gender dysphoria*?

A condition associated with marked psychosexual distress due to discrepancy between an individual's perception of his/her own gender and the assigned sex of rearing is termed as "gender dysphoria." It may or may not be associated with DSDs.

63. *What are the factors that guide gender assignment in patients with genital ambiguity*?

Type of DSD, appearance of external genitalia, differentiation of internal genitalia, age at presentation, feasibility of genital reconstructive surgery, need for lifelong gonadal steroid replacement, risk for gonadal malignancy, potential for fertility, and psychosocial development of the patient are the factors that should be considered prior to gender assignment in an individual with ambiguous genitalia. A patient with 46,XX DSD due to 21α-hydroxylase deficiency CAH should be reared as female as they have future prospects for normal pubertal development and fertility. Patients with 46,XY DSD due to 5α-reductase type 2 deficiency can be reared as male because these patients experience significant virilization and sex reversal (female to male) during peripubertal period. Patients with 46,XY DSD due to 17β-HSD3 deficiency are usually reared as female as they do not have genital ambiguity; however, few patients with 17β-HSD3 deficiency have genital ambiguity and can be reared as male as they experience significant virilization during peripubertal period. Patients with 46,XY complete gonadal dysgenesis or complete androgen insensitivity are usually raised as female. The gender assignment in patients with partial androgen insensitivity, androgen biosynthetic defects, and 46,XY partial gonadal dysgenesis is based on phallic length, feasibility of genital reconstructive surgery, and gender role. The decision of sex assignment in patients with MGD and ovotesticular DSD should be individualized (Figs. 9.20 and 9.21).

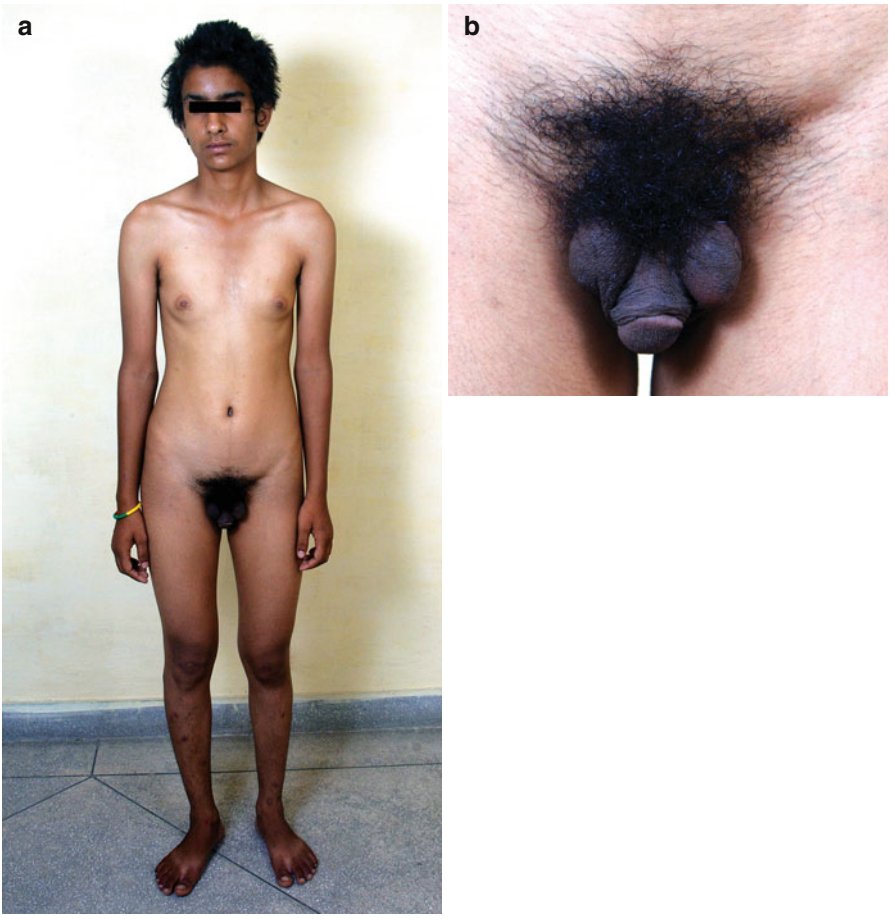

Fig. 9.20 (**a**) An individual with OT-DSD reared as male. (**b**) External genitalia showing Tanner pubic hair stage P_3, phallus, and bifid scrotum. The patient underwent bilateral testicular prosthesis implantation after corrective surgery

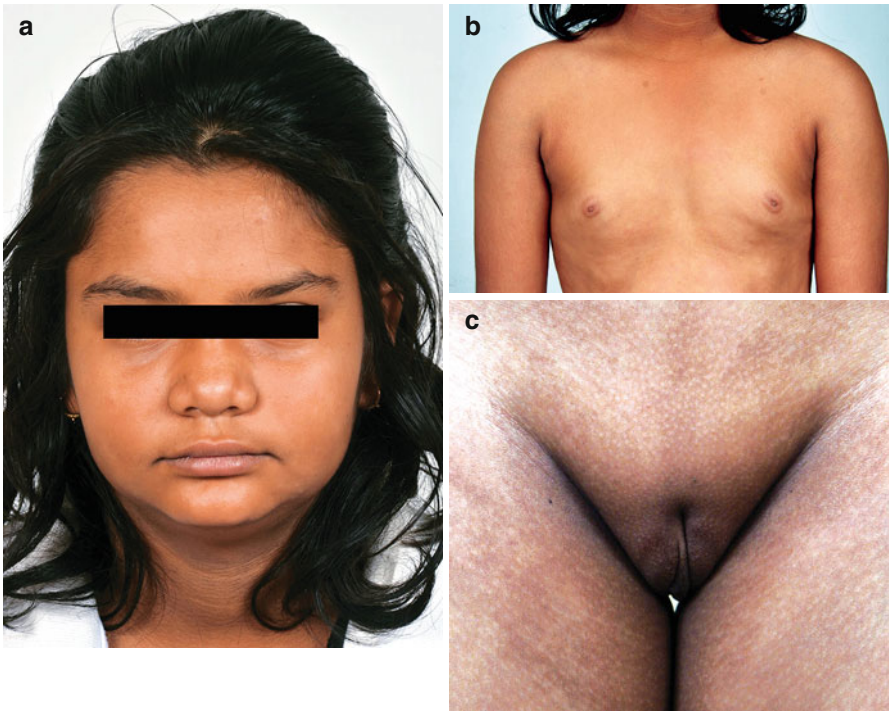

Fig. 9.21 (**a**, **b**) An individual with OT-DSD who is reared as female. She underwent bilateral gonadectomy and phallic resection during prepubertal period and is on estrogen replacement. (**c**) External genitalia showing normal female phenotype

64. *When should surgery for hypospadias be performed in a child with 46,XY DSD*?

In a child with 46,XY DSD and hypospadias, corrective surgery should ideally be performed at 6–12 months of age. This is because gender identity of an individual is established by 2–3 years of life, and, therefore, early genital surgery is associated with better psychosocial and emotional outcome. Further, surgery during this period has favorable outcome because of pliability of genital tissues, reduced pro-inflammatory cytokine response, and lesser surgical complications. Genital reconstruction surgery is usually performed as a one-stage procedure. However, proximal hypospadias and coexisting chordee require a two-stage repair.

65. *When to consider gonadectomy in a patient with DSD*?

Decision to perform gonadectomy in a patient with DSDs is based on the sex of rearing and the future risk of development of germ cell tumors. The risk of germ cell malignancy is high in patients with 46,XY gonadal dysgenesis (complete or partial), 45,X/46,XY mixed gonadal dysgenesis, and in patients with PAIS having non-scrotal testes; hence, gonadectomy is recommended at diagnosis. Patients with CAIS and ovotesticular DSD are at low risk for the development of germ cell malignancy. Despite this fact, gonadectomy is recommended in patients with CAIS as almost all these individuals are reared as females. However, it is controversial whether to perform gonadectomy during childhood or after puberty. In patients with ovotesticular DSD, gonadectomy may be required depending on the sex of rearing. The risk of malignant germ cell tumors in ovarian tissue in patients with OT-DSD is not known, while the risk is 3–4 % for testicular tissue; hence, individuals having OT-DSD require periodic surveillance. Patients with Turner syndrome having Y-cell line are at intermediate risk for development of malignant germ cell tumors; hence, gonadectomy is recommended at diagnosis.

Further Readings

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Congenital Adrenal Hyperplasia

10

10.1 Case Vignette

A 15-day-old child was brought by her parents for repeated vomiting, excessive crying and jitteriness, and poor feeding. She was a product of non-consanguineous marriage and was delivered by induced labor at 33 weeks of gestation due to maternal complications (pregnancy-induced hypertension). Her birth weight was 1.9 Kg and she was assigned female gender. She was investigated and found to have hyponatremia and hyperkalemia (Na^+ 119 mEq/L, K^+ 8.1 mEq/L) and was diagnosed to have hypoaldosteronism. She was initiated with fludrocortisone 50 μg/day. With this treatment, her symptoms subsided but she failed to thrive and progressively became darker; however, serum electrolyte abnormalities were resolved. She was taken to an endocrinologist at 5 months of age, and she was suspected to have congenital adrenal hyperplasia (CAH) and was investigated accordingly. Her unstimulated serum 17α-hydroxyprogesterone [17(OH)P] was 120 ng/ml and was diagnosed to have CAH due to 21α-hydroxylase deficiency, and dexamethasone was added to fludrocortisone. This therapy resulted in weight gain and decrease in pigmentation, and patient became more active. At 2 years of age, she was referred to our institute. Her height was 81 cm (10^{th} percentile, target height 158 cm), weight 12 Kg (25^{th} percentile), and she had a Tanner staging of $A_{.}$, P_1, B_1. She did not have Cushing's stigmata. Examination of the external genitalia showed posterior labial fusion, hyperpigmented labioscrotal folds, isolated clitoromegaly, and the gonads were not palpable. Two distinct openings were seen at perineum. The karyotype was 46,XX. Serum 17(OH)P was 26.8 ng/ml. Dexamethasone was replaced with hydrocortisone (7.5 mg/day) and

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fludrocortisone (100 μg/day) was continued. The annual follow-up of the patient with clinical and biochemical parameters is depicted in the table given below.

Parameters	2 years	3 years	4 years	5 years	6 years	7.5 years[a]	8 years	12 years	13 years
Height (cm)	81	93	100	105	115	125	128	153	154
Weight (Kg)	12	14.5	17	17	24	28	30	50	54
Tanner stage	$A\_P_1B_1$	$A\_P_2B_1$	$A\_P_2B_1$	–	$A\_P_3B_1$	$A\_P_3B_3$	$A\_P_3B_3$	$A_+P_5B_4$	$A_+P_5B_4$
Bone age (year)	–	7 years	8 years	–	–	10	–	–	14
17(OH)P (ng/ml)	26.8	250	0.5	0.1	–	0.78	–	174	90
T (nmol/L)	2.7	–	0.36	0.3	0.06	0.09	–	16.8	5.7
Hydrocortisone (mg/day)	7.5	10	6	6	7	10	10	17.5	0.25[b]
Fludrocortisone (μg/day)	100	100	100	100	100	100	100	150	150

T testosterone
[a]LH 0.3 mIU/ml, E_2 42 pg/ml
[b]Hydrocortisone was substituted with dexamethasone 0.25 mg/day

At the age of 3 years, she had appearance of pubic hair along with growth spurt with a steep rise in 17(OH)P which was suggestive of gonadotropin-independent precocious puberty (GIPP) that required increase in the dose of hydrocortisone. Between the age of 3–5 years, her growth velocity was appropriate for her age, and there was no progression of Tanner staging, but she had cushingoid facies. Serum 17(OH)P was suppressed and testosterone was in prepubertal range; therefore, the dose of hydrocortisone was reduced at this point of care. Between the age of 5–7.5 years, she had spurt in growth velocity followed by initiation of thelarche. Serum 17(OH)P continued to remain suppressed, while serum LH increased to pubertal range 0.3 mIU/ml, which was suggestive of gonadotropin-dependent precocious puberty (GDPP). However, in the next 6 months, patient did not have progression of pubertal events. From the age of 8 year onward, she had progression of pubertal events with serum LH of 1.5 mIU/ml, and she was initiated with leuprolide 3.75 mg at monthly interval. With this therapy, her growth velocity was approximately 6 cm/year, and there was no progression of breast development till the age of 12 years. At the age of 12 years, leuprolide was discontinued, and after 3 months, she had menarche and her Tanner staging was A_+, P_5, B_4. Six months later, she presented with worsening of hyperpigmentation, secondary amenorrhea, and deepening of voice. Serum 17(OH)P was 174 ng/ml, testosterone 16.8 nmol/L, LH 0.07 mIU/ml, FSH <0.5 mIU/ml, estradiol 73.1 pg/ml, and ACTH 384 pg/ml. Ultrasonography of pelvis showed uterine size 4 × 3 cm, endometrial thickness 5 mm, and ovarian volume 3.5 ml each. Hydrocortisone dose was increased to 17.5 mg/day and fludrocortisone to 150 μg/day. After 3 months of this therapy, 17(OH)P was 90 ng/ml and testosterone 5.7 nmol/L. She was switched to

dexamethasone 0.25 mg/day and fludrocortisone 150 μg/day, and with this therapy, 17(OH)P decreased to 40 ng/ml. Hydrocortisone was added at a daily dose of 10 mg (in three divided doses) along with dexamethasone and fludrocortisone (Fig. 10.1).

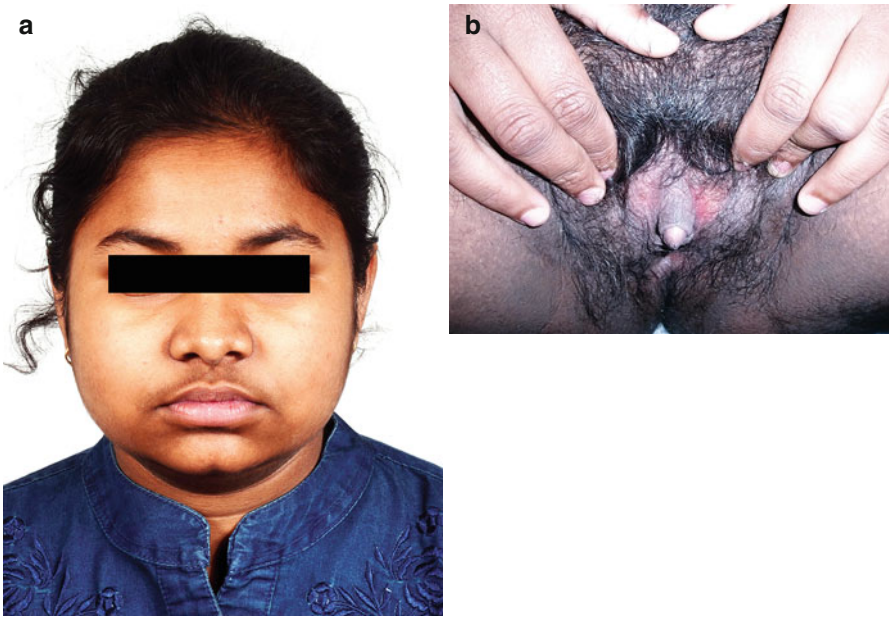

Fig. 10.1 (**a**) A 13-year-old female with CAH due to 21α-hydroxylase deficiency. (**b**) External genitalia shows isolated clitoromegaly with posterior labial fusion

10.2 Stepwise Analysis

The index patient presented at the age of 15 days with features of salt crisis. The differential diagnosis of recurrent episodes of vomiting in a neonate raises a suspicion of neonatal sepsis, gastroenteritis, and hypertrophic pyloric stenosis. However, all these disorders are associated with hyponatremia and hypokalemia. The concurrent presence of hyponatremia with hyperkalemia in an infant with recurrent episodes of vomiting suggests the possibilities of CAH, congenital adrenal hypoplasia, and pseudohypoaldosteronism. The index patient had very high serum 17(OH)P and hyperkalemia, which conclusively established the diagnosis of salt-wasting variant of classical CAH due to 21α-hydroxylase deficiency. Serum 17(OH)P >100 ng/ml in an infant with salt crisis obviates the need for ACTH stimulation test. The other salt-wasting variant of CAH associated with mild elevation of 17(OH)P includes 3βHSD type 2 deficiency which is usually associated with genital ambiguity in both genders. The salt crisis in neonates with CAH usually manifests at 1–2 weeks of life despite the enzyme deficiency present since birth. This occurs because of transplacental

passage of maternal progesterone which exerts partial mineralocorticoid agonistic activity in the presence of aldosterone deficiency, which progressively wanes thereafter, as circulating progesterone is metabolized by second week of life. The index patient was assigned female gender possibly because she had mild genital virilization (clitoromegaly with posterior labial fusion, Prader stage 2), and further this would not have been well evident at birth in a premature child. The index child was initiated with only fludrocortisone that resulted in resolution of symptoms and correction of electrolyte abnormalities; however, she failed to grow and continued to become dark. This is expected because fludrocortisone supplementation alone does not correct glucocorticoid deficiency and at recommended doses will not suppress CRH-ACTH axis, thereby resulting in failure to thrive (as CRH is an anorexigenic peptide) and progressive darkening of skin. Later, replacement with dexamethasone was initiated which resulted in clinical improvement but led to decrease in height to 10^{th} percentile. This is because dexamethasone is 80 times more potent in inhibiting epiphyseal growth plate activity as compared to hydrocortisone; therefore, hydrocortisone is preferred in the management of CAH instead of dexamethasone, particularly in growing children. Hence, in the index case, at 2 years of age, dexamethasone was substituted with hydrocortisone. Fludrocortisone is to be replaced not only in infants who present with salt crisis but also in those who have simple virilizing CAH. This is because infants with CAH, even simple virilizing, do have subclinical aldosterone deficiency which results in decreased intravascular volume, thereby leading to increased ADH secretion, which in turn stimulates ACTH production and consequent worsening of hyperandrogenemia and hyperpigmentation. Increased angiotensin II as a result of increased plasma renin activity in these infants also contributes to growth and proliferation of adrenal cortex. Further, rising level of 17(OH)P exerts anti-mineralocorticoid activity in the presence of mild aldosterone deficiency (simple virilizing CAH). Therefore, supplementation with fludrocortisone even in simple virilizing CAH ameliorates all these biochemical abnormalities and reduces the doses of hydrocortisone. During third year of life, she experienced growth spurt with appearance of pubic hair accompanied with marked rise in serum 17(OH)P suggestive of GIPP. The dose of hydrocortisone was further increased and it resulted in decrease in growth velocity, nonprogression of Tanner staging, and suppression of 17(OH)P and testosterone. This indicates overtreatment with glucocorticoids as serum 17(OH)P was suppressed to 0.5 ng/ml. The recommended target for serum 17(OH)P is to maintain its level between 4 and 12 ng/ml. This cutoff is higher than the normal reference range, because the dose of glucocorticoid required to suppress 17(OH)P to normal range is higher and detrimental to growth plate, thereby, resulting in growth suppression and development of metabolic syndrome. Therefore, the dose of hydrocortisone was reduced. In addition, monitoring of serum androstenedione and testosterone (in female) may also be useful to guide the therapy. Between the age 4–7.5 years, serum 17(OH)P continued to remain suppressed, and she experienced another spell of growth spurt along with thelarche (B_3). This suggests the onset of GDPP which was further corroborated by serum LH of 0.3 mIU/ml. The growth spurt preceded the onset of thelarche, and it is a usual phenomenon during pubertal development in a growing girl child which was observed in the index patient. Children with CAH who are either untreated or undertreated experience GIPP, and

optimal treatment often subsequently results in induction of GDPP. This happens because rapid reduction of androgens with optimal glucocorticoid treatment results in withdrawal of inhibitory effects (of androgens) on androgen-primed GnRH–gonadotropin axis. Patient was started on GnRH agonist, which was continued for the next 2 years. With this therapy, the growth velocity remained in prepubertal range (6 cm/year), and after 3 months of discontinuation of GnRH agonist, she had menarche with Tanner stage $A_{+,}$ $P_{5,}$ B_4. Six months later, she presented with worsening of hyperpigmentation, secondary amenorrhea, and deepening of voice, and serum 17(OH)P and testosterone were markedly elevated. Sudden worsening of clinical symptoms related to androgen excess is usually observed during peripubertal period. This is a consequence of increased ACTH drive as a result of augmented cortisol clearance during peripubertal period. This occurs due to increased IGF1 during peripubertal period, as serum IGF1 inhibits 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), thereby decreasing t½ of cortisol. This mandates the increase in dose and frequency of hydrocortisone administration. Other options in this scenario include the use of potent glucocorticoids (e.g., dexamethasone or prednisolone; either alone or in combination with hydrocortisone), modified release preparations of hydrocortisone, and the use of hydrocortisone infusion pump. Hydrocortisone replacement in reverse circadian rhythm has also been tried without any substantial benefits. In the index patient, dexamethasone alone could not control 17(OH)P and testosterone; therefore, hydrocortisone was also added to dexamethasone akin to the "basal–bolus" regimen in diabetes.

10.3 Clinical Rounds

1. *What is congenital adrenal hyperplasia*?

   Congenital adrenal hyperplasia (CAH) includes a group of autosomal recessive disorders due to deficiency of enzymes required for cortisol synthesis in adrenal cortex and consequently results in adrenal hyperplasia due to adrenocorticotropic hormone (ACTH) overdrive.

2. *What are the enzymes involved in cortisol biosynthesis*?

   The enzymes involved in cortisol biosynthesis include side-chain cleavage (20,22 desmolase or CYP11A1), 17α-hydroxylase (CYP17A1), 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2), 21α-hydroxylase (CYP21A2), and 11β-hydroxylase (CYP11B1). In addition, steroidogenic acute regulatory protein (StAR) is required for the transport of cholesterol into mitochondria for cortisol synthesis. All these enzymes are cytochrome P450 dependent except 3β-HSD2. Inherited deficiencies of any of these enzymes or StAR protein result in CAH. 21α-hydroxylase deficiency is the most common cause of CAH and accounts for 95 % of patients. The enzymes involved in cortisol biosynthesis are depicted in the figure given below (Fig. 10.2).

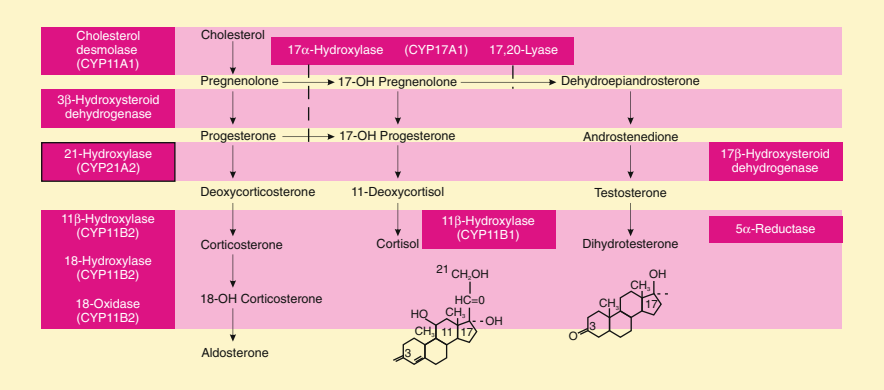

Fig. 10.2 Adrenal steroidogenesis

3. *What are the enzymes common for adrenal and gonadal steroidogenesis*?

   The enzymes which are common for adrenal and gonadal steroidogenesis include side-chain cleavage (20,22 desmolase or CYP11A1), 17α-hydroxylase (CYP17A1), and 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2). Hence, deficiency of these enzymes not only results in impaired cortisol synthesis but also defective gonadal steroidogenesis.

4. *How to classify CAH due to 21α-hydroxylase deficiency*?

   Depending on the residual enzymatic activity, CAH due to 21α-hydroxylase deficiency is classified as classical or non-classical variants. The classical variant is further subclassified as salt-wasting (75 %) and simple virilizing (25 %) forms. Patients with salt-wasting variant have residual 21α-hydroxylase enzymatic activity <1 %; those with simple virilizing have 1–5 % residual enzymatic activity and non-classical variant have 20–50 % (Fig. 10.3).

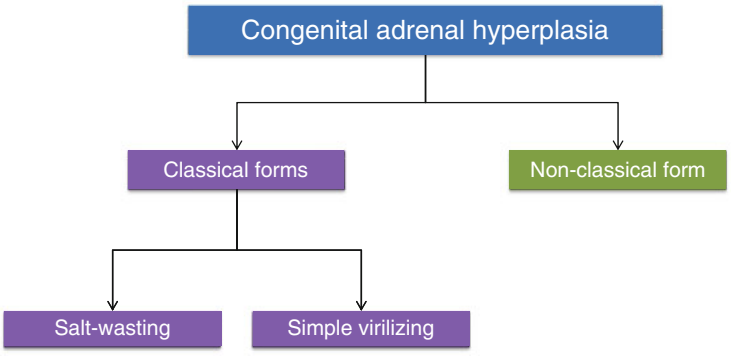

Fig. 10.3 Classification of CAH due to 21α-hydroxylase deficiency

5. *Why is 21α-hydroxylase deficiency the most common cause of CAH*?

   Humans have two CYP21A genes, a pseudogene (CYP21A1P) and the active gene (CYP21A2), which are located on the short arm of chromosome 6. The active gene encodes for 21α-hydroxylase enzyme, while pseudogene produces a truncated protein without any enzymatic activity. The presence of >95 % homology between functional gene and pseudogene allows recombination between these two genes. CYP21A genes are located on the short arm of chromosome 6 within the region of major histocompatibility locus (HLA class III), a site with very high rate of recombination. The genetic recombination is an event that occurs during meiosis as well as during mitosis and produces a new combination of alleles in the offspring which is different from each parent. The presence of >95 % homology between active gene and pseudogene, and location at a site with high rate of recombination, explains the increased prevalence of CAH due to 21α-hydroxylase deficiency.

6. *What is the difference between homozygote*, *heterozygote, and compound heterozygote*?

   An individual who has same mutation in each allele of a gene is said to be homozygote, while an individual with a mutation in one allele of a gene (and the other allele is normal) is said to be heterozygote. An individual with different mutation in each allele of a gene is said to be compound heterozygote.

7. *What is the importance of compound heterozygosity in CAH*?

   Compound heterozygosity is present in the majority (60–75 %) of patients with CAH. The clinical phenotype of a compound heterozygote individual with CAH is determined by the less severely mutated allele. For example, if an individual receives different mutation from each parent (e.g., exon 1 mutation from father and exon 8 mutation from mother), the individual is said to be compound heterozygote for that disease. Assuming that mutation in exon 1 allele is a severe mutation (known to be associated with salt-wasting) and mutation in exon 8 allele is a mild mutation (known to be associated with non-classical CAH), the individual will manifest as non-classical phenotype of CAH.

8. *What is the genotype–phenotype correlation in patients with CAH due to 21α-hydroxylase deficiency*?

   The genotype–phenotype correlation in CAH can be considered in terms of biochemical or clinical phenotype. There is a good correlation between genotype and biochemical phenotype as patients with severe mutations have <5 % residual enzyme activity with stimulated serum 17α-hydroxyprogesterone 17(OH)P level >100 ng/ml, whereas those with less severe mutations have 20–50 % residual enzyme activity with stimulated serum 17(OH)P level 10–100 ng/ml. Hence, stimulated serum 17(OH)P is an excellent test to establish the diagnosis of

CAH, and genetic mutations are usually not required to confirm the diagnosis of CAH. Although, there is a good correlation between genotype and biochemical phenotype, there is poor correlation between genotype and clinical phenotype in terms of urogenital sinus virilization (severity of genital ambiguity). This is probably due to variability in androgen sensitivity among individuals as a result of differences in the number of CAG repeats in the androgen receptor. The number of CAG repeats has an inverse correlation with androgen sensitivity. In addition, the electron donor enzyme P450 oxidoreductase (POR) may also modulate the clinical phenotype, as one variant of POR has been shown to reduce the activity of 17α-hydroxylase, thereby decreasing the androgen biosynthesis.

9. *What are the different types of mutations implicated in CAH due to 21α-hydroxylase deficiency*?

   The various types of mutations seen in patients with CAH due to 21α-hydroxylase deficiency include large base pair deletions, nonsense mutation, and missense mutations. Large base pair deletions and nonsense mutations of CYP21A gene manifest as salt-wasting form of CAH as these mutations result in <1 % residual enzymatic activity. Missense mutations may result in either simple virilizing forms of disease or in non-classical form based on the particular mutations. Large base pair deletions are due to defects in genetic recombination during meiosis and accounts for 20 % of mutations in patients with CAH, while transfer of deleterious genetic material from pseudogene to active gene during mitosis occurs in 75 % of patients.

10. *How does a child with salt-wasting crisis present*?

    A child with salt-wasting CAH manifests with failure to thrive, lethargy, recurrent vomiting, dehydration, and hypotension. The biochemical abnormalities include hyperkalemia, hyponatremia, and metabolic acidosis.

11. *Why does salt crisis in a patient with classical CAH develop 1–2 weeks after birth*?

    The salt crisis in neonates with CAH usually manifests at 1–2 weeks of life despite the enzyme deficiency is present since birth. Progesterone plays a key role in prevention of salt crisis in initial few days of life. Progesterone in high concentration exerts agonistic activity at mineralocorticoid receptor in the presence of aldosterone deficiency. Though progesterone has a weak mineralocorticoid receptor agonistic activity, in aldosterone-sufficient state, it exerts anti-mineralocorticoid activity. As infants with salt-wasting CAH have aldosterone deficiency, transplacental passage of maternal progesterone in newborn prevents salt crisis by mineralocorticoid activity of progesterone.

12. *What are the differential diagnoses to be considered in a neonate with salt-wasting crisis*?

    One of the characteristic features of salt-wasting crisis is vomiting and hypotension. However, neonates with congenital hypertrophic pyloric stenosis, gastric volvulus, gastroenteritis, sepsis, and meningitis can also present with intractable vomiting and hypotension. All these disorders are characterized by the presence of hypokalemia, and the presence of hyperkalemia and metabolic acidosis in a newborn with vomiting and hypotension should alert the possibility of salt-wasting crisis due to CAH, congenital adrenal hypoplasia, congenital hypoaldosteronism (aldosterone synthase deficiency), or pseudohypoaldosteronism (aldosterone resistance).

13. *Why is hypotension severe in salt-wasting children with CAH*?

    Aldosterone deficiency manifests as salt-wasting; however, the manifestations of salt-wasting are amplified by the coexisting cortisol deficiency present in patients with CAH. This is because cortisol is required for maintenance of vasoreactivity to circulating vasopressors including angiotensin II and catecholamines. Cortisol is also essential for the synthesis of catecholamine in adrenal medulla; hence, catecholamine insufficiency also contributes to hypotension in patients with CAH. In addition, the accumulation of 17(OH)P which has mineralocorticoid antagonistic properties also contribute to severe hypotension.

14. *Is salt-wasting crisis more common in females with CAH due to 21α-hydroxylase deficiency*?

    No. Salt-wasting crisis due to CAH is more commonly diagnosed in 46,XX newborns as compared to 46,XY neonates. This is because during an episode of salt crisis, the presence of genital ambiguity in a 46,XX newborn alerts the clinician to consider a possibility of CAH, whereas the diagnosis of salt crisis due to CAH is often missed in a 46,XY neonate due to the absence of genital ambiguity, and many of 46,XY neonate succumb to salt crisis. The presence of genital hyperpigmentation and hyperkalemia are the clues to suspect CAH in a 46,XY neonate with salt-wasting crisis.

15. *How to approach a neonate with salt-wasting crisis*?

    Any child presenting with vomiting, dehydration, and hypotension should be suspected to have salt crisis, and serum electrolytes, blood glucose, and blood gas analysis should be urgently performed in these children. The presence of hyperkalemia and metabolic acidosis suggest a diagnosis of salt-wasting crisis.

In this scenario, samples for 17(OH)P, cortisol and aldosterone, and plasma renin activity should be sent, and therapy with hydrocortisone and intravenous fluids should be initiated empirically, pending the result of these investigations. The distinguishing characteristics of various causes of salt-wasting based on the results of biochemical investigations are summarized in the table given below.

Disorders	17(OH)P	Serum cortisol	Plasma aldosterone	Plasma renin activity
Classical CAH (salt-wasting)	Elevated	Low	Low	Elevated
Congenital adrenal hypoplasia	Low	Low	Low	Elevated
Congenital hypoaldosteronism	Normal	Normal	Low	Elevated
Pseudohypoaldosteronism	Normal	Normal	Elevated	Elevated

16. *How to treat a neonate with salt-wasting crisis due to CAH*?

Prompt recognition and management is essential to prevent mortality associated with salt-wasting crisis. The management of salt-wasting crisis includes intravenous fluids preferably containing isotonic dextrose–saline and intravenous hydrocortisone at doses of 100 mg/m^2 in divided doses. Administration of dextrose with saline is preferred rather than saline, as neonates with salt crisis are prone to hypoglycemia due to cortisol deficiency as cortisol is also required for gluconeogenesis. Isotonic saline promptly corrects intravascular volume depletion and helps in restoring eukalemia. Once the child is hemodynamically stable and starts accepting oral feed, therapy with oral hydrocortisone (10–15 mg/m^2 in divided doses) and fludrocortisone (100 μg twice daily) can be initiated along with oral salt supplementation (4–8 mmol/Kg). Newborns require higher doses of fludrocortisone as they are aldosterone resistant.

17. *What are the features of virilization of external genitalia in a 46,XX newborn*?

The extent of virilization in a 46,XX newborn due to prenatal androgen exposure may vary from Prader stage 1–5 depending on the time of exposure, severity of androgen excess, and sensitivity to androgens. Urogenital sinus differentiation is complete by 12th week of intrauterine life; hence, the androgen exposure prior to 12 weeks results in labioscrotal fusion along with clitoromegaly, while exposure after 12 weeks results in isolated clitoromegaly. In addition, the severity of androgen excess and sensitivity to androgens also determine the extent of virilization (Fig. 10.4).

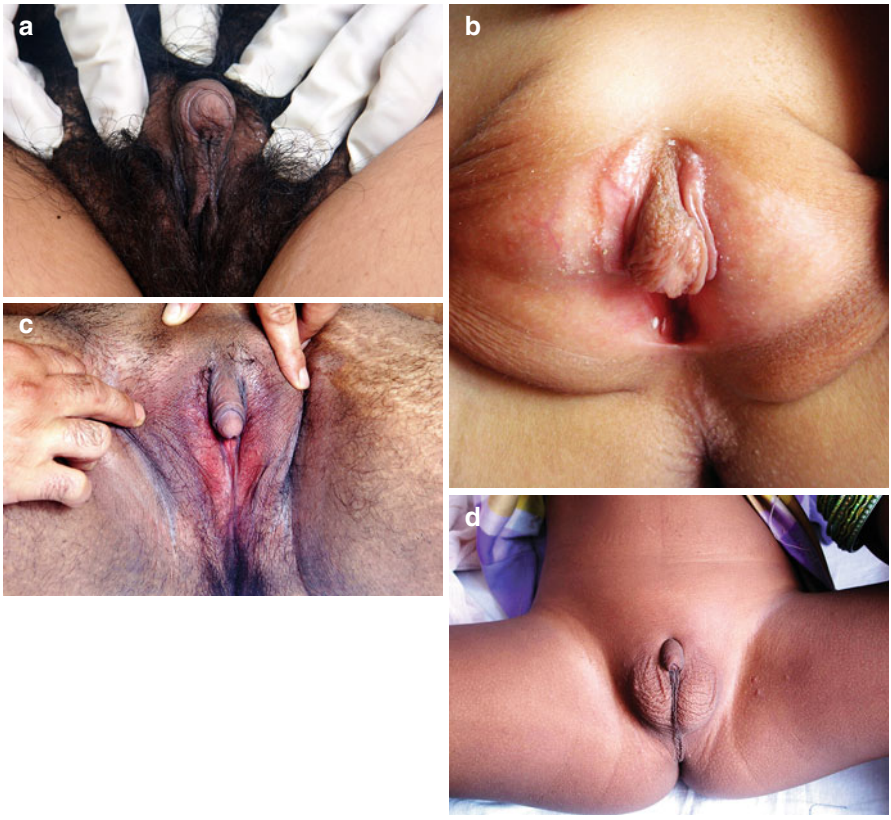

Fig. 10.4 Varying degree of labioscrotal fusion in children with CAH due to 21α-hydroxylase deficiency. (**a**) Isolated clitoromegaly. (**b**) Posterior labial fusion with clitoromegaly. (**c**) Labioscrotal fusion with penoscrotal hypospadias. (**d**) Complete labioscrotal fusion and apparently male genitalia with absent testes

18. *How to assess the degree of virilization in a 46,XX newborn*?

The degree of virilization in a 46,XX newborn can be assessed by using Prader staging, which is shown in the figure given below (Fig. 10.5).

Stage 1: Normal female genitalia with clitoromegaly
Stage 2: Partial posterior labial fusion with clitoromegaly
Stage 3: Labioscrotal fusion so that there is a single opening in the perineum, and clitoromegaly
Stage 4: Fusion of labioscrotal folds so that the single opening is at the base of the phallic structure
Stage 5: Appearance like a male external genitalia

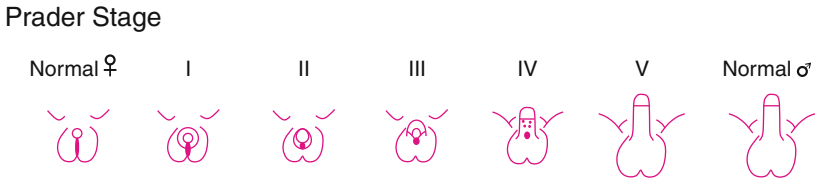

Fig. 10.5 Prader staging for virilization of external genitalia in a 46,XX newborn

19. *How to assess the posterior labial fusion*?

Posterior labial fusion is objectively assessed by the measurement of anogenital ratio, which is calculated by the distance between the anus and posterior fourchette divided by distance between the anus and base of phallus. If the ratio is >0.5, it suggests posterior labial fusion.

20. *What is the fate of the urogenital sinus*?

In a developing embryo, the primitive Wolffian and Mullerian ducts are attached to the cloaca. This is followed by differentiation of the cloaca into the urogenital sinus anteriorly (along with Wolffian and Mullerian ducts) and rectum posteriorly. In females, regression of Wolffian ducts occurs between 8 and 12 weeks; and the urogenital sinus starts differentiating into the lower part of the urinary bladder, urethra, and lower one-third of the vagina by 8–9 weeks; and two distinct openings, urethral and vaginal opening, are appreciable at perineum by 16–17 weeks. In males, regression of Mullerian ducts occurs between 7 and 11 weeks, and the urogenital sinus develops into the lower part of the urinary bladder, urethra, and prostate by 8–12 weeks (Fig. 10.6).

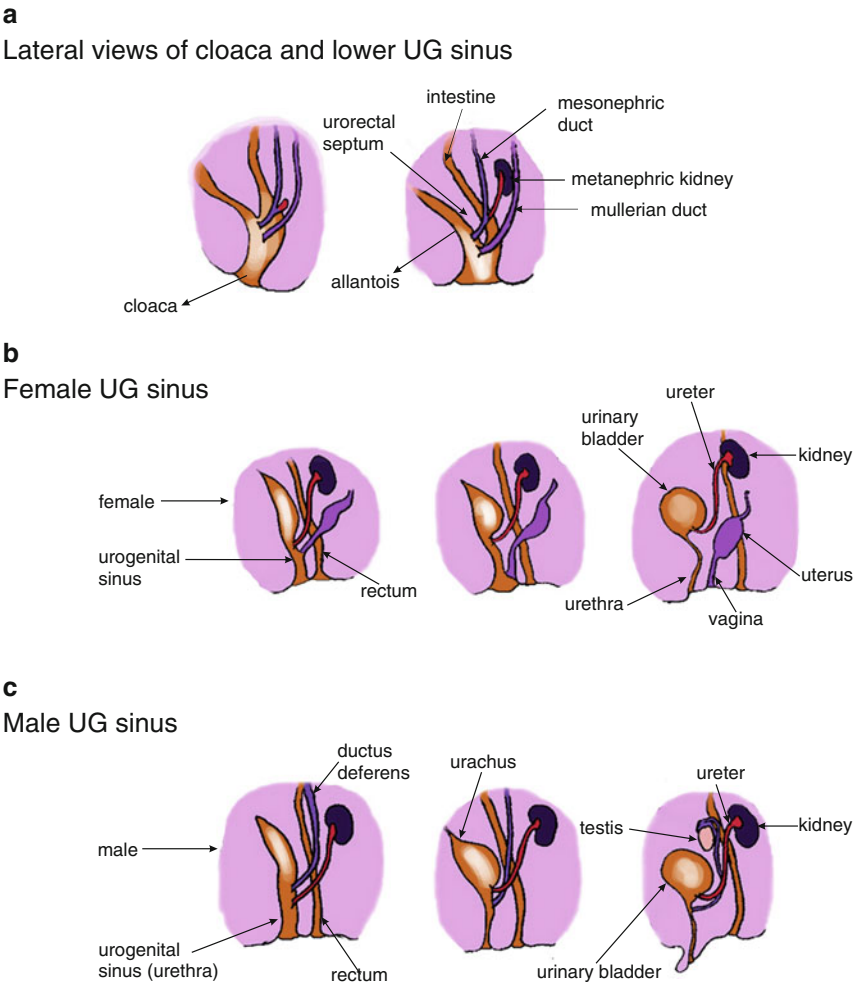

Fig. 10.6 (**a**) Lateral view of the cloaca. (**b**) Female urogenital sinus. (**c**) Male urogenital sinus

21. *What are the urogenital sinus abnormalities seen in a 46,XX newborn with CAH due to 21α-hydroxylase deficiency*?

In the absence of androgens, the urogenital sinus differentiates into urethra and lower one-third of the vagina during organogenesis; however, on exposure to circulating androgens, the urogenital sinus differentiates into prostate and urethra. In a female embryo, the urogenital sinus differentiates into urethra and vagina with two distinct openings at the perineum. However, in a female embryo with CAH, exposure to high levels of adrenal androgens during urogenital development (7–12 weeks of intrauterine life) prevents differentiation of the urogenital sinus into the urethra and lower one-third of the vagina and results in persistence of the urogenital sinus. This is clinically evident as single common opening for urethra and Mullerian derivatives in a patient with CAH. However, depending on the level and sensitivity to androgens, the site of Mullerian duct opening into the urogenital sinus can be located near the perineal surface (low vaginal confluence) or away from it (high vaginal confluence) (Fig. 10.7).

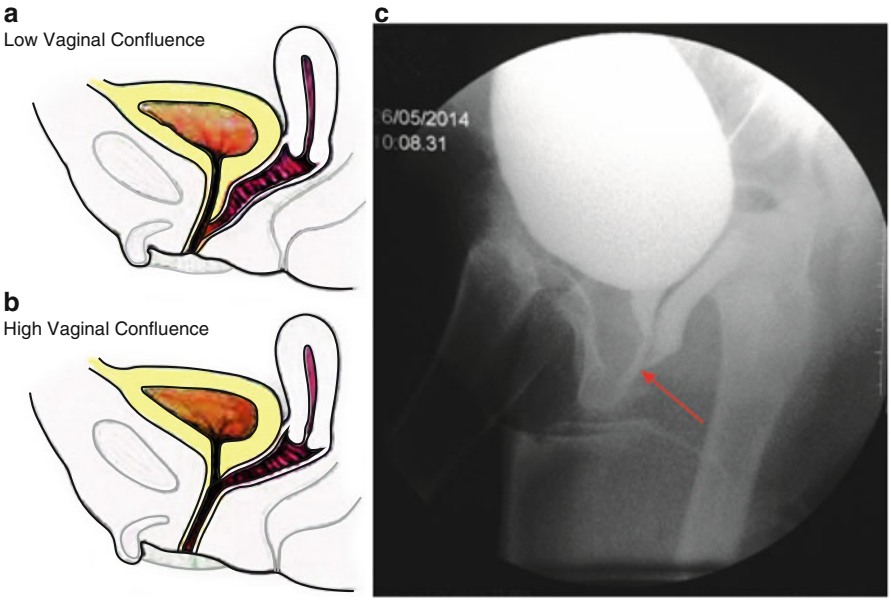

Fig. 10.7 (**a**, **b**) Urogenital sinus with low vaginal confluence and high vaginal confluence. (**c**) Sinogram of a patient with ovotesticular DSD showing low vaginal confluence (*red arrow*)

22. *Why is it important to differentiate between low- and high vaginal confluence*?

The differentiation between low- and high vaginal confluence can be made by performing either genitoscopy or urogenitogram. This is important in determining the nature and timing of genital reconstructive surgery. Children with low

vaginal confluence should undergo vaginoplasty and perineal reconstruction (with or without clitoroplasty) at an early age. The surgical reconstruction of high vaginal confluence is technically more challenging, and the optimal timing for surgery in children with high vaginal confluence is not defined.

23. *Why does a female fetus with CAH lack development of Wolffian structures despite exposure to high levels of androgens during period of genital differentiation*?

Differentiation of external genitalia to male phenotype depends on the exposure to circulating androgens (predominantly dihydrotestosterone), whereas differentiation of Wolffian structures is mediated by the paracrine action of androgens (predominantly testosterone) from the testes. In a female fetus with CAH, exposure to high levels of circulating adrenal androgens results in the differentiation of external genitalia to male phenotype during the period of genital differentiation. As the source of androgen excess in female fetus with CAH is adrenal and only paracrine action of testosterone from testes can result in stabilization of Wolffian structures; therefore, female fetus with CAH will not develop Wolffian structures.

24. *What are the features of post-natal virilization in a 46,XX child*?

Post-natal exposure to androgens in a girl child will result in clitoral enlargement, pubarche, acne, and deepening of voice. However, as the urogenital sinus has already completed differentiation, androgen exposure in the post-natal period does not result in genital virilization beyond Prader stage 1.

25. *Why pubarche does not occur during infancy despite prenatal androgen excess in CAH*?

CAH is characterized by a high level of androgens during intrauterine and post-natal period. This results in virilization of external genitalia in newborn girls and penile enlargement in newborn boys. However, pubic hair appears only after the age of 2–4 years. This is because priming of pilosebaceous unit (and hence pubarche) require prolonged and persistent exposure to androgens as compared to virilization of the urogenital sinus which require only short-term exposure.

26. *What are the manifestations of simple virilizing CAH due to 21α-hydroxylase deficiency*?

Girls with simple virilizing CAH due to 21α-hydroxylase deficiency manifest with genital ambiguity at birth, whereas affected boys present with premature pubarche and penile enlargement between 2–4 years of age. Patients with simple virilizing CAH do not manifest salt-wasting crisis as 1–2 % of residual 21α-hydroxylase activity is sufficient to maintain near-normal level of aldosterone. However, if the diagnosis is delayed, girls usually present at peripubertal

age with hirsutism, acne, primary amenorrhea, deepening of voice, and short stature, whereas boys present with short stature, premature appearance of facial hair, and testicular enlargement due to testicular adrenal rest tumors (TART).

27. *What are the manifestations of non-classical CAH*?

Females with non-classical CAH (NCCAH) due to 21α-hydroxylase deficiency may manifest with premature pubarche during childhood or with hirsutism (60 %), oligomenorrhoea (54 %), and cystic acne (33 %) during peripubertal period or with infertility in adulthood. However, many women with NCCAH are asymptomatic. Males with NCCAH may manifest with premature pubarche during childhood and, rarely, with asymmetrical testicular enlargement due to TART in adults.

28. *What are the differences between NCCAH and polycystic ovarian disease*?

The differences between NCCAH and polycystic ovarian disease are summarized in the table given below.

Parameters	**NCCAH**	**PCOD**
Prevalence	1 in 1,000	1 in 10–20
Family history	Usually present	May be present
Menstrual irregularity	54 %	80 %
Hirsutism	60 %	50–60 %
Cystic acne	More common	Less common
Phenotype	Lean	Commonly obese
Features of insulin resistance	Absent	Commonly present
Clitoromegaly	Uncommon	Rare
Stimulated 17(OH)P	10–100 ng/ml	<10 ng/ml
Ultrasonography of ovaries	Usually normal	>12 follicles of size 2–9 mm or ovarian volume >10 ml

29. *How to establish a diagnosis of CAH due to 21α-hydroxylase deficiency*?

Estimation of serum 17(OH)P is recommended for the diagnosis of CAH due to 21α-hydroxylase deficiency. A baseline serum 17(OH)P of >100 ng/ml confirms the diagnosis of classical CAH due to 21α-hydroxylase deficiency and does not require ACTH stimulation test. However, ACTH-stimulated 17(OH)P should be performed in those with a baseline serum 17(OH)P <100 ng/ml. A stimulated serum 17(OH)P >100 ng/ml confirms the diagnosis of classical CAH, a value between 10 and 100 ng/ml establishes the diagnosis of NCCAH, and a value <10 ng/ml suggests that the individual is either carrier or is not affected. Once the diagnosis of classical CAH is confirmed, estimation of plasma renin activity helps to differentiate simple virilizing and salt-wasting forms of CAH. Further, estimation of plasma renin activity also helps in monitoring of a child on therapy.

30. *In a newborn with ambiguous genitalia, what are the possibilities to be considered when serum 17(OH)P is only mildly elevated*?

In a newborn with ambiguous genitalia, if the stimulated serum 17(OH)P is mildly elevated (10–100 ng/ml), CAH due to causes other than 21α-hydroxylase deficiency should be considered. These include 11β-hydoxylase and cytochrome P450-oxidoreductase (POR) deficiency. The diagnosis of 11β-hydoxylase can be confirmed by the estimation of 11-deoxycortisol, and POR deficiency by estimation of serum/urinary steroids (pregnenolone, progesterone, and 17(OH)P metabolites) with gas chromatography/mass spectroscopy. Further, genetic analysis can be performed, if required.

31. *What are the causes of elevated serum 17(OH)P other than CAH*?

The disorders associated with increased 17(OH)P include polycystic ovarian disease, adrenocortical carcinoma, and the use of drugs like ketoconazole and spironolactone.

32. *What is the rationale of neonatal screening for CAH*?

The incidence of classical CAH is approximately 1 in 16,000–20,000. Newborn screening for CAH is recommended as the disease is potentially fatal and mortality rate due to salt-wasting is approximately 4–10 % in the unscreened population. Further, CAH can be easily diagnosed by a simple blood test [serum 17(OH)P], and early diagnosis and therapy improves outcome. The greatest benefit of neonatal screening is prevention of salt-wasting crises, especially in a male child who may otherwise be missed due to lack of genital ambiguity. This is evidenced by the female preponderance in studies performed prior to the introduction of neonatal screening program for CAH and advent of equal male to female ratio, thereafter. In addition, neonatal screening helps in early and appropriate gender assignment and allows timely initiation of therapy to prevent progression of virilization and morbidity associated with surgical intervention.

33. *How to perform neonatal screening for CAH*?

Neonatal screening for CAH is performed by estimation of 17(OH)P as a two-tier procedure; first-tier using immunoassay and second-tier using liquid chromatography-tandem mass spectrometry (LC-MS/MS) performed on dried blood spot on filter paper (Guthrie card). The first-line screening test should be performed on day 3 of life in a term newborn as serum 17(OH)P levels are usually elevated in neonates during first 2 days of life [neonatal surge of 17(OH)P] as well as in preterm newborn. If the first-line screening test demonstrates elevated 17(OH)P levels, second-tier test should be employed. Neonates with elevated 17(OH)P and symptoms or hyponatremia/hyperkalemia should be considered to have CAH and treated accordingly. However, those neonates with elevated 17(OH)P who are asymptomatic should be subjected to ACTH

stimulation (250 µg) test to confirm the diagnosis of CAH. The role of genetic analysis in neonatal screening is limited. A scheme for neonatal screening of CAH is depicted in the figure given below (Fig. 10.8).

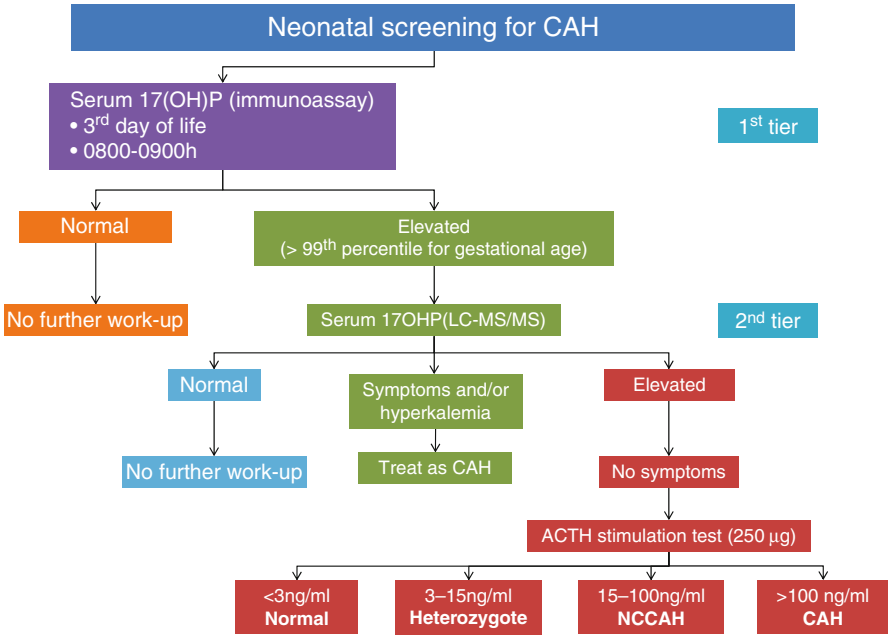

Fig. 10.8 Newborn screening for CAH

34. *What is the rationale of two-tier screening program for CAH*?

The incidence of classical CAH is 1 in 16,000–20,000. Although the cutoff of 17(OH)P in the first-tier screening provides a very high sensitivity (98.9 %) and specificity (99.6 %), the positive predictive value is very low (1 %). This means 99 out of 100 positive tests are false positive. Hence, to improve the positive predictive value, a second-tier test (LC-MS/MS) is employed. Estimation of 17(OH)P by LC-MS/MS is expensive, labor intensive, and time consuming; therefore, it is not preferred as a first-line screening test, when the test needs to be carried out on a large number of samples.

35. *What are the fallacies of estimation of 17(OH)P as a screening test for CAH*?

Serum 17(OH)P is elevated in the first 2 days of life as a result of physiological neonatal surge; hence, estimation of 17(OH)P is unreliable as a screening test,

if done within 48h of life. In addition, preterm babies and those with neonatal sepsis have elevated 17(OH)P, whereas newborns who have received prenatal corticosteroid treatment to induce lung maturation may have low 17(OH)P levels. Therefore, gestational age-specific 17(OH)P cutoffs should be used in preterm babies and those with sepsis or have received corticosteroids should be evaluated later. Estimation of 17(OH)P does not distinguish 21α-hydroxylase deficiency from 11β-hydroxylase deficiency. Finally, the first-tier screening uses immunoassays for 17(OH)P which has marked cross-reactivity with other steroid metabolites.

36. *What is the role of ACTH stimulation test in neonatal screening for CAH*?

It has been shown in various large scale neonatal screening programs for CAH that the positive predictive value (PPV) of first-tier screening test using 17(OH)P by immunoassay is 0.8–1.07 % and PPV of second-tier test using 17(OH)P by LC-MS/MS is 7.3 %. Therefore, confirmatory test for the diagnosis of CAH is required. This is accomplished by performing ACTH stimulation test (250 μg) which is considered as a gold standard for the diagnosis of CAH. However, ACTH stimulation test is not required in neonates with symptoms suggestive of CAH.

37. *How to improve the outcome of neonatal screening program for CAH*?

The outcome of neonatal screening program for CAH can be improved by estimation of other steroid metabolites by LC-MS/MS. A ratio of the sum of serum 17(OH)P and 21-deoxycortisol divided by cortisol has been shown to improve the PPV of second-tier test to 100 % when a cutoff of 0.53 was taken. 21-deoxycortisol is a metabolite derived from 17(OH)P by the action of enzyme 11β-hydroxylase, and 21-deoxycortisol levels are elevated in patients with 21α-hydroxylase deficiency.

38. *What is the role of genotyping in the neonatal screening for CAH*?

Genetic analysis has been suggested as a second-tier test in place of hormonal profiling by LC-MS/MS. This is because >90 % of children with CAH due to 21α-hydroxylase deficiency carry one of the ten common mutations of 21α-hydroxylase gene, and genotyping can be performed on the same dried blood spot used for the first-tier screening. However, the test is very expensive, requires expertise, and currently limited to detect CAH due to 21α-hydroxylase deficiency. Genetic analysis has not been validated as a second-tier test in comparison to hormone profiling by LC-MS/MS in large studies. Although a negative genotyping makes a diagnosis of CAH highly unlikely, a positive genotyping needs further confirmation by stimulated 17(OH)P estimation as the positive predictive value of genotyping is only 18 %.

39. *A couple has a child with classical CAH. What is the probability of having a child with classical CAH in the subsequent pregnancy*?

The couple had a child with classical CAH and both parents are apparently normal. This suggests that both parents are likely to be carriers. As CAH is an autosomal recessive disorder, the probability of having a baby with classical CAH in the subsequent pregnancy is one in four. Rarely, 1–2 % of patients with CAH may have spontaneous mutations, which are not present in either of the parents. Therefore, stimulated 17(OH)P and genotyping should be performed in both parents to predict the outcome (Fig. 10.9).

Fig. 10.9 Inheritance pattern of CAH in a couple who are carriers for the disease

40. *A woman with classical CAH is planning pregnancy. What is the probability of having a child with classical CAH*?

Since the woman has classical CAH, she has severe mutation in both alleles of the 21α-hydroxylase gene. If her partner is not harboring any mutations, then all their children will be carriers and none of them will develop CAH. Hence, the probability of having a child with classical CAH depends on the likelihood of her partner being a carrier for a gene for classical CAH. The frequency of carriers for classical CAH is 1 in 60 in general population, as disease frequency is 1:16,000. The probability of having a child with classical CAH when a woman has classical CAH and her partner is carrier for classical CAH is 50 % (one in two) as depicted in the figure given below. Therefore, the probability of having a child with classical CAH in the index patient is 1 in 120 (Fig. 10.10).

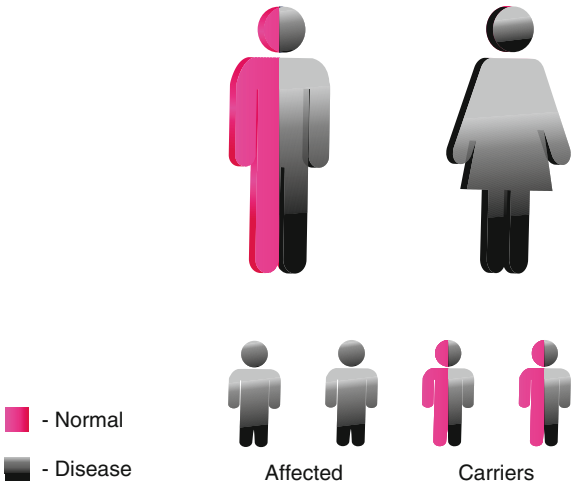

Fig. 10.10 Inheritance pattern of CAH in a couple where mother has classical CAH and father is a carrier

41. *A woman with classical CAH is planning pregnancy. What is the probability of having a child with non-classical CAH*?

    If a woman has classical CAH, the likelihood of having a child with non-classical CAH depends on the probability of the woman's partner being a carrier for a gene for non-classical CAH. The frequency of carrier for non-classical CAH is 1 in 16 in general population as disease frequency is 1:1,000. Therefore, the probability of having a child with non-classical CAH when a woman has classical CAH and her partner is carrier for gene for non-classical CAH is 50 % (one in two) as depicted in the figure given below. Therefore, the probability of having a child with non-classical CAH in the index patient is 1 in 32 (Fig. 10.11).

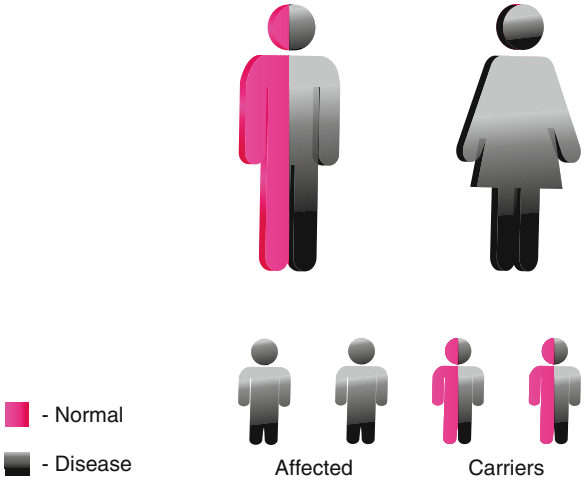

Fig. 10.11 Inheritance pattern of CAH in a couple where mother has classical CAH and father is carrier for NCCAH

42. *A woman with non-classical CAH is planning pregnancy. What is the probability of having a child with classical CAH*?

Non-classical CAH can be either due to the presence of *severe* and *mild* mutant alleles or two *mild* mutant alleles of 21α-hydroxylase gene. If the woman has non-classical CAH, the possibility of having a child with classical CAH arises only if she has NCCAH due to *severe* and *mild* mutant allele and her partner is a carrier for the gene for classical CAH. As approximately 75 % of patients with non-classical CAH have one *severe* and *mild* mutant allele, the probability of the index patient having one *severe* and *mild* mutant allele is three in four. The frequency of carrier for classical CAH is 1 in 60 in general population. The probability of having a child with classical CAH when a woman has non-classical CAH (with *severe* and *mild* mutant allele) and her partner is a carrier for classical CAH is 25 % (one in four) as shown in the Fig. 10.12. Therefore, the probability of having a child with classical CAH in the index patient is 1 in 320, i.e., (3:4) × (1:60) × (1:4).

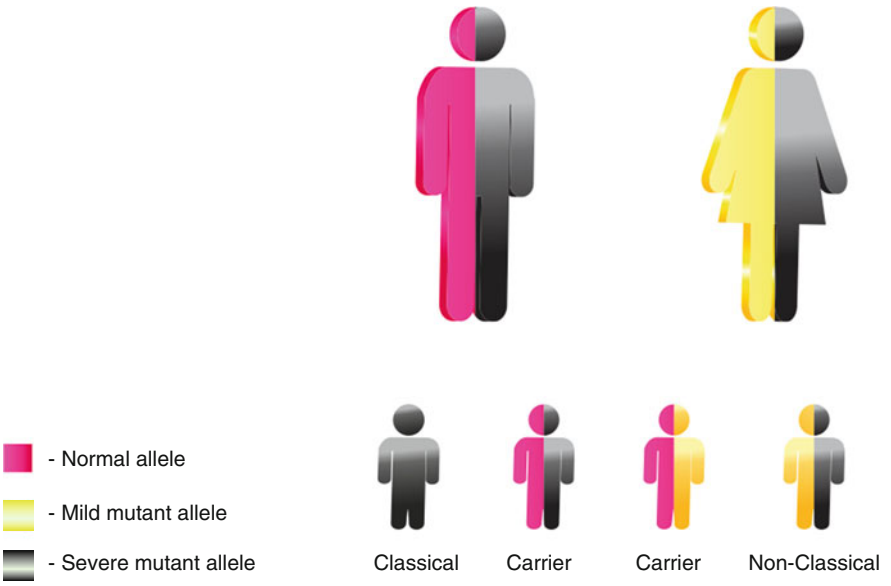

Fig. 10.12 Inheritance pattern of CAH in a couple where mother has non-classical CAH (with severe and mild mutant allele) and father is carrier for classical CAH

43. *A woman with non-classical CAH is planning pregnancy. What is the probability of having a child with non-classical CAH*?

If a woman has non-classical CAH, the likelihood of having a child with non-classical CAH depends on the genotype of the woman (whether she has *severe* and *mild* mutant alleles or two *mild* mutant alleles of 21α-hydroxylase gene) and partner (whether he is a carrier for gene for classical or non-classical CAH). The various possibilities in this scenario are given in the table below.

Female genotype	Male genotype	Risk of NCCAH
Severe and mild mutation	Severe and normal (carrier for classical CAH)	(1:60) × (1:4) = 1 in 240
	Mild and normal (carrier for NCCAH)	(1:16) × (1:2) = 1 in 32
Mild and mild mutation	Severe and normal (carrier for classical CAH)	(1:60) × (1:2) = 1 in 120
	Mild and normal (carrier for NCCAH)	(1:16) × (1:2) = 1 in 32

44. *When does cortisol biosynthesis start in intrauterine life*?

Although the fetal hypothalamic–pituitary–adrenocortical (HPA) axis is functional only by 15 weeks of intrauterine life, it has been shown that embryonic adrenal gland can synthesize cortisol as early as 8–9 weeks of intrauterine life. This is due to the expression of the enzyme 3β-HSD2, which is obligatory for cortisol biosynthesis. This early synthesis of cortisol is important because it suppresses ACTH production from the pituitary and, consequently, inhibits the production of adrenal androgens, thereby preventing virilization of external genitalia in a normal female fetus. The cortisol biosynthesis during this period is independent of CRH-ACTH axis; however, fetal adrenal androgen production during this period is ACTH dependent as evidenced by the presence of genital virilization in affected female fetuses with CAH. The expression of enzyme 3β-HSD2 in adrenal gland (and hence cortisol synthesis) declines after 12 weeks till about 24 weeks of intrauterine period. Therefore, cortisol levels in fetus during mid-gestation are approximately ten times lower than that found in maternal serum.

45. *What is the rationale behind prenatal therapy in CAH*?

The aims of prenatal therapy for CAH is to prevent the virilization of female fetus, reduce the need for genital reconstruction surgery, and minimize adverse psychological distress associated with genital ambiguity. This is accomplished by administering dexamethasone to the mother as soon as the pregnancy is confirmed. Dexamethasone is used for prenatal therapy as it is not metabolized by placental 11β-hydroxy steroid dehydrogenase type 2(11β-HSD2) and, hence, freely crosses placental barrier, whereas other glucocorticoids like prednisolone and hydrocortisone are readily metabolized by placental 11β-HSD2. Dexamethasone inhibits fetal hypothalamo–pituitary–adrenocortical axis and thereby suppresses fetal adrenal androgen production and consequently prevents fetal virilization.

46. *When to initiate dexamethasone as prenatal therapy for CAH*?

Treatment with dexamethasone should be initiated as soon as pregnancy is confirmed as exposure of female embryo to adrenal androgens between 7 and 12 weeks of intrauterine life results in virilization of the urogenital sinus and consequent genital ambiguity. Dexamethasone is administered at a dose of 20 μg/Kg/day in divided doses with a maximum dose of 1.5 mg/day, and the preconceptional weight is used for calculation of the dose. After genetic analysis by chorionic villous sampling at 10–12 weeks, the need for further continuation of dexamethasone can be reconsidered. In a male fetus and unaffected female fetus, it is discontinued, while in an affected female fetus, dexamethasone is continued. Maternal urinary estriol may be used to monitor adequacy of therapy (DHEA and DHEAS are converted to estriol in fetal zone of adrenal cortex). Estimation of 17(OH)P is not useful for prenatal diagnosis or monitoring of therapy.

47. *Why is prenatal therapy for CAH not recommended*?

In a woman with a child having CAH, the probability of having another child with CAH in the subsequent pregnancy is one in four (and that of having a female child with CAH is one in eight) as CAH is an autosomal recessive disorder. Hence, to prevent genital ambiguity in one female child, seven children need to be treated unnecessarily. The efficacy of prenatal dexamethasone therapy is only 80–85 %. In addition, prenatal dexamethasone therapy is associated with adverse fetal and maternal outcomes. Prenatal therapy results in exposure of fetus to very high levels of glucocorticoids (approximately 60 times greater than levels in a normal fetus). The fetal risks associated with dexamethasone therapy include intrauterine growth retardation, orofacial congenital malformations, impaired verbal working memory in childhood, and, possibly, future risk of cardiovascular events. The maternal risks include weight gain, edema, and, possibly, higher incidence of hypertension and hyperglycemia. Hence, prenatal dexamethasone therapy is still considered as experimental.

48. *How to treat a child with classical CAH due to 21α-hydroxylase deficiency*?

Children with classical CAH due to 21α-hydroxylase deficiency should be treated with hydrocortisone at doses of 10–15 mg/m^2/day in three divided doses and fludrocortisone at doses of 100–200 μg/m^2/day. In addition, oral salt (1–2 g/day) should also be administered during early infancy. High-dose glucocorticoids should be administered in these children during periods of stress like infection, trauma, or surgery. Genital reconstructive surgery should be considered at an appropriate age based on the severity of ambiguity.

49. *Why is hydrocortisone preferred over other glucocorticoids in the management of CAH in children*?

Hydrocortisone is preferred over other glucocorticoids like prednisolone and dexamethasone as it is physiological, has short duration of action, and exerts minimal detrimental effects on epiphyseal growth plate. It has been shown that at equivalent doses (in terms of glucocorticoid activity), prednisolone has 10–15-fold higher and dexamethasone 70–80-fold higher growth inhibitory effects, as compared to hydrocortisone. However, if the dose of hydrocortisone exceeds 20 mg/m^2/day in infants and 15–17 mg/m^2/day in adolescents, it may also exert growth-suppressive effects.

50. *What is the rationale of mineralocorticoid therapy in addition to glucocorticoids in patients with simple virilizing CAH due to 21α-hydroxylase deficiency*?

Patients with simple virilizing CAH due to 21α-hydroxylase deficiency have 1–5 % residual enzymatic activity, which is sufficient to prevent salt-wasting crisis. However, these patients have subclinical aldosterone deficiency as evidenced by elevated plasma renin activity (PRA) and predisposition for salt crisis during stress. Subclinical aldosterone deficiency results in chronic depletion of sodium and extracellular fluid volume. This results in activation of renin–angiotensin axis and consequently increased angiotensin II levels, leading to increased secretion of ACTH from the pituitary gland via increasing the secretion of CRH as well as ADH. Angiotensin II is also a potent adrenocortical growth promoter. In addition, the chronic extracellular volume depletion also results in increased ADH secretion and further promotes ACTH drive to adrenal gland. This increased ACTH drive mandates a higher dose of glucocorticoids to normalize adrenal androgens. Hence, therapy with fludrocortisone results in lowering of glucocorticoid doses and improves growth potential (Fig. 10.13).

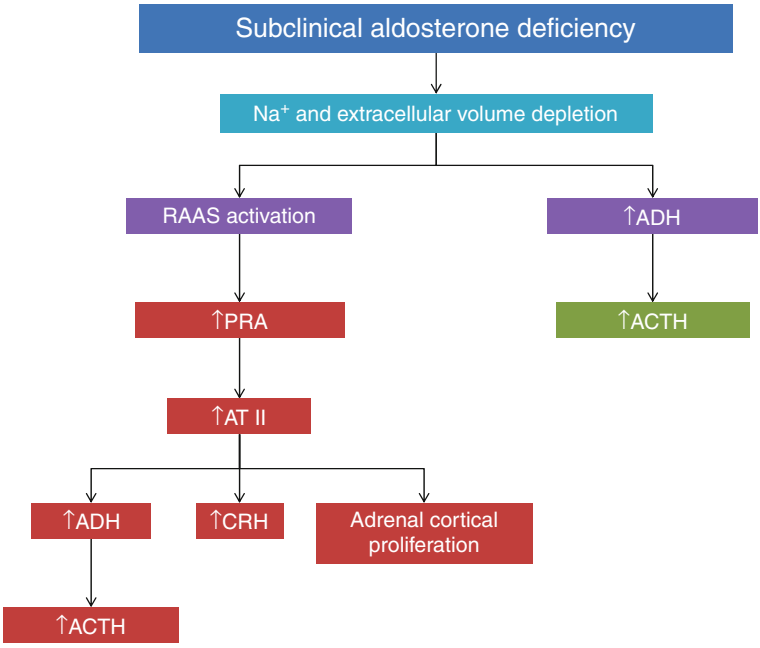

Fig. 10.13 Consequences of subclinical aldosterone deficiency in a child with simple virilizing CAH

51. How to monitor a child with CAH due to 21α-hydroxylase deficiency?

The clinical parameters to be monitored in a child with CAH include growth velocity, weight, regression/progression of features of virilization (clitoromegaly, pubic hair, and acne), blood pressure, and stigma of glucocorticoid excess at three monthly intervals. In addition, bone age should be assessed annually after 2 years of age. Growth velocity is a sensitive parameter for assessment of adequacy of therapy as decreased growth velocity suggests overtreatment, while increased growth velocity suggests undertreatment. Biochemical monitoring includes serum 17(OH)P, androstenedione, testosterone, plasma renin activity (PRA), and serum potassium and sodium. Sample for biochemical evaluation should be taken in the morning between 0800 and 0900h without discontinuing the morning dose of hydrocortisone. Serum 17(OH)P should be targeted between 4 and 12 ng/ml as an attempt to normalize serum 17(OH)P results in overtreatment with glucocorticoids. Androstenedione and testosterone should be maintained in age- and gender-specific normal range. PRA and potassium should be monitored as the requirement of fludrocortisone decreases with advancing age, and the levels are to be maintained within the reference range.

52. *What is the rationale for the estimation of androstenedione and testosterone in children with CAH due to 21α-hydroxylase deficiency*?

Although estimation of serum 17(OH)P is a sensitive test for the diagnosis of CAH due to 21α-hydroxylase deficiency, it is a suboptimal parameter for monitoring the efficacy of therapy when used alone. This is because serum 17(OH)P levels has only modest correlation with circulating androstenedione and testosterone as circulating levels of 17(OH)P are 100–1,000 times higher in patients with CAH as compared to normal individuals. In addition, serum 17(OH)P levels show high variability as compared to androstenedione and testosterone. Hence, estimation of androstenedione and testosterone should be used in conjunction with serum 17(OH)P for monitoring therapy of children with CAH. Normalization of androstenedione and testosterone is a biochemical marker of optimal therapy, whereas normalization of 17(OH)P suggests overtreatment. Estimation of DHEA or DHEAS is not recommended as they are easily suppressible with glucocorticoids and the levels poorly correlate with circulating 17(OH)P.

53. *How to monitor an adult with CAH due to 21α-hydroxylase deficiency*?

In children with CAH, prevention of salt-wasting crisis, optimization of linear growth, and attainment of final adult height are the major concerns, whereas in adults, achievement of fertility, prevention of metabolic syndrome, and osteoporosis are important issues. The clinical parameters to be monitored in an adult with CAH include blood pressure, signs of glucocorticoid excess, and regression/progression of virilization (in females). Biochemical monitoring includes estimation of serum 17(OH)P, testosterone, androstenedione, plasma renin activity, and serum potassium and sodium. However, serum testosterone has limited utility in men as it is predominantly produced from the testes. Serum 17(OH)P should be targeted between 4 and 12 ng/ml, whereas androstenedione and testosterone (the later in females) should be maintained in the age- and gender-specific normal range. In addition, testicular ultrasonography, measurement of gonadotropins, and semen analysis in men and serum progesterone in women should be performed while planning fertility. Beside this, periodic monitoring with fasting plasma glucose, lipid profile, and DXA should also be performed in adults with CAH.

54. *What are the causes of increase in serum 17(OH)P in a child with CAH due to 21α-hydroxylase deficiency who was previously well controlled*?

Compliance to therapy must be ensured prior to further investigation in a child with CAH who was previously well controlled. The most common cause of increase in 17(OH)P in a child on therapy is onset of puberty. In addition, development of adrenal rest tumors in gonads and evolution of ACTH-independent adrenal nodular hyperplasia may also result in increase in serum 17(OH)P levels.

55. *What are the causes of testicular enlargement in a boy with CAH due to 21α-hydroxylase deficiency*?

Bilateral symmetrical testicular enlargement in a boy with CAH due 21α-hydroxylase deficiency suggests reactivation of hypothalamo–pituitary–testicular axis (either as a part of GDPP due to delayed initiation of therapy or normal puberty), whereas unilateral/asymmetrical testicular enlargement suggests the development of testicular adrenal rest tumor.

56. *What is testicular adrenal rest tumor*?

In a developing embryo, adrenogonadal primordium differentiates into adrenal and gonadal tissue. During differentiation, few adrenal cells are admixed with gonadal tissue and are termed as "adrenal rest cells." Despite ectopic location, these cells retain the characteristics of adrenal tissue including responsiveness to ACTH. Therefore, in the presence of ACTH excess these adrenal rest cells may proliferate leading to adrenal rest cells tumor in testes (TART) and ovary (OART). Although CAH is the most common cause of TART, it may be seen in any disorder associated with ACTH excess, e.g., primary adrenal insufficiency, ACTH-dependent Cushing's syndrome and Nelson's syndrome (Fig. 10.14).

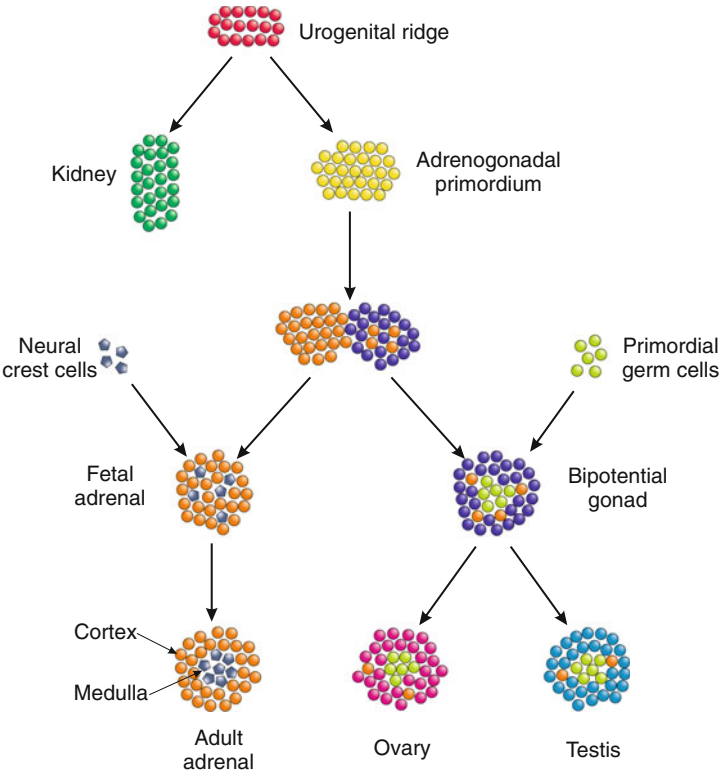

Fig. 10.14 Embryogenesis of adrenals, gonads and adrenal rest cells in gonads

57. *What are the characteristics of TART associated with CAH due to 21α-hydroxylase deficiency*?

TART are benign unencapsulated tumors located within rete testis. There is high variability in the reported prevalence of TART (0–94 %) in CAH, possibly because of varying degree of disease control among the selected population and the method used for the detection of tumor. These tumors are usually not palpable as they are small and deep-seated in rete testis, but are palpable when the size exceeds >2 cm. Ultrasonography (USG) is a good modality as it can detect adrenal rest tumors of even 2 mm size. TART is visualized as hypoechoic lesion on USG (stage 2 onwards) and are typically multiple, bilateral, and eccentrically located within the rete testis. As ultrasound is a highly sensitive imaging tool for the identification of TART, other imaging modalities like MRI do not provide any additional information.

58. *How to stage TART*?

The different stages of TART are summarized in the table given below (Claahsen Vander Grinten classification).

Stage	Histology	Comments
Stage 1	Adrenal rest cell in rete testis	Identified only by histopathology
Stage 2	Hypertrophy and hyperplasia of adrenal rest cells	Can be identified by USG
		Excellent response to glucocorticoids
Stage 3	Further cell growth and compression of rete testis	Moderate response to glucocorticoids
		May require surgery for fertility
Stage 4	Fibrosis and focal lymphocytic infiltrates	Require surgery for fertility
Stage 5	Parts of the tumor are replaced by adipose tissue	Irreversible testicular damage

59. *What are the consequences of TART in a patient with CAH*?

The most important long-term consequence of TART is infertility. This occurs due to obstructive azoospermia resulting from compression of efferent ductules from seminiferous tubule by the adrenal rest tumor located in rete testes. Long-standing disease leads to peritubular fibrosis and irreversible testicular damage, including impaired Leydig cell function. Intensive therapy with glucocorticoids usually leads to the regression of TART in stage 2 and 3, whereas surgical intervention may be beneficial in stage 4 (Fig. 10.15).

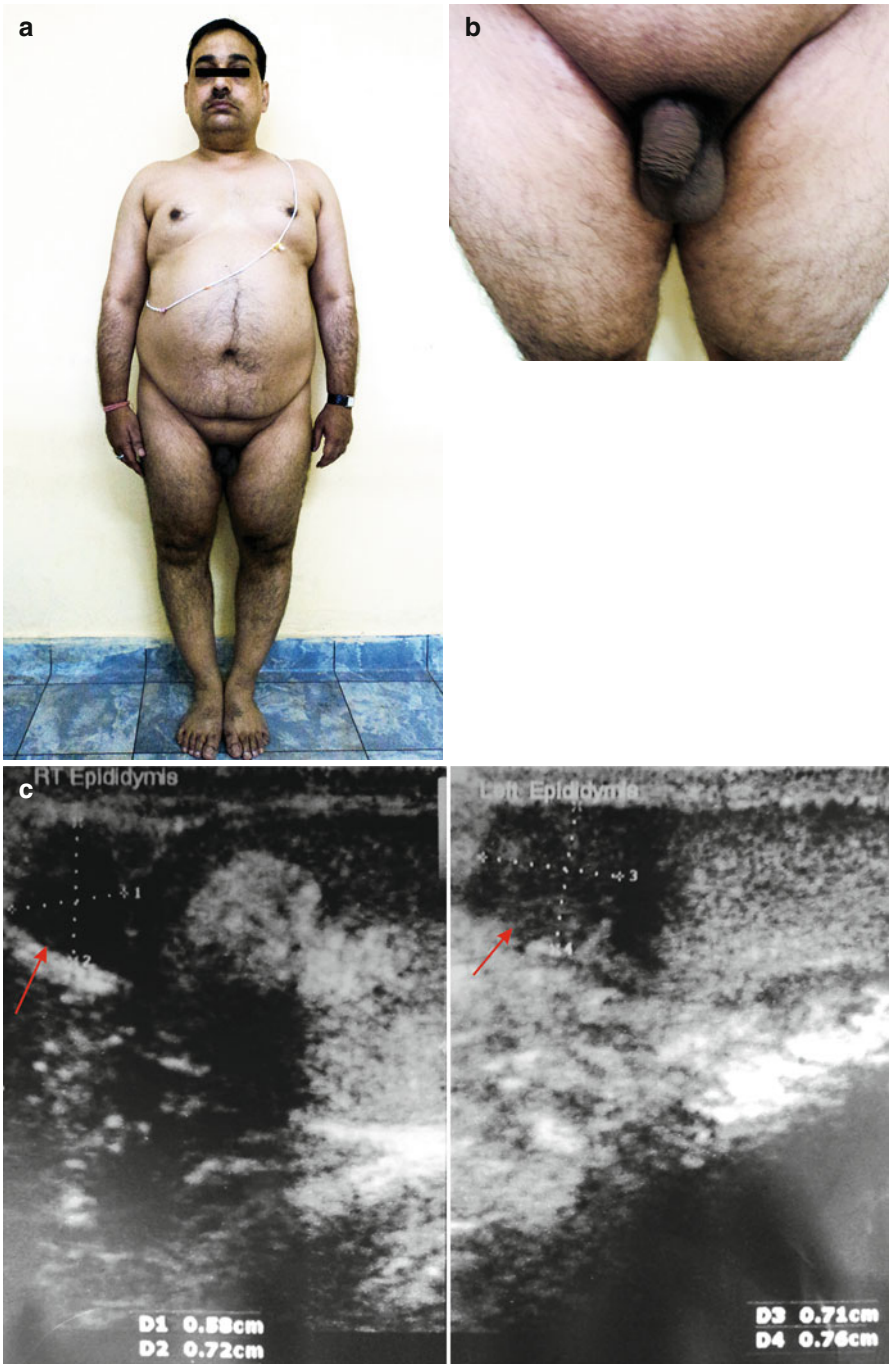

Fig. 10.15 A 35-year-old male presented with primary infertility. (**a**) He was short and had history of early pubarche and was diagnosed to have CAH due to 21α-hydroxylase deficiency. (**b**) Testicular volume was 15 ml bilaterally, and (**c**) ultrasonography showed hypoechoic areas (*red arrows*) suggestive of TART

60. *How to differentiate between TART and Leydig cell tumor*?

TART and Leydig cell tumor mimics each other, and failure to consider a diagnosis of TART in a male with CAH having testicular mass may result in inadvertent orchidectomy. The important differences between these disorders are summarized in the table given below.

Parameters	TART	Leydig cell tumor
Presentation	Infertility	Gynecomastia
Cell of origin	Adrenal rest cell located in testes	Leydig cells
Laterality	Bilateral >80 %	Bilateral in only 3 %
Reinke crystalloids	Absent	Present in 35–40 %
Malignant potential	Absent	Present (10 %)
Treatment	Glucocorticoids in initial stages	Surgery
	Surgery in later stages	

61. *What are the causes of decreased growth velocity in a child with CAH on treatment*?

Untreated children with CAH initially have accelerated growth velocity and subsequently premature epiphyseal closure results in short final adult height. Optimal therapy with glucocorticoids results in suppression of ACTH-mediated adrenal androgen production and, consequently, normalization of growth velocity. The most common cause of reduced growth velocity in a child with CAH on therapy is overzealous treatment with glucocorticoids. This commonly occurs when therapy is aimed to maintain serum 17(OH)P in the normal range rather than in the recommended range (4–12 ng/ml). This is because the dose of glucocorticoids required to maintain serum 17(OH)P in the recommended range is lower than the dose which have adverse effects on epiphyseal growth plate. In addition, aggressive treatment with GnRH analogue in patients of CAH with GDPP can also result in suboptimal growth velocity. Further, children with CAH with markedly advanced bone age at presentation also have decreased growth velocity despite optimal treatment.

62. *How to optimize the growth potential in a child with classical CAH*?

To maximize final adult height in a child with classical CAH, optimal therapy with glucocorticoids and mineralocorticoids should be ensured targeting 17(OH)P, androstenedione, and testosterone in the defined range. However, even with optimal therapy, final adult height is compromised by −1 to −2SD as these children experience phases of hyperandrogenemia and glucocorticoid excess. The various strategies which have been tried to promote height potential in these children include the use of recombinant growth hormone (rhGH), the combined use of GnRH agonists with rhGH, or a combination of flutamide and testolac-

tone. The rationale of rhGH therapy in these individuals is to counteract the effects of glucocorticoids on linear growth. Therapy with rhGH and GnRH agonists or rhGH alone has been shown to increase height by 1 SDS (7 cm). In a small study, it has also been shown that combination therapy with flutamide and testolactone for 2 years resulted in restoration of normal growth velocity and deceleration in bone maturation. However, therapy with these agents is considered experimental as limited data is available regarding their efficacy and safety and may be considered if the predicted adult height of a child is ≤−2.25 SD.

63. *When to suspect the onset of GDPP in a child with CAH on therapy with glucocorticoids*?

Onset of thelarche in girls and testicular enlargement in boys are the clinical clues to suspect the onset of GDPP after initiation of glucocorticoid therapy for CAH. Those children with CAH who have advanced bone age (>11–12 years) at initiation of therapy are at a higher risk for the development of GDPP, as the advanced bone age is a surrogate evidence of maturation of hypothalamo–pituitary–gonadal axis. The onset of GDPP can be confirmed by estimation of basal LH (≥0.3 μIU/ml) and LH response to GnRH (≥5–8 μIU/ml). Timely recognition of GDPP is important because untreated children with GDPP have compromised final adult height. In addition, psychosocial distress associated with progression of puberty can also be prevented.

64. *Why does gonadotropin-dependent precocious puberty occur in children with CAH*?

The onset of gonadotropin-dependent precocious puberty in children with CAH commonly occurs after the initiation of therapy, especially in those where therapy was delayed. This is because abrupt lowering of persistently elevated sex steroids after initiation of therapy (for long-standing untreated CAH) results in premature reactivation of a primed HPG-axis.

65. *What are the causes of reduced growth velocity in a child with CAH who developed GDPP and is on therapy with glucocorticoids and GnRH agonists*?

In this scenario, the reduced growth velocity can be due to either overtreatment with glucocorticoids or GnRH agonists. Estimation of serum 17(OH)P, androstenedione, testosterone/estradiol, and LH helps in differentiating between the two. A serum 17(OH)P level below the recommended range suggests overtreatment with glucocorticoids. In a child with reduced growth velocity while on therapy with glucocorticoids and GnRH agonists, suppressed testosterone/estradiol and LH, while 17(OH)P in the recommended

range suggests overtreatment with GnRH agonists. This is because prepubertal gonads produce minor quantities of gonadal steroids, which promotes GH-dependent IGF1 generation. In addition, coexisting disorders like hypothyroidism and celiac disease should also be considered.

66. *Are there any alterations in adrenomedullary function in children with CAH due to 21α-hydroxylase deficiency*?

Although the embryological origin of adrenal cortex (mesonephros) and medulla (neural crest) are different, paracrine actions of cortisol is essential for normal development and function of adrenal medulla. Cortisol not only maintains the integrity of chromaffin cells in adrenal medulla but also potentiates the activity of the enzyme phenyl-N-methyl transferase, which catalyzes the conversion of norepinephrine to epinephrine. Exogenous glucocorticoid administration does not improve the adrenomedullary dysfunction as high intra-adrenal levels of cortisol are required for normal adrenomedullary function, and this cannot be achieved with exogenous glucocorticoid therapy. The clinical implication of adrenomedullary dysfunction in patients with CAH due to 21α-hydroxylase deficiency include propensity to develop hypoglycemia and severe hypotension.

67. *What are the medical options available in difficult to manage CAH*?

In a child with difficult to treat CAH, various therapeutic strategies have been employed. These include administration of higher doses of hydrocortisone (15–20 mg/m^2/day), higher dose of hydrocortisone at night (reverse circadian regimen), and combination of hydrocortisone along with prednisolone and/or dexamethasone (basal–bolus). The use of supraphysiological doses of hydrocortisone results in adverse effects particularly reduced growth velocity, insulin resistance, and osteoporosis. Administration of hydrocortisone in reverse circadian regimen has not yielded any beneficial effects. Combination of hydrocortisone (in divided doses) and prednisolone/dexamethasone (at bedtime) has been tried; however, limited data is available regarding the efficacy and safety of these regimens. Slow-release preparations of hydrocortisone (e.g., Chronocort) and continuous subcutaneous hydrocortisone infusion (CSHI) via pump have been tried in children with difficult to control CAH and shown to be effective. Abiraterone acetate is an oral 17α-hydroxylase inhibitor which has been shown to be effective in reducing the androgens levels, when added to the glucocorticoid regimen. In addition, its use is also associated with reduction in doses of glucocorticoids. There is limited data regarding the use of antiandrogens like flutamide (androgen receptor antagonist) or finasteride (5α-reductase inhibitor) in patients with CAH.

68. *What is the role of bilateral adrenalectomy in patients with CAH*?

Bilateral adrenalectomy is a therapeutic option in patients with CAH who are difficult to manage despite optimal medical therapy. Patients with severe virilization and infertility and those who experience adverse effects on glucocorticoid therapy are candidates for bilateral adrenalectomy. The beneficial effects of bilateral adrenalectomy include reduction in glucocorticoid doses, regression of features of virilization, improved quality of life, and easier monitoring. There is reduction in doses of glucocorticoid as aim of therapy after bilateral adrenalectomy is to provide replacement doses of glucocorticoids rather than suppression of ACTH. Regression of features of virilization and improvement in fertility outcome occur as the source of adrenal androgens is eliminated. However, patients who are subjected to bilateral adrenalectomy are at increased risk for adrenal crisis and development of adrenal rest tumors. The reappearance of virilization in a female or the presence of asymmetric testicular enlargement in a male after bilateral adrenalectomy suggests the development of adrenal rest tumors in the ovary (OART), or in the testis (TART), respectively.

69. *What are the long-term risks associated with untreated simple virilizing CAH*?

The long-term risks associated with untreated simple virilizing CAH include short final adult height, infertility, virilization and secondary polycystic ovarian disease, bilateral adrenal nodular hyperplasia, and testicular/ovarian adrenal rest tumors. Rarely, adrenocortical carcinoma, adrenal myelolipoma, and corticotroph hyperplasia have been reported in these patients (Fig. 10.16).

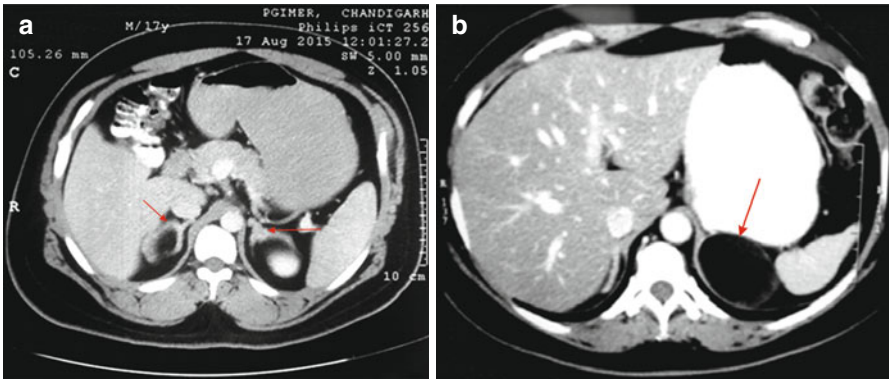

Fig. 10.16 CT abdomen showing (**a**) bilateral adrenal hyperplasia (*red arrows*) in a child with classical CAH due to 21α-hydroxylase deficiency. (**b**) Left adrenal myelolipoma (*red arrow*) in another patient with CAH due to 21α-hydroxylase deficiency

70. *When to treat a patient with non-classical CAH due to 21α-hydroxylase deficiency*?

Children with NCCAH having early pubarche and advanced bone age (>2.5 SD over chronological age) should be treated with glucocorticoids. However, therapy should only be continued till the peripubertal age to avoid the untoward consequences of glucocorticoids. Adolescent females with NCCAH who have hirsutism/acne and menstrual irregularity may require short-term glucocorticoid treatment. However, therapy with oral contraceptives and/or antiandrogens are equally/more effective. Adult women with NCCAH who have menstrual irregularities and features of virilization can be treated with oral contraceptives and/or antiandrogens. However, if fertility is a concern, short-term therapy with glucocorticoids is indicated to suppress adrenal androgens as well as to target serum progesterone to <0.6 ng/ml. The preferred glucocorticoid in this scenario is dexamethasone administered at bedtime (0.25 mg to 0.5 mg/day). Males with NCCAH who have TART and/or infertility should be treated with glucocorticoids.

71. *What are the causes of infertility in CAH*?

Women with CAH have impaired fertility due to chronic anovulation as a consequence of hyperandrogenemia, secondary polycystic ovarian disease, and high levels of circulating progesterone. In addition, inadequate introitus, hostile cervical mucus (as consequence of progesterone excess), and reduced sexual activity also contribute. In men, infertility may be due to suppression of gonadotropins as a result of elevated adrenal androgens or due to the presence of testicular adrenal rest tumors.

72. *What are the hypertensive variants of CAH*?

21α-hydroxylase deficiency is the most common cause of CAH and is associated with salt-wasting crisis in approximately three-fourth of patients with classical CAH. However, CAH due to 11β-hydoxylase and 17α-hydroxylase enzyme deficiencies are associated with hypertension. Deficiency of the enzyme 11β-hydoxylase results in accumulation of deoxycorticosterone acetate (DOCA). Although DOCA is a weak mineralocorticoid, high concentration of DOCA in patients with 11β-hydoxylase deficiency results in hypertension and hypokalemia. Deficiency of the enzyme 17α-hydroxylase results in hypertension and hypokalemia due to diversion of precursor metabolites (pregnenolone and progesterone) to mineralocorticoid synthetic pathway, resulting in higher levels of DOCA. However, plasma aldosterone level is low, due to DOCA-mediated inhibition of RAAS and consequent decrease in angiotensin II which downregulates aldosterone synthase (CYP11B2). Finally, overtreatment with glucocorticoids and mineralocorticoids is also a common cause of hypertension in patients with CAH.

73. *What are the characteristic features of CAH due to 11β-hydoxylase deficiency*?

11β-hydoxylase deficiency is the second most common cause of CAH with an incidence of 1 in 100,000. Girls with 11β-hydoxylase deficiency manifest with genital ambiguity at birth without salt-wasting crisis. Hypertension, the cardinal differentiating feature of 11β-hydoxylase deficiency from 21α-hydroxylase deficiency, is not present in the neonatal period. It usually manifests between 3 and 4 years of age as distal renal tubules are resistant to the action of mineralocorticoids in early childhood. Boys with 11β-hydoxylase deficiency present at 2–4 years of age with premature pubarche, penile enlargement (GIPP), and hypertension. The levels of serum 17(OH)P are elevated; however, the 17(OH)P values are much lower than those found in classical CAH due to 21α-hydroxylase deficiency. The diagnosis of 11β-hydoxylase deficiency can be confirmed by the estimation of 11-deoxycortisol, which is invariably elevated, and by genetic analysis (Fig. 10.17).

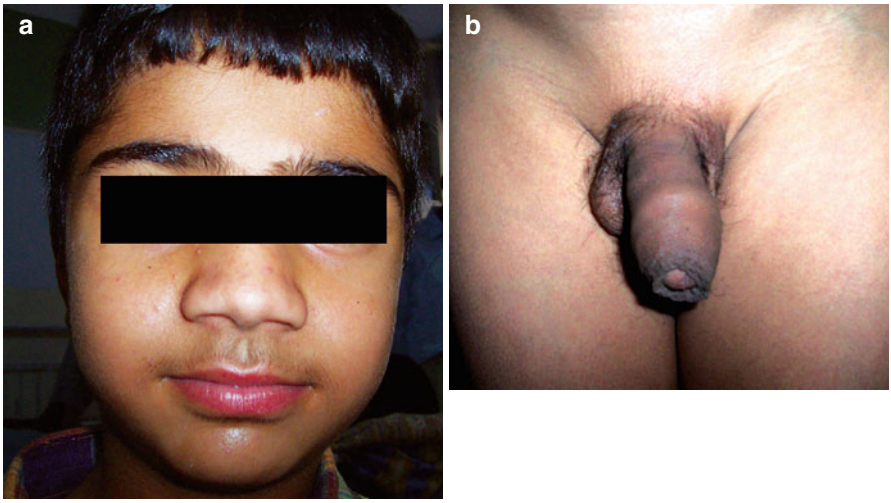

Fig. 10.17 (**a**) Acne and upper lip hair in a 4-year-old boy with CAH due to 11β-hydoxylase deficiency. (**b**) Pubic hair Tanner stage P2, testicular volume 2 ml with disproportionate penile enlargement suggestive of gonadotropin-independent precocious puberty. Child had hypertension which responded to glucocorticoids

74. *Can patients with 11β-hydoxylase deficiency have salt-wasting crisis*?

Although 11β-hydoxylase deficiency is characteristically associated with hypertension, some children with 11β-hydoxylase deficiency may have salt-wasting crisis during infancy. The exact mechanism remains elusive; however, the proposed mechanisms include accumulation of natriuretic metabolites of pregnenolone like 16-hydroxy pregnenolone (which counteract the mineralocorticoid effect of DOCA) and end-organ resistance to mineralocorticoids. Interestingly, salt-wasting crisis can occur after initiation of glucocorticoid

therapy, even in those who have hypertension prior to therapy. This paradox occurs because after initiation of glucocorticoid therapy, there is inhibition of ACTH drive for adrenal steroidogenesis, which results in nonavailability of precursor metabolites required for mineralocorticoid synthesis. Therefore, after initiation of glucocorticoids, some children may experience fall in blood pressure and require addition of fludrocortisone.

75. *A 17-year-old tall girl presented with primary amenorrhea, hypertension, and absent secondary sexual characteristics. What is the likely diagnosis*?

The presence of hypertension in an adolescent girl without any sign of pubertal development should raise the suspicion of CAH due to 17α-hydroxylase deficiency (CYP17A1). Deficiency of 17α-hydroxylase enzyme results in hypertension and hypokalemia due to diversion of precursor metabolites to mineralocorticoid synthetic pathway and accumulation of deoxycorticosterone (DOCA) and corticosterone. Primary amenorrhea and absent pubertal development is due to impaired estradiol synthesis in the ovary, as the enzyme is expressed both in the adrenal and gonads. Patients with 17α-hydroxylase deficiency do not manifest adrenal insufficiency, as corticosterone has modest glucocorticoid activity. The disorder is characterized by hypokalemia, decreased plasma renin activity and aldosterone, and elevated FSH and LH with low estradiol levels (hypergonadotropic hypogonadism). The diagnosis is confirmed by estimation of pregnenolone, progesterone, deoxycorticosterone, and corticosterone which are invariably elevated, or by genetic analysis. Boys with CAH due to 17α-hydroxylase deficiency present with hypertension and complete female external genitalia or rarely, with genital ambiguity depending on the residual enzyme activity.

76. *What are the variants of CAH associated with genital ambiguity in a boy*?

The variants of CAH associated with genital ambiguity in a boy include 17α-hydroxylase deficiency and 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2) deficiency, as these enzymes are expressed in both testes and adrenals. These disorders can be differentiated by the presence of hypertension and cryptorchidism (usually intra-abdominal) in 17α-hydroxylase deficiency and the presence of salt-wasting crisis with normally located testes in 3β-HSD2 deficiency. In addition, boys with CAH due to cytochrome P450 oxidoreductase (POR) deficiency also have genital ambiguity.

77. *What are the characteristic features of CAH due to cytochrome P450 oxidoreductase* (*POR*) *deficiency*?

The POR protein is essential for electron transport from NADPH for optimal functioning of various microsomal enzymes like CYP21A2 (21α-hydroxylase), CYP17A1 (17α-hydroxylase and 17,20-lyase), CYP19A1 (aromatase), and CYP51A1 (lanosterol 14α-demethylase). CAH due to POR deficiency is characterized by genital ambiguity, skeletal malformations, and history of maternal virilization during pregnancy. The genital ambiguity in 46,XY newborns is due

to reduced synthesis of testosterone as a consequence of impaired activity of CYP17A1 in testes, whereas genital ambiguity in 46,XX newborn is a result of reduced aromatization of fetal adrenal androgens because of impaired activity of CYP19A1 (aromatase) and synthesis of androgens via "backdoor pathway." One of the peculiar features of CAH due to POR deficiency is that virilization is nonprogressive after birth. The maternal virilization is also a result of impaired activity of CYP19A1 and synthesis of androgens via "backdoor pathway." Skeletal malformations are the characteristic manifestation of this variant of CAH and are due to impaired action of CYP51A1, which is required for sterol synthesis in the bone tissue. These malformations include midfacial hypoplasia, craniosynostosis, radiohumeral synostosis, and bowing of the femur and resemble Antley–Bixler syndrome. Mineralocorticoid deficiency is uncommon in these patients; however, they may manifest glucocorticoid deficiency.

78. *What is "backdoor pathway" of androgen synthesis*?

In humans there are two classical pathways for the synthesis of androgens in testes and adrenals. The classical Δ^5 pathway involves conversion of 17-hydroxypregnenolone to DHEA and to testosterone, while the Δ^4 pathway involves conversion of 17-hydroxyprogesterone to androstenedione and to testosterone. The classical Δ^5 pathway predominates in both testes and adrenals. In addition to these classical pathways, there is also an alternative pathway of androgen biosynthesis which is termed as "backdoor pathway." This pathway involves conversion of 17-hydroxyprogesterone to dihydrotestosterone (DHT) without the formation of two major intermediates, DHEA or testosterone. The "backdoor pathway" is functional during fetal life and in early infancy. The "classical and backdoor pathways" are depicted in the figure given below (Fig. 10.18).

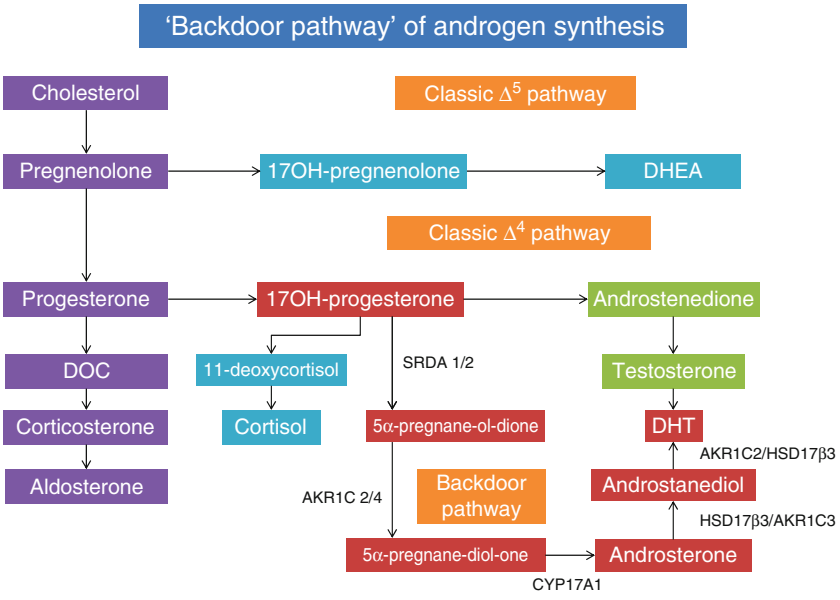

Fig. 10.18 Backdoor pathway of androgen biosynthesis

79. *What is the clinical relevance of "backdoor pathway" in CAH*?

P450 oxidoreductase is required for the electron transfer to 17α-hydroxylase (CYP17A1), 21α-hydroxylase (CYP21B1), and aromatase (CYP19A1). Therefore, 46,XY individuals with CAH due to POR deficiency manifest with under virilization of external genitalia and cortisol deficiency. However, 46,XX individuals with POR deficiency also have genital ambiguity in addition to cortisol deficiency. The presence of genital ambiguity in patients with 46,XX individuals can be explained by androgen synthesis through "backdoor pathway." In addition, "backdoor pathway" has also been shown to be functional in newborns and children with 21α-hydroxylase deficiency. This may partly explain the variability between genotype and phenotype (virilization of external genitalia) in these patients.

80. *What are the characteristics of CAH due to 3β-HSD2 deficiency*?

The enzyme 3β-HSD is required for the conversion of Δ^5 steroids to Δ^4 steroids and is expressed in all three layers of adrenal cortex. There are two isoforms of enzyme in humans, 3β-HSD1 and 3β-HSD2. The enzyme 3β-HSD1 is expressed in the placenta, skin, breast, and adipocytes, whereas 3β-HSD2 is expressed in the adrenal, testes, and ovary. Classical CAH due to 3β-HSD2 manifests with salt-wasting and genital ambiguity in both genders; however, genital ambiguity is rare and less severe (isolated mild clitoromegaly) in females. Therefore, girls with classical CAH due to 3β-HSD2 may succumb to salt-wasting crisis as the diagnosis is not considered due to lack of genital ambiguity. This is in contrast to CAH due to 21α-hydroxylase deficiency wherein boys are underdiagnosed due to the absence of genital ambiguity. The diagnosis of CAH due to 3β-HSD2 is established by an ACTH-stimulated serum 17-hydroxypregnenolone >100 nmol/L or elevated Δ^5 to Δ^4 steroid ratio.

81. *What are the causes of glucocorticoid-remediable hypertension*?

Glucocorticoid excess, whether endogenous or exogenous, is associated with hypertension. On the contrary, in certain disorders, hypertension associated with "mineralocorticoid excess/activity" resolves with glucocorticoids. These disorders include congenital adrenal hyperplasia (CAH) due to 11β-hydroxylase and 17α-hydroxylase deficiency, glucocorticoid resistance syndrome (GRS), familial hyperaldosteronism type 1 (glucocorticoid-remediable aldosteronism, GRA/FH type 1), and apparent mineralocorticoid excess syndrome (AME). The defects in these disorders include increased ACTH drive either due to cortisol deficiency (e.g. CAH) or resistance (e.g. GRS), or increased ACTH sensitivity (e.g. familial hyperaldosteronism type 1) or specificity-spillover of cortisol action on mineralocorticoid receptor (e.g. AME).

82. *How to assign the gender in a newborn with 46,XX CAH due to 21α-hydroxylase deficiency*?

A newborn with 46,XX CAH due to 21α-hydroxylase deficiency after confirmation of diagnosis should be counseled for gender assignment as a female irrespective of degree of virilization because fertility potential is invariably preserved in these children. Appropriate reconstruction surgery is indicated in those who have external genitalia Prader stage ≥3, at suitable time. However, sometimes in clinical practice either because of parental insistence or social stigma these children are reassigned as male sex particularly those who have severe virilization of external genitalia (Prader stage ≥4). If the decision is taken to rear them as a male, appropriate reconstruction surgery as well as implantation of artificial testes along with bilateral oophorectomy and hysterectomy should be performed prepubertally. Further at the age of puberty, the child should be supplemented with testosterone for optimal virilization (Figs. 10.19 and 10.20).

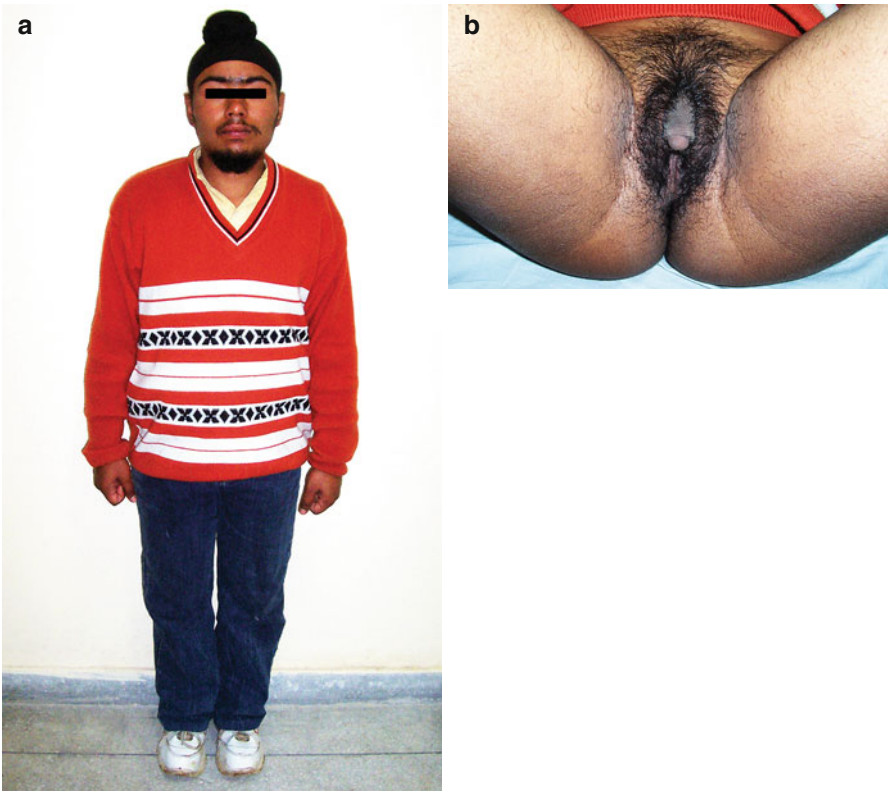

Fig. 10.19 (**a**, **b**) A 15-year-old 46,XX simple virilizing CAH due to 21α-hydroxylase deficiency presented with apparent male external genitalia (Prader stage 4). Patient had gender identity, role, and orientation as a male and preferred to continue with same gender identity and role

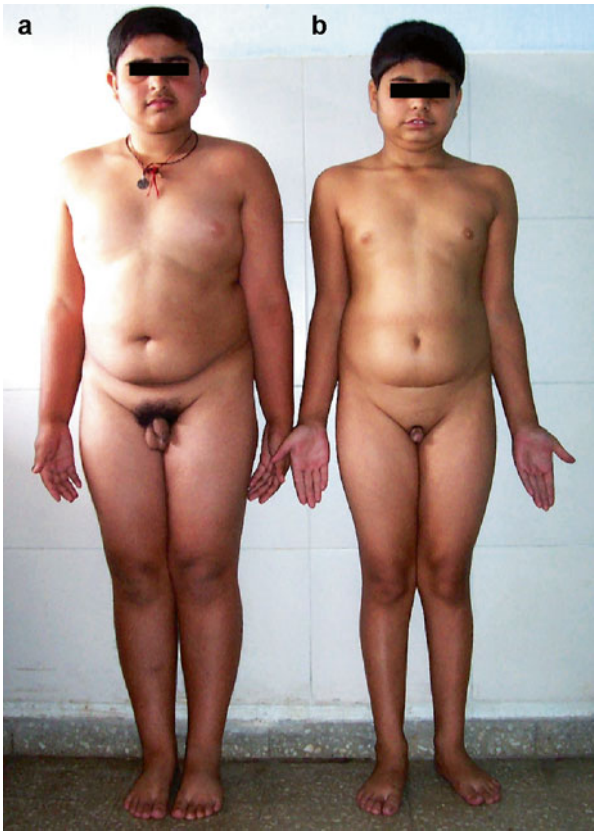

Fig. 10.20 Siblings with CAH due to 21α-hydroxylase deficiency (**a**) 46,XY (**b**) 46,XX reared as male

83. *What are the appropriate timings for genital reconstruction surgery in a girl with genital ambiguity due to CAH*?

In girls with CAH who have severe genital virilization (Prader stage ≥3), clitoral recession should be considered along with perineal reconstruction during infancy. Vaginal reconstruction in these children will depend on the position of vaginal confluence; if high vaginal confluence is present, surgery may be delayed till puberty, whereas with low vaginal confluence, surgery may be done simultaneously along with clitoroplasty. Children with Prader stage 1 and 2 should be kept under close surveillance as clitoromegaly may regress after intensive treatment with glucocorticoids.

Further Readings

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Multiple Endocrine Neoplasia 11

11.1 Case Vignette

A 42-year-old male presented with prominence of both the eyeballs for the last 6 months. He was detected to have hypertension 7 years earlier and for that he was receiving telmisartan, atenolol, and amlodipine. He had history of recurrent pain abdomen and dyspeptic symptoms for the last 5 years, and he frequently used to take proton pump inhibitors to relieve these symptoms. He had no history of headache, vomiting, or visual disturbances. There was no history of anorexia, constipation, bone pains, polyuria, graveluria, or renal stone disease. However, he had history of tightening of rings, palmar sweating, decreased libido and erectile dysfunction, and reduced frequency of shaving. There was history of fatigue and progressive increase in weakness for the last 2–3 years, and he was diagnosed to have iron deficiency anemia. There was no history of symptoms suggestive of thyrotoxicosis, chronic obstructive airway disease, or chronic kidney or liver disease. He was a nonsmoker and nonalcoholic. On examination, his height was 169 cm, weight 90 Kg, BMI 31.3 Kg/m^2, blood pressure 130/80 mmHg, and pulse rate 96 bpm and had multiple skin tags and grade 3 acanthosis nigricans. He did not have other cutaneous markers like collagenoma, angiofibroma, and lipoma. There was bilateral mild proptosis and he had palmar sweating and seborrhea. He did not have goiter and deep tendon reflexes were normal. His sexual maturation score was A_4, P_3, testicular volume 20 ml (bilateral), and sparse facial and body hair. He also had bilateral lipomastia. Visual fields, visual acuity, and optic disk were normal. Other systemic examination was unremarkable. On investigation, hemoglobin was 9.8 g/dl with normal leucocytes and platelet counts, and liver and renal function tests were normal. Corrected serum calcium was 11.4 mg/dl, phosphorus 2.4 mg/dl, alkaline phosphatase 104 IU/L, iPTH 1,098 pg/ml (N 15–65), and 25(OH)D 6 ng/ml (N 30–70). An 0800h serum cortisol was 262 nmol/L (N 171–536), prolactin 9,291 ng/ml (N 4–15.2), free T_4 0.5 ng /dl (N 0.8–1.8), TSH 2.92 μIU/ml (N 0.27–4.2), LH 0.21 mIU/ml (N 1.7–8.6), FSH 0.26 mIU/ml (N 1.5–12.4), and testosterone 0.206 nmol/L (N 9.9–27). Serum IGF1 was 363 ng/ml (N 101–267, age matched), basal serum GH 15 ng/ml, and nadir serum GH after glucose load 5.6 ng/ml. Serum gastrin level was 284 pg/ml (N 13–115), basal acid output

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40 mEq/L (N <15), fasting plasma insulin 10.2 μIU/ml, C-peptide 3.19 ng/ml with corresponding plasma glucose 98 mg/dl, and HbA1c 5.2% (N <5.7%). One microgram ACTH stimulation test showed a peak cortisol response 455 nmol/L (N >550 nmol/L). Twenty-four-hour urinary metanephrine was 93 μg (N <350) and normetanephrine 161 μg (N <600). CEMRI sella showed a 5×4.6×3.2 cm sellar–suprasellar mass with right parasellar extension. The CECT abdomen showed small enhancing focal lesion in the head of the pancreas, circumferential wall thickening in D1 and D2 segment of the duodenum, and bilateral adrenal gland enlargement. Endoscopic ultrasonography showed two lesions in the pancreas (8×8 mm in the body and 6×4 mm in the tail of the pancreas). DOTANOC–PET CT showed somatostatin receptor expressing lesions in the sella turcica, thyroid gland, and posterior to the thyroid gland (parathyroid gland); however, focal non-avid hypodense lesions were identified in the pancreas and in the adrenal gland. Upper gastrointestinal endoscopy showed multiple superficial ulcers in D1 and D2 segment of the duodenum. Ultrasonography of the neck showed 1×0.9 cm size heterogeneous lesion with central vascularity in antero-inferior aspect of left lobe of the thyroid gland. Sestamibi parathyroid scan showed uptake in the right superior and the left superior and inferior parathyroid glands. SPECT–CT fusion image showed increased tracer uptake in the left superior parathyroid gland. The patient was initiated with L-thyroxine and cabergoline. Patient underwent transsphenoidal surgery (TSS) and resection of the pituitary tumor was carried out. Postoperatively, patient was continued with L-thyroxine supplementation and hydrocortisone was added. Cabergoline was also continued as there was large residual tumor postoperatively. After 3 months of pituitary surgery, he underwent bilateral neck exploration, and three enlarged parathyroid glands were identified (left superior and inferior parathyroid, and right inferior parathyroid gland) and were excised. Simultaneously, open laparotomy was also performed; intraoperative ultrasonography confirmed the lesions in the head and body of the pancreas, and these were excised accordingly. Postoperatively 3 months after TSS, serum prolactin was 23 ng/ml (on cabergoline) and GH was non-suppressible (nadir GH 1.3 ng/ml) after glucose load, however, IGF 1 was normalized (226 ng/ml; N 101–267, age matched). Repeat CEMRI sella revealed 1.5×2.8×2.8-cm sellar–suprasellar mass with parasellar extension. After parathyroid surgery with one gland in situ, serum calcium and phosphorus was normalized (9.3 mg/dl and 3.3 mg/dl, respectively), serum iPTH was 46.6 pg/ml, and serum gastrin was undetectable (<10 pg/ml). Histopathology of the sellar mass confirmed pituitary adenoma and showed diffuse positivity for GH and prolactin on immunohistochemistry (IHC). Parathyroid gland histology showed parathyroid adenoma in all three resected glands. Pancreatic tumor histology was consistent with neuroendocrine tumor. Genetic analysis for MEN1 gene demonstrated duplication of C1546 gene in exon 10 and frame-shift mutation at P-Arg 516 consistent with MEN1 syndrome. The pedigree of the index patient is shown in the figure given below. The patient was continued with cabergoline and received external beam radiotherapy (EBRT) for residual pituitary lesion. However, serum GH still remained non-suppressible after glucose load with elevated IGF1; therefore, he was initiated with octreotide LAR (20 mg once a month). He did not have features of hungry bone syndrome postoperatively. He was continued with proton pump inhibitor along with calcium carbonate and calcitriol (Fig. 11.1).

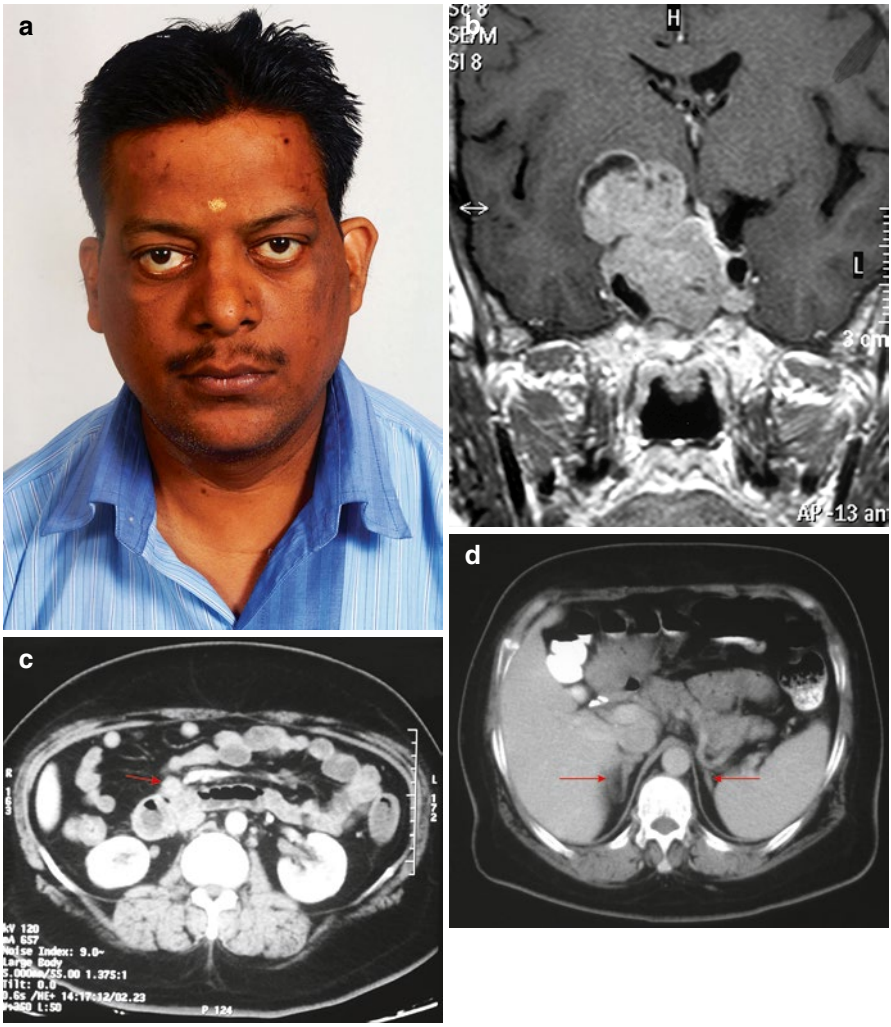

Fig. 11.1 (**a**) A 42-year-old male presented with proptosis. (**b**) CEMRI sella showing giant pituitary adenoma. (**c**) CECT abdomen showing 1 × 1 cm isodense lesion in the head of the pancreas. (**d**) CECT abdomen depicting bilateral adrenal enlargement. (**e**) ^{99m}Tc sestamibi scan displaying tracer uptake in the *right superior*, *left superior*, and inferior parathyroid glands. (**f**) SPECT-CT fusion image showed increased tracer uptake in the *left superior* parathyroid gland. (**g**) Chromatogram demonstrated duplication of C1546 gene in exon 10 and frame-shift mutation at P-Arg 516 consistent with MEN1 syndrome

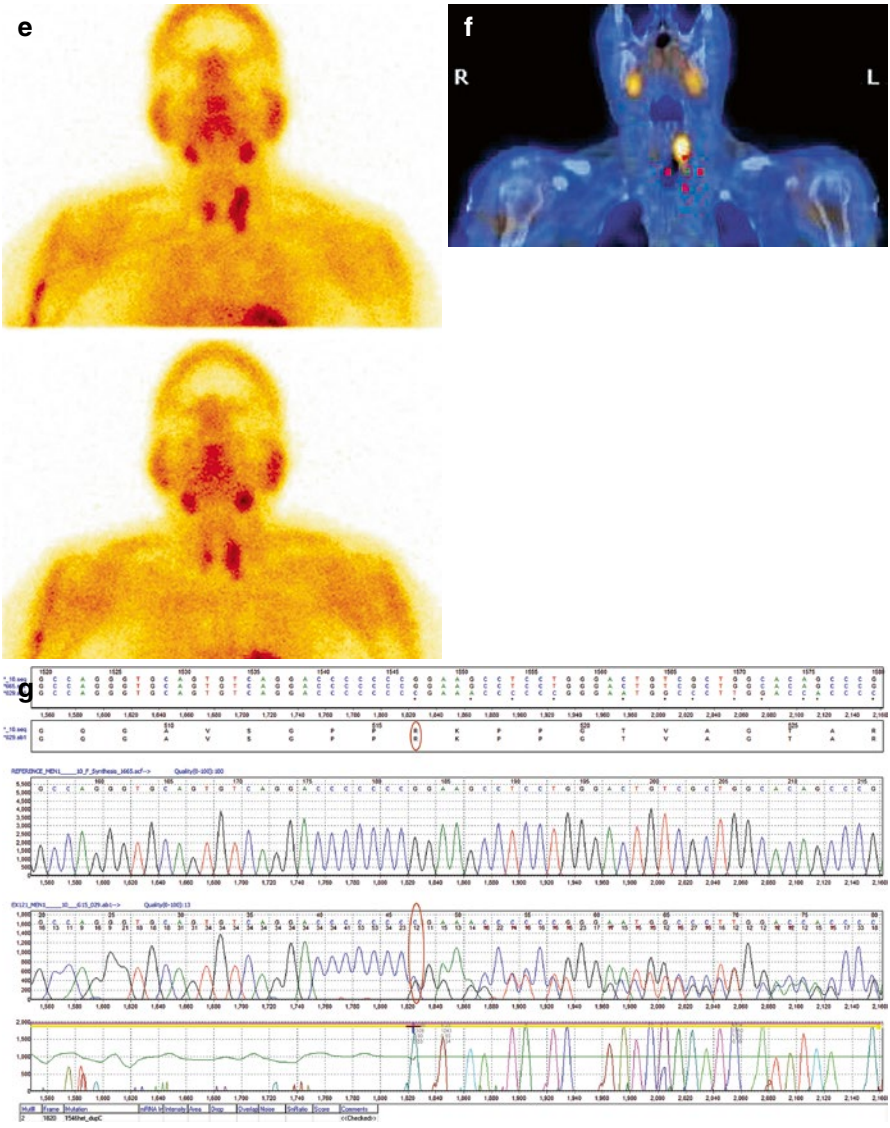

Fig. 11.1 (continued)

11.2 Stepwise Analysis

In the index patient with this clinical and biochemical profile, the diagnosis of multiple endocrine neoplasia type 1 (MEN1) was considered, and genetic analysis confirmed MEN1 syndrome. He had involvement of the pancreas, pituitary, parathyroid, and adrenal glands. The former three endocrine organs had evidence of hormone hypersecretion, while adrenal glands were enlarged but nonfunctional. The initial presentation of the index patient with proptosis and hypertension is an unusual manifestation of MEN1. Proptosis has been described with endocrine disorders including autoimmune thyroid disease (Graves' and Hashimoto's thyroiditis), Cushing's syndrome and acromegaly, and rarely with primary hyperparathyroidism (PHPT). The cause of proptosis in a patient with acromegaly is due to GH–IGF1-mediated extraocular muscle hypertrophy. The reason for proptosis in a patient with PHPT remains elusive; however, orbital wall osteitis fibrosa cystica may manifest rarely as asymmetrical proptosis in a patient with PHPT. The index patient had hypertension for the last 7 years and was requiring three antihypertensive drugs for the control of blood pressure. Both GH excess and PHPT can result in hypertension. GH excess-mediated hypertension is because of augmented cardiac output due to increased left ventricular muscle mass, GH-dependent Na^+ and water reabsorption from distal convoluted tubule (with inhibition of renin–angiotensin–aldosterone axis), decreased NO synthase activity, and concurrent insulin resistance. PHPT-related hypertension is due to increased sympathetic activity, PTH-mediated left ventricular hypertrophy and hypercalcemia-associated increased reactivity to circulating vasoconstrictors, and possibly microvascular injury due to hypercalcemia. The index patient had persistent symptoms of acid-peptic disease which may be attributed to the presence of gastrinoma, and further these symptoms were aggravated by PHPT-associated hypercalcemia-induced gastrin secretion from G-cell of the antrum of stomach. Anemia in a patient with acromegaly is rare, and if present, possibility of a bleeding colonic polyp/carcinoma should be considered. However, the presence of anemia in the index patient can be explained by multiple gastrointestinal ulcers and PTH-mediated marrow fibrosis. The most common pituitary adenoma associated with MEN1 syndrome is prolactinoma (20 %) followed by somatotropinoma (10 %). The index patient had mamosomatotropinoma, which is rare in patients with MEN1, and represent either a stem cell adenoma, mixed GH and prolactin-secreting adenoma (i.e., admixture of isolated GH and prolactin-secreting cells in the same adenoma), or mamosomatotroph cell adenoma (single cell having both GH and prolactin granules). Treatment for mamosomatotropinoma include D_2 receptor agonists and, if required, surgical excision of the tumor followed by somatostatin analogues for residual disease, if any. The index patient received cabergoline and underwent TSS as it was giant adenoma (>4 cm). As there was significant residual disease, he received radiotherapy followed by somatostatin analogue and cabergoline therapy. Despite being large and invasive pituitary macroadenomas in MEN1 syndrome, pituitary carcinoma is rare. PHPT is the most common (95 %) manifestation of MEN1 syndrome, and in 85 % of patients with MEN1, PHPT is the presenting manifestation, and most patients present <25 years of age. Hypercalcemia in PHPT with MEN1 syndrome is usually mild, and patients are often asymptomatic as was seen in the index patient; however, in our patient, it was

detected at the age of 42 years. Multiglandular parathyroid involvement is characteristic of MEN1 syndrome either as parathyroid adenoma or hyperplasia or as a combination of both. Preoperative imaging is of limited value as the sensitivity to localize multiglandular disease by any imaging modality, whether ultrasonography or sestamibi scan, is only 55–65 % as compared to 80–95 % for localization of single-gland disease. This is because ultrasonography cannot detect small-sized adenomas/hyperplasic gland, whereas localization of abnormal gland by sestamibi scan depends on mitochondrial content of the abnormal gland which may be variable in multiglandular disease. Therefore, patients with MEN1 should be subjected for bilateral neck exploration irrespective of localization. Further, 3.5 gland excision is recommended to prevent the recurrence of disease as the presence of multiglandular disease may be synchronous or metachronous. In the index patient, ultrasonography did not localize any abnormal gland, whereas sestamibi scan localized three abnormal parathyroid glands. On bilateral neck exploration, three abnormal parathyroid glands were identified and excised accordingly. Like other neoplasias, parathyroid carcinoma is rare in MEN1 syndrome. Approximately 40 % of patients with MEN1 syndrome have gastrinomas, and 60 % of these gastrinomas are present in the duodenum, while the rest are present in the pancreas. The index patient had increased basal acid output and hypergastrinemia, and there were two hypodense lesions in the head and body of the pancreas suggestive of gastrinomas. Surgical enucleation of these lesions resulted in normalization of serum gastrin levels. Bilateral symmetrical/asymmetrical nonfunctional adrenomegaly is the characteristic feature of MEN1 syndrome and has been reported in approximately 40 % of patients. However, single or multiple adenomas have also been reported, but only 10 % of patients with adrenal lesions have hormonal hypersecretion. Adrenal lesions >4 cm require surgical excision. Occurrence of multiple endocrine neoplasia in a same patient, either synchronous or metachronous, has been attributed to loss-of-function of MEN1 gene, which is a tumor suppressor gene. In our patient, duplication of C1546 gene in exon 10 and frame-shift mutation at P-Arg 516 was found, and it was consistent with MEN1 syndrome.

11.3 Clinical Rounds

1. *What is multiple endocrine neoplasia*?

   Multiple endocrine neoplasia (MEN) is a heterogeneous group of autosomal dominant inherited disorder characterized by the presence of tumors involving ≥2 endocrine organs, either synchronous or metachronous, in a same patient. The clinical manifestations of MEN syndrome depend on the involvement of endocrine organs and the hormone/s secreted by them.

2. *What are the types of MEN syndrome*?

   Multiple endocrine neoplasia is conventionally classified into type 1 and type 2 MEN syndrome. Type 2 is further subclassified into 2A and 2B (also called as

type 3). Recently, type 4 MEN syndrome has also been described. This classification is based on involvement of specific endocrine organs in each particular type of MEN syndrome. The disorders which are associated with multiple endocrine as well as non-endocrine neoplasia like Carney's complex, Von Hippel–Lindau disease, and neurofibromatosis type 1 are classified under multiple endocrine and other organ neoplasia (MEON) syndrome.

3. *What are the endocrine organs involved in different MEN syndrome*?

The endocrine organs involved in different MEN syndrome are summarized in the table given below.

MEN syndrome	Endocrine organs involved	Associated features
MEN 1	Parathyroid: hyperplasia/ adenoma	Lipoma, collagenoma, angiofibroma, gastric carcinoid, meningioma
	Pituitary: prolactinoma, somatotropinoma	
	Pancreas: gastrinoma, insulinoma	
	Adrenal: nonfunctional adrenal hyperplasia and adenoma	
	Thyroid: thyroid nodule, MNG	
MEN 2A	Thyroid: MTC	Cutaneous lichen amyloidosis
	Parathyroid: hyperplasia/ adenoma	Hirschsprung disease
	Adrenal: pheochromocytoma	
MEN 2B (MEN3)	Thyroid: MTC	Mucosal neuroma, marfanoid habitus, and slipped capital femoral epiphysis
	Adrenal: pheochromocytoma	
MEN 4	Parathyroid: adenoma	Reproductive organ neoplasia
	Pituitary: adenoma	
	Pancreas: NET	

MNG multinodular goiter, *MTC* medullary thyroid carcinoma, *NET* neuroendocrine tumor

4. *What is the prevalence of different endocrine organ involvement in MEN1*?

Parathyroid gland is the most common endocrine organ involved, and parathyroid hyperplasia/adenoma is seen in 95 % of patients with MEN1 syndrome. Pancreatic neuroendocrine tumors (NET) occur in 40–70 % of MEN1 patients and comprise of gastrinoma (40 %), insulinoma (10 %), glucagonoma (<1 %), vasoactive intestinal polypeptidoma (VIPoma, <1 %), and nonfunctioning NET (20–55 %). Anterior pituitary tumors occur in 30–40 % of patients of MEN 1 and consists of prolactinoma (20 %), somatotropinoma (10 %), corticotropinoma (<5 %), and NFPA (<5 %). In addition, patients with MEN1 may develop adrenocortical tumor (40 %), angiofibroma (85 %), lipoma (30 %), collagenoma

(70 %), and gastric neuroendocrine tumors (10 %). Rarely, pheochromocytoma (<1 %) and thymic NET (<2 %) have also been described.

5. *Who should be screened for MEN1*?

   Screening for MEN1 should be performed in a patient with ≥2 MEN1-associated endocrine tumors (parathyroid, pancreatic, and pituitary tumor), asymptomatic first-degree relatives of an individual with MEN1 mutation, or patients who have ≥2 MEN1-associated endocrine tumors that are not part of classical triad of parathyroid, pancreatic, or pituitary tumor (e.g., pancreatic and adrenal). In addition, patients with PHPT <30 years of age, multiglandular parathyroid disease, gastrinoma, and multiple pancreatic neuroendocrine tumor should also be screened for MEN1 syndrome.

6. *When to suspect MEN1 syndrome in a patient who presents with single endocrine gland involvement*?

   Primary hyperparathyroidism (PHPT) is the most common and the earliest manifestation of MEN1 syndrome. Therefore, PHPT in a young individual (<30 years) or multiglandular parathyroid involvement should raise a suspicion of MEN1 syndrome. In addition, patients with acid peptic disease who have multiple ulcerations at unusual sites (second part of the duodenum and jejunum), resistance to proton pump inhibitor therapy, or recurrence of peptic ulcer disease after surgery should raise a suspicion of gastrinoma. The probability of having MEN1 syndrome in a patient with gastrinoma is approximately 25 %, and hence, every patient with gastrinoma should be evaluated for MEN1 syndrome. In addition, patients with multiple pancreatic NET at any age should also be evaluated for MEN1 syndrome. Pituitary tumor occurs in 15–50 % of patients with MEN1 syndrome and can manifest as early as 5 years of age or as late as in ninth decade with a mean age of 38 years. On the contrary <3 % of patients with pituitary tumor have MEN1. Therefore, screening for MEN1 in a patient with isolated pituitary tumor is not rewarding.

7. *A 21-year-old man presented with pathological fracture of shaft of the femur. His biochemical profile showed corrected serum calcium 11.5 mg/dl, phosphate 2.1 mg/dl, alkaline phosphatase 315 IU/L, iPTH 300 pg/ml, and 25(OH)D 20 ng/ml. Ultrasonography showed left and right inferior parathyroid adenoma. His family history was noncontributory. How to approach further*?

   The clinical and biochemical profile of the index patient suggest the diagnosis of primary hyperparathyroidism (PHPT). Young age of onset and the presence of multiglandular disease in a patient with PHPT mandate screening for MEN1 syndrome. The investigations required for the detection of other endocrine organ involvement include estimation of serum gastrin, fasting plasma glucose, insulin and C-peptide, prolactin, and chromogranin A. However, it should be further corroborated with genetic analysis for *MEN1* gene mutation. Preoperative

imaging is not helpful in these individuals, and bilateral neck exploration should be performed in these patients. The genetic analysis in the index patient showed frame-shift mutation in *MEN1* gene, the patient underwent bilateral neck exploration and 3.5 glands were removed, and the histopathology of the excised gland showed parathyroid adenoma in one gland and the rest showed hyperplasia. The biochemical workup for other endocrine organ involvement was noncontributory. However, the patient should be followed up annually or with any new onset of symptom, as the multiple endocrine organ involvement may evolve over time.

8. *How to follow up a patient of MEN1 who has a single endocrine gland involvement at presentation*?

The majority of endocrine tumors associated with MEN1 are benign, while nonfunctioning pancreatic neuroendocrine tumors are usually malignant and contribute to increased morbidity and mortality in these patients. Therefore, periodic imaging is recommended to identify the disease at the earliest possible in those individuals who have MEN1 mutation. The biochemical tests are recommended to identify functional tumors and imaging for nonfunctional tumor/s. The time schedule for recommended work up and imaging modalities for patients with MEN1 are summarized in the table given below.

Endocrine tumor	Age to screen (years)	Biochemistry (annually)	Imaging
Parathyroid	8	Calcium and iPTH	–
Gastrinoma	20	Gastrin	–
Insulinoma	5	Fasting glucose and C-peptide	–
Nonfunctional pancreatic NET	<10	Chromogranin A	MRI, CT, or endoscopic ultrasound (annually)
Anterior pituitary	5	Prolactin, IGF1	MRI (3 yearly)
Adrenal	<10	Unless symptomatic or tumor size >1 cm	CT (annually)
Thymic and bronchial carcinoid	15	–	CT or MRI (annually)

9. *What are the characteristics of pituitary tumor associated with MEN1 syndrome*?

Pituitary tumors are present in 30–40 % of patients with MEN1 syndrome. On the contrary, 3 % of patients with pituitary tumor have MEN1 syndrome. These tumors are more common in women and are usually macroadenomas (85 %). Further, they have aggressive behavior and are usually resistant to medical treatment. Most common pituitary tumor in MEN1 syndrome is prolactinoma (20 %), followed by somatotropinoma (10 %), and rarely corticotropinoma and nonfunctioning

pituitary adenoma. Fifteen percent of patients with MEN1 syndrome may present with prolactinoma as the first manifestation. Despite aggressive nature of these tumors, pituitary carcinoma is rare. D_2 receptor agonists are effective in patients with prolactinoma; however, patients with somatotropinoma require transsphenoidal surgery followed by somatostatin analogues and/or ϒ-knife for residual disease. On immunohistochemistry, plurihormonal expression is more frequent in MEN1-associated pituitary tumor compared with non-MEN1 pituitary tumor.

10. *What are the characteristics of insulinoma associated with MEN1 syndrome*?

Insulinoma may be the first presenting manifestation of MEN1 in 10% of patients. The age of presentation of MEN1-associated insulinoma is <40 years (usually <20 years), while the majority of patients with non-MEN1 insulinoma present beyond 40 years of age. In 10% of patients with MEN1, insulinoma is associated with other pancreatic NET. They are usually single, size >5 mm, and benign. Biochemical testing and imaging modalities for the diagnosis are similar as in patients with sporadic insulinoma.

11. *What are the cutaneous manifestations of MEN1 syndrome*?

Cutaneous manifestations in patients with MEN1 syndrome include lipomas, facial angiofibromas, and collagenomas. Lipomas are present in 30% of patients and are frequently multiple. The usual sites of distribution of lipoma include subcutaneous tissue, visceral tissue, pleura, and retroperitoneum. They characteristically do not recur after surgery. Angiofibromas and collagenomas are present in 70–80% of patients with MEN1 syndrome. These cutaneous markers may appear even prior to the endocrine organ involvement and may be a clue to the diagnosis during presymptomatic phase in relatives of patients with MEN1 syndrome (Fig. 11.2).

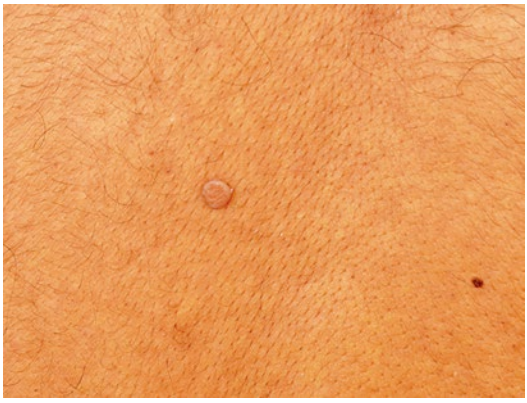

Fig. 11.2 Collagenoma in a patient with MEN1 syndrome

12. *A 30-year-old woman presented with upper abdominal pain and recurrent episodes of vomiting for the last 2 years. She is on pantoprazole (40 mg twice daily) with no relief in her symptoms. Upper gastrointestinal endoscopy revealed multiple ulcerations in the stomach and duodenum. How to proceed further?*

    Multiple gastrointestinal ulcerations particularly at unusual sites (beyond second part of the duodenum) and resistance to proton pump inhibitors should raise a suspicion of gastrinoma in this patient. The initial biochemical evaluation includes estimation of fasting serum gastrin after appropriate precaution (omission of proton pump inhibitors for at least 7 days). Serum gastrin level >150 pg/ml and increased basal gastric acid secretion (gastric pH <2) establishes the diagnosis. Mild hypergastrinemia has also been reported due to hypercalcemia as a result of primary hyperparathyroidism which is usually associated with gastrinoma in MEN1 syndrome. Provocative tests may be required in patients with mild hypergastrinemia to differentiate between antral G-cell hyperplasia and gastrinomas.

13. *In the above patient, the fasting serum gastrin level was 267 pg/ml and basal gastric pH <2. CECT abdomen was normal; however, endoscopic ultrasound revealed a 0.5 × 0.5 cm mass lesion at second part of the duodenum. What to do next?*

    High fasting serum gastrin levels and very low gastric pH suggest the diagnosis of gastrinoma. CT scan is usually normal as these are very small tumors and endoscopic ultrasound (EUS) is more yielding. In the index patient, EUS localized the lesion in second part of the duodenum. The common sites of gastrinomas are second part of the duodenum, antrum of the stomach, and sometimes in the pancreas. Approximately 40 % of patients with MEN1 have gastrinomas, and 20 % of patients with gastrinomas have MEN1 syndrome. Therefore, all patients with gastrinomas should be evaluated for MEN1. PHPT is the most common-presenting manifestation of MEN1 (85 %), and gastrinomas are usually associated with PHPT. Serum calcium profile and iPTH were performed in this patient and it was suggestive of PHPT.

14. *What are the characteristics of gastrinomas with MEN1 syndrome?*

    The gastrinomas associated with MEN1 syndrome are usually small (<5 mm) and are frequently multiple. Over 80 % of gastrinomas are found within the arbitrary triangle, known as "gastrinoma triangle" formed by confluence of cystic and common bile duct superiorly, junction of second and third portion of the duodenum inferiorly, and junction of the neck and body of the pancreas medially. Gastrinomas associated with MEN1 are more often present in duodenal wall than in the pancreas. The duodenal gastrinomas usually arise deep in the mucosa, adapt indolent course but frequently metastasize to peripancreatic lymph node and rarely to the

liver. Additional distinguishing feature in MEN1-related gastrinoma is the higher incidence of concurrent gastric carcinoids as compared to sporadic gastrinoma.

15. *How to manage a patient with gastrinoma in MEN1 syndrome*?

Duodenal gastrinomas are usually small, but multiple; therefore, surgical cure is usually difficult. Whipple's pancreaticoduodenectomy results in cure rate of 65 %, but it is associated with higher operative mortality and long-term complications which include weight loss, diabetes mellitus, and malabsorption. Hence, medical treatment is preferred which includes proton pump inhibitors and somatostatin analogues, and streptozotocin-based chemotherapy in those with metastatic disease. Though pancreatic gastrinomas are rare, but tumor size >2 cm mandates surgical resection.

16. *What are the characteristics of nonfunctioning pancreatic neuroendocrine tumor in MEN 1 syndrome*?

Nonfunctioning pancreatic neuroendocrine tumor (NET) is present in 20–55 % of patients with MEN1 syndrome, and is increasingly recognized with better imaging modalities. These tumors are usually recognized late in the course of disease due to the absence of clinical manifestations. The majority of these tumors are malignant and result in high morbidity and mortality. Endoscopic ultrasound is the most sensitive modality to localize these pancreatic neuroendocrine tumors, whereas somatostatin receptor scintigraphy is useful to detect metastatic disease. Surgical resection is recommended for tumor >1 cm in size or tumor <1 cm but rapidly growing (doubling of tumor size over 3–6 months interval). Tyrosine kinase inhibitors and mTOR inhibitor (mammalian target of rapamycin) have been found to be useful.

17. *What is MEN1 gene*?

MEN1 is a tumor suppressor gene which is located on chromosome 11q13. It consists of 10 exons which encodes 610 amino acid protein termed as Menin that regulates transcription, genome stability, cell division, and proliferation. Inheritance of a germ-line *MEN1* mutation predisposes an individual to develop a tumor after acquisition of somatic mutation which may be a point mutation or more commonly a deletion. This results in loss of heterozygosity in the involved tissue, thereby leading to tumor formation (Knudson hypothesis). The first-degree relatives of the patients with MEN1 have 50 % risk of developing the disease and can often be identified by *MEN1* mutational analysis.

18. *What is multiple endocrine neoplasia type 2 syndrome*?

Multiple endocrine neoplasia type 2 (MEN2) syndrome is an autosomal dominant inherited disorder characterized by multiple endocrine organ involvement of neural crest origin. Classically, the MEN2 syndrome is subdivided into MEN2A and MEN2B, and with the current classification, familial medullary thyroid carcinoma is also considered as part of MEN2 syndrome.

19. *What are the components of MEN2A syndrome*?

MEN2A syndrome consists of medullary thyroid carcinoma, pheochromocytoma, and PHPT. Medullary thyroid carcinoma (MTC) is usually present in almost all patients (100 %), whereas pheochromocytoma is present in 30–50 % and PHPT in 10–20 % of patients with MEN2A syndrome. The dermatological manifestation of MEN2A syndrome is cutaneous lichen amyloidosis which usually occurs in 30 % of patients and is invariably associated with codon 634 mutations of *RET* proto-oncogene. It is a precocious marker of future development of MTC. In addition, Hirschsprung disease (7 %) is also associated with MEN2A syndrome (Fig. 11.3).

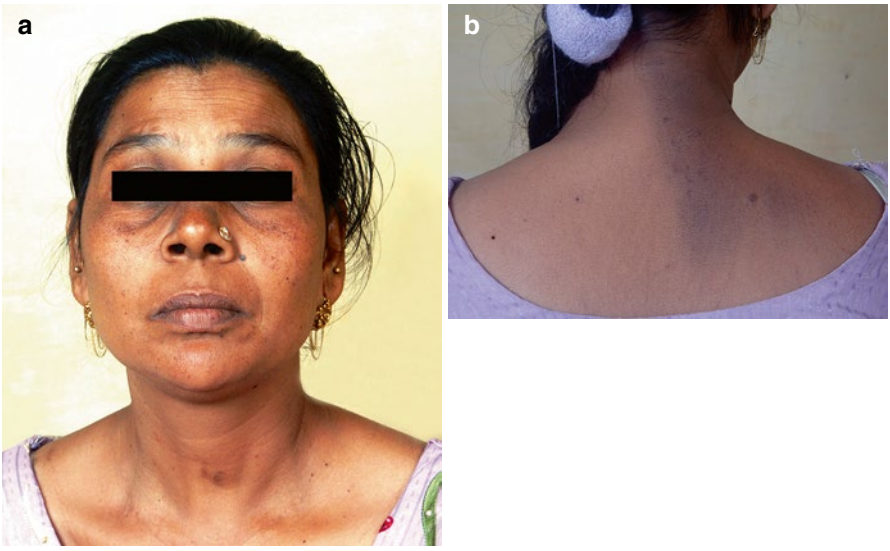

Fig. 11.3 (**a**) A patient with MEN 2A syndrome with a scar in the neck. (**b**) Note the cutaneous lichen amyloidosis over the nape of the neck in the same patient

20. *How to suspect MEN2A syndrome*?

MTC is the most common and the earliest presenting manifestation of MEN2A syndrome. The presence of diffuse or nodular goiter and recurrent diarrhea in a euthyroid young individual (<35 years of age) should raise a suspicion of MTC. Seventy percent of patients may have cervical lymph node metastasis even at presentation. Therefore, MTC is an important differential diagnosis in a young individual with goiter and cervical lymphadenopathy. Pheochromocytoma usually follows MTC; however, it may be the initial presenting manifestation in 13–27 % of individuals. Young age of onset, milder symptoms, bilateral adrenal lesions, and predominantly epinephrine-secreting tumor are the characteristic features of MEN2A-associated pheochromocytoma compared to sporadic pheochromocytoma. In addition, MEN2A-associated pheochromocytoma is invariably benign. PHPT in MEN2A is usually mild and asymptomatic and presents many years later after the diagnosis of MTC.

21. *A 20-year-old male presented with nodular goiter and cervical lymphadenopathy. Thyroid function test was normal and FNAC from thyroid showed MTC. How to proceed further*?

The index patient has MTC which may be sporadic or hereditary. Approximately 20 % of patients with MTC have either MEN2 or familial MTC (FMTC). Therefore, every patient of MTC should be screened for familial syndromes. The work up protocol for a patient with suspected MTC is depicted in the flow-diagram given below (Fig. 11.4).

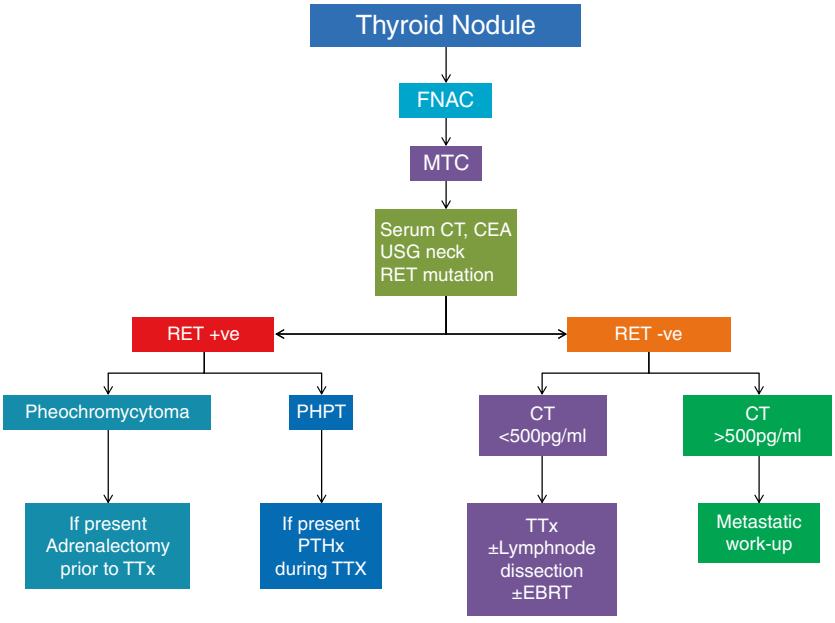

Fig. 11.4 Approach to a patient with suspected MTC

22. *What are the causes of nodular goiter with cervical adenopathy*?

The differential diagnosis for a nodular goiter with cervical adenopathy includes papillary, medullary and anaplastic thyroid carcinoma, thyroid lymphoma, and tuberculosis of the thyroid gland. Sometimes, subacute thyroiditis and pyogenic thyroid abscess can also lead to thyromegaly with cervical adenopathy.

23. *What are the causes of high serum calcitonin other than MTC*?

The disorders associated with high serum calcitonin levels other than MTC include chronic renal failure, Hashimoto's thyroiditis, PHPT, prostate and lung cancer, mastocytosis, and neuroendocrine tumors. However, lack of rise in serum calcitonin levels in response to calcium and pentagastrin differentiates these disorders from MTC. Further, the use of DPP4 inhibitors, GLP1 agonists, and proton pump inhibitors is associated with increased serum calcitonin levels. Ingestion of food also results in raised serum calcitonin levels (entero-calcitonin axis). The presence of heterophile antibodies interfere with the assay, thereby may result in falsely elevated serum calcitonin. Therefore, serum calcitonin level should be measured in the fasting state, and detailed drug history should be elicited before interpreting high serum calcitonin levels.

24. *What are the causes of low serum calcitonin in patients with MTC*?

Low serum calcitonin levels in patients with MTC may occur as a result of "hook effect" (prozone phenomenon) or poorly differentiated tumor. "Hook effect" can be obviated by measuring the blood sample after dilution. Differentiated parafollicular C-cells produce high levels of serum calcitonin, whereas poorly differentiated cells produce very low quantity of serum calcitonin and high levels of carcinoembryonic antigen (CEA). Therefore, CEA levels may be helpful in monitoring the progression of disease in patients with poorly differentiated MTC. Further in a patient with neck mass and a FNAC proven MTC, the presence of low serum calcitonin should raise a suspicion of other neuroendocrine tumor (e.g., paraganglioma) after careful exclusion of "hook effect."

25. *What is C-cell hyperplasia*?

C-cell hyperplasia (CCH) represents multicentric, clonal proliferation of parafollicular C-cells and precedes the development of MTC. The diagnosis of C-cell hyperplasia requires the presence of seven C-cells per cluster surrounded by a normal follicle and extension of C-cells beyond the junction of the upper third and lower two-thirds of the lateral lobes of the thyroid gland. Patients with CCH should be evaluated for MEN2 syndrome after exclusion of secondary causes of CCH, which include PHPT, renal failure, and Hashimoto's thyroiditis.

26. *What is RET proto-oncogene*?

RET (REarranged during Transfection) proto-oncogene is the only gene known to be associated with MEN2 syndrome. This gene is located on chromosome 10q11.2 and encodes receptor tyrosine kinase family. Constitutive activation of this receptor and consequent downstream signaling result in unrestricted cell growth and proliferation. Cells-derived from the neural crest, branchial arch, and urogenital system express *RET* proto-oncogene. Gain-of-function mutations of *RET* proto-oncogene result in tumorigenesis in these organs. Almost all patients with MEN2A, MEN2B, and FMTC have *RET* proto-oncogene germ-line mutations, and approximately 50 % of patients with sporadic MTC have somatic *RET* mutations.

27. *Who should undergo genetic testing for RET proto-oncogene*?

Genetic testing for *RET* mutations should be offered to the following individuals:

(a) Patient with isolated MTC at any age
(b) Patient with two of the following endocrine organ involvement (e.g., MTC, PHPT, or pheochromocytoma)
(c) Patients with familial MTC
(d) First-degree relatives of patients with MEN 2
(e) Children with phenotype of MEN2B
(f) Patients with cutaneous lichen amyloidosis (CLA) and Hirschsprung disease (HD)
(g) Patients with intestinal ganglioneuromatosis

28. *How to suspect MEN 2B syndrome*?

Early age of onset (infancy) and aggressive disease at presentation are the usual features of MTC associated with MEN2B. MTC occurs in all patients with MEN2B. Approximately, three-fourths of patients with MEN2B are sporadic, while the rest are familial. However, all affected individuals have *RET* mutations. Pheochromocytomas occur in approximately 50 % of individuals and are often bilateral, which may be synchronous or metachronous. Parathyroid adenoma/hyperplasia virtually does not occur in MEN2B. Nevertheless, the other characteristic features which help in recognition of MEN2B syndrome during infancy and early childhood include mucosal neuromas, narrow long facies, marfanoid habitus, kyphoscoliosis, and slipped capital femoral epiphysis (Fig. 11.5).

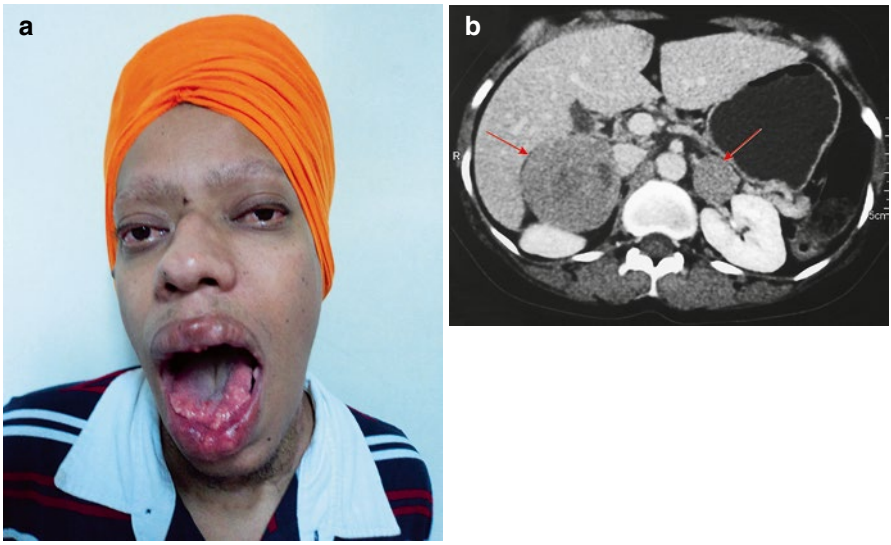

Fig. 11.5 (**a**) A 16-year-old boy presented with multiple mucosal neuromas, medullary thyroid carcinoma, and (**b**) bilateral asymmetrical pheochromocytoma (*red arrows*)

29. *What are the mucosal neuromas*?

Neuromas are encapsulated nerve sheath tumors which are composed of well-differentiated Schwann cell, while neurofibromas are unencapsulated fusiform nerve sheath tumors which are composed of Schwann cell, perineural cell, and fibroblast in a collagen matrix. The neuromas in MEN2B are present on mucosal surfaces including dorsal surface of the tongue, palate, and pharynx and along with the whole digestive tract. Neuromas of the eyelids may cause thickening and eversion of upper eyelid margins, and thickened corneal nerves may be seen by slit lamp examination. Neuromas of the lips give a typical blubbery lip appearance (Fig. 11.6), and palpable submucosal nodules may be present at vermilion border of the lip. Mucosal neuromas are present in almost in all patients with MEN2B syndrome. Approximately, 80 % of the individuals may have gastrointestinal symptoms even during infancy due to diffuse ganglioneuromatosis.

Fig. 11.6 Blubbery lips and mucosal neuromas in a patient with MEN2B syndrome. Note the scar in the neck after surgery for MTC

30. *What are the common RET mutations associated with different variants of MEN 2 syndrome*?

The *RET* mutations associated with different variants of MEN2 syndrome are summarized in the table given below.

MEN 2A	MEN 2B	FMTC
Codon 634 (exon 11) – 85 %	Codon 918 (exon 16) – 95 %	Exon 10 and 11
Codon 609, 611, 618, and 620 (exon 10), codon 630 (exon 11) – 15 %	Codon 883 (exon 15) – 5 %	Codon 768, 790, 791 (exon 13)
		Codon 804, 844 (exon 14)
		Codon 891 (exon 15)

31. *What is the importance of RET mutational analysis in the management of MEN 2 syndrome*?

The *RET* genotype analysis predicts the aggressiveness of MTC, and this information is useful in the surgical management of MTC. The different *RET*

mutational analysis associated with risk for MTC and age of prophylactic surgery in these patients is summarized in the table given below.

Mutations	Age of prophylactic surgery	Age to screen for pheochromocytoma	Age to screen for PHPT
Codon 883, 918, 804–806 (highest risk)	<1 year of age	8 years of age	–
Codon 634 (higher risk)	<5 years of age	8 years of age	8 years of age
Codon 609, 611, 618, 620 (lesser risk)			
Codon 321, 531, 532 (least risk)	>5 years of age	20 years of age	20 years of age

A basal and/or stimulated calcitonin and neck ultrasound examination should be performed annually in individuals where prophylactic surgery for MTC is not contemplated.

32. *What is MEN4 syndrome*?

Approximately, 3 % of patients with PHPT and pituitary adenoma who simulates MEN1 syndrome but are negative for *MEN1* gene mutation are reclassified as MEN4 syndrome. These patients have *CDKN1B* gene mutation instead of *MEN1* gene mutation. Other tumors described in MEN4 syndrome include pancreatic neuroendocrine tumors and adrenal tumors. The distinguishing features of MEN4 from MEN1 syndrome include the presence of reproductive organ tumors like testicular cancer and neuroendocrine cervical carcinoma.

33. *What is multiple endocrine and other organ neoplasia* (*MEON*)?

Disorders which are associated with multiple endocrine as well as non-endocrine neoplasia like Carney's complex, Von Hippel–Lindau disease, and neurofibromatosis type 1 are classified under multiple endocrine and other organ neoplasia (MEON) syndrome.

34. *What is Carney's complex*?

Carney's complex is an autosomal dominant inherited disorder, characterized by small pigmented cutaneous and mucosal lesions (lentigines), cardiac and cutaneous myxomas, and the presence of multiple endocrine neoplasias including primary pigmented nodular adrenal disease (PPNAD), pituitary adenoma, and nodular goiter. In addition, non-endocrine tumors like fibroadenoma of the breast and testicular tumors have also been reported in these patients.

35. *What is the molecular mechanism for the development of multiple neoplasias in patients with Carney's complex*?

Protein kinase A (PRKA) is an enzyme which is ubiquitously expressed and is involved in cell growth and proliferation in cyclic AMP-responsive tissues. The PRKA, a heterotetramer, consists of two regulatory (R) and two catalytic (C) subunits. Stimulation of adenylyl cyclase through G protein-coupled receptor (GPCR) activation leads to cAMP synthesis, which in turn results in dissociation of regulatory subunits from the catalytic subunits. The free catalytic subunits lead to activation of downstream pathway (e.g., cyclic AMP-responsive element-binding protein 1, i.e., CREB) which promotes cell growth and proliferation. *PRKAR1A* gene encodes the regulatory subunit of the PRKA enzyme, and its mutation leads to structural defects in the regulatory subunits. The defective regulatory subunits fail to bind with catalytic subunits, thereby unrestrained catalytic subunit activity results in tumorigenesis in cAMP-responsive tissues in patients with Carney's complex. This is depicted in the figure given below (Fig. 11.7).

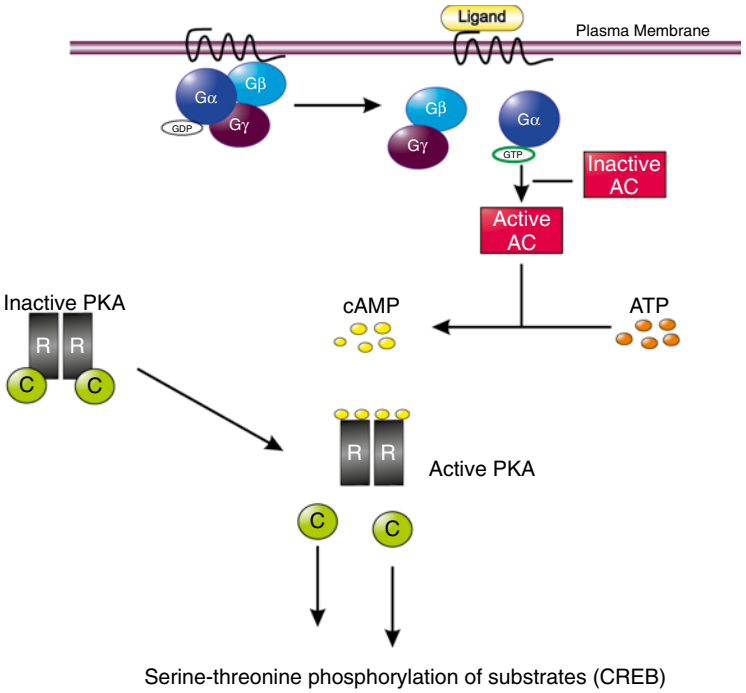

Fig. 11.7 Molecular mechanism of PRKA-mediated cell growth and proliferation

36. *What is Von Hippel–Lindau disease*?

Von Hippel–Lindau disease is an autosomal dominant inherited disorder characterized by retinal and central nervous system hemangioblastomas, pheochromocytoma/paraganglioma, pancreatic islet cell tumor, and pancreatic cystadenoma. In addition, renal cell carcinoma, endolymphatic sac tumors, and epididymal or broad ligament cystadenomas have also been described in these patients. Retinal angiomas are the most common manifestation of VHL and present as acute vision loss (due to bleed). CNS hemangioblastomas usually occur in the cerebellum, brain stem, and spinal cord and clinically manifest as compressive symptoms depending on the site of involvement. Bilateral pheochromocytoma and/or paragangliomas, which are usually multiple, is a characteristic feature of VHL. Pancreatic islet cell tumors are usually nonsecretory, whereas pancreatic cystadenoma may present with features of biliary obstruction. The treatment for retinal angiomas includes laser photocoagulation, while surgical resection is the treatment of choice for pheochromocytoma and pancreatic tumors. For symptomatic CNS hemangioblastomas, either surgical resection or stereotactic radiotherapy may be useful (Fig. 11.8).

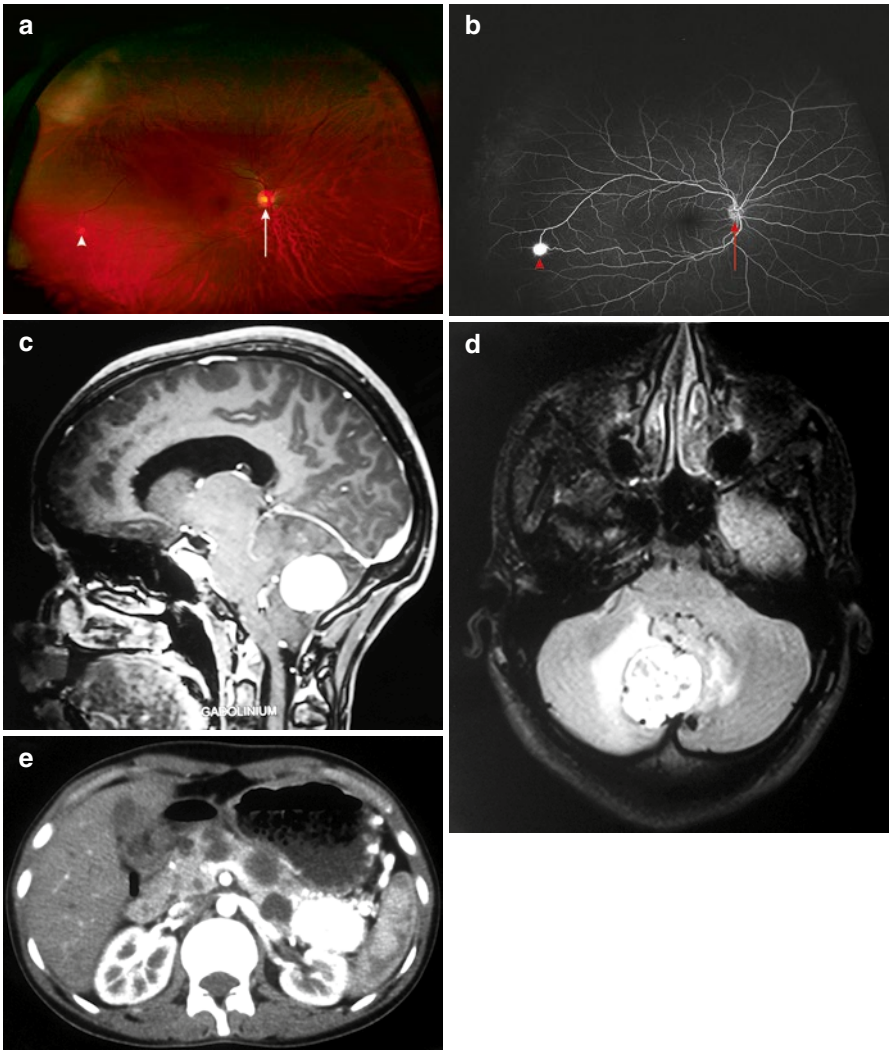

Fig. 11.8 (**a**, **b**) Fundus photograph and fluorescein angiography in a patient with VHL showing retinal angioma (*arrowheads*) and *arrows* represents optic disk. (**c**) Sagittal and (**d**) axial CEMRI brain showing hyperintense lesion in the cerebellum suggestive of cerebellar hemangioblastoma. (**e**) CECT abdomen showing multiple hypodense lesions in the head and body of the pancreas suggestive of cystadenoma and enlarged right kidney which was histologically consistent with renal cell carcinoma

37. *How does VHL gene mutation result in tumorigenesis*?

VHL is a tumor suppressor gene that encodes a protein which in normoxic state leads to ubiquitylation of hypoxia-inducible factor (HIF), thereby resulting in its proteasomal degradation and prevents cell growth and proliferation. Loss-of-function mutation of VHL gene results in stabilization of HIF which activates downstream signaling pathways including VEGF, PDGF, and TGF-α and thereby promoting angiogenesis, cell growth, and proliferation leading to tumorigenesis. The clinical implication of this knowledge is the use of tyrosine kinase inhibitors (such as sorafenib and sunitinib) in patients with VHL syndrome, as these drugs inhibit the VEGF and PDGF receptor tyrosine kinase and consequent tumorigenesis.

38. *When to suspect a diagnosis of neurofibromatosis type 1*?

Neurofibromatosis type 1 (NF1) is a neurocutaneous syndrome characterized by constellation of following features, and the presence of two of these features is required to establish the diagnosis.

1. Six or more café au lait macules >5 mm in longest diameter in prepubertal and >15 mm in longest diameter in postpubertal individuals
2. The presence of neurofibromas ≥2 or plexiform neurofibroma ≥1
3. Freckling in the axillary or inguinal area
4. Optic glioma
5. Lisch nodules ≥2
6. Skeletal dysplasia (sphenoid wing) and pseudoarthrosis
7. A first-degree relative with NF1

39. *What are the endocrine manifestations of NF1*?

Endocrine neoplasias associated with NF1 include pheochromocytoma, hypothalamic/optic nerve glioma, PHPT, duodenal carcinoids, and rarely MTC. Approximately, 15 % of children with NF1 may harbor optic nerve glioma which may present as gonadotropin-dependent precocious puberty, and 2 % of patients with NF1 have concurrent pheochromocytoma.

40. *What is the molecular basis for tumors associated with neurofibromatosis type 1*?

Neurofibromatosis type 1 is associated with mutations of *NF1* gene, a tumor suppressor gene, which is located on chromosome 17q11.2. *NF1* gene encodes a protein neurofibromin, which is expressed in many tissues including the brain, neural crest-derived tissues, kidney, and spleen. Neurofibromin stimulates intrinsic GTPase activity which in turn inhibits the ras p21 family. Normally,

ras activates a number of downstream signaling pathways including the mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) pathways. Loss-of-function mutations of NF1 gene result in inactivation of GTPase activity, thereby leading to unrestrained activity of ras signaling pathway and consequent tumorigenesis in neurofibromin-responsive tissues.

Further Readings

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Diabetes in the Young

12

12.1 Clinical Rounds

1. *How to define diabetes in the young?*

   Diabetes in the young is defined as onset of diabetes at or below 30 years of age. The differential diagnosis of diabetes is challenging in younger individuals as compared to the middle aged and elderly. This is because diabetes in an individual <30 years of age could be due to type 1 diabetes, type 2 diabetes, maturity-onset diabetes of the young (MODY), latent autoimmune diabetes of adults (LADA), fibro-calculus pancreatic diabetes (FCPD), lipodystrophic diabetes, or rarely, neonatal diabetes mellitus (NDM), whereas diabetes in middle-aged and elderly individuals is usually due to type 2 diabetes mellitus. Further, therapeutic strategies are different in younger individuals depending on the etiology of diabetes.

2. *How do islet cells develop during embryogenesis?*

   During embryogenesis, the pancreas develops from the foregut. Outpouching from the foregut results in formation of ventral and dorsal buds: the former develops into posterior part of the head of the pancreas, while the latter forms anterior part of the head, body, and tail of the pancreas. Endocrine pancreas develops from undifferentiated ductal epithelial cell derived from these buds under the influence of transcription factors (e.g., PDX 1 and ISL1). The development of endocrine pancreas is exclusively dependent on exocrine pancreas. α- and β-cells appear by 8–9 weeks of gestation, and in early gestation, α-cells are more abundant than β-cells. However, the number of β-cells progressively increases during second and third trimester, and the proportion of α-to β-cell is almost equal in a newborn.

A. Bhansali et al., *Clinical Rounds in Endocrinology*,
DOI 10.1007/978-81-322-2815-8_12

3. *What is the topography of islet cells in the pancreas?*

The word *islet* means small island, which originated from the French word *islette* and refers to clusters of cells. A healthy adult human pancreas weighs approximately 68 g (range 45–120 g) and consists of approximately one million islets, and each islet contains about 3,000–4,000 cells. The endocrine pancreas constitutes approximately 1–2 % of the total weight of the pancreas, while the rest (98 %) is contributed by the exocrine pancreas. The details about islet cells in the pancreas are summarized in the table given below.

Islet cells	Cell proportion (%)	Cell distribution	Hormone secreted
α-cells	40	Neck, body, and tail of the pancreas	Glucagon
β-cells	50	Neck, body, and tail of the pancreas	Insulin, amylin
δ-cells	8–10	–	Somatostatin
PP-cells	<1	Head of the pancreas	Pancreatic polypeptide
ε-cells	<1	–	Ghrelin

4. *How was the word "insulin" derived?*

The word "insulin" was derived from a Latin word *insula* which means island. The island in this context refers to the cluster of cells in the pancreas ("*Insula*"), and the product of *insula* was termed as "insulin." Both *insula* and *islette* refers to island which means cluster of cells and are of Latin and French in origin, respectively.

5. *What is the insulin secretory profile in a normal individual?*

Insulin is secreted in a pulsatile pattern in a healthy adult. This consists of rapid burst at a frequency of every 4 min and ultradian oscillations every 15–20 min. During the prandial phase, the oscillations are exacerbated in amplitude and frequency and are appreciated as first and second phase of insulin secretion. The pulsatile insulin secretion in postabsorptive period (basal) contributes approximately 50 % of total daily insulin secretion and regulates hepatic glucose output and suppresses lipolysis during inter-prandial and fasting state. The meal-related insulin secretion during first phase initiates just prior to ingestion of food and lasts for 5–15 min, while the second phase of insulin secretion starts thereafter and continues for 120 min. The first-phase insulin secretion occurs due to release of preformed granules, whereas the second phase is due to neogenesis of insulin granules. The first-phase insulin secretion suppresses the hepatic glucose output in immediate postprandial period, whereas the second phase of insulin secretion promotes peripheral glucose uptake in skeletal muscle and adipocytes.

6. *What is glucose–glucagon axis?*

In physiology, declining glucose levels result in stimulation of glucagon secretion, whereas rising glucose levels lead to suppression of glucagon secretion. This tightly regulated phenomenon responsible for the maintenance of glucose homeostasis may be referred as "glucose–glucagon axis." The key regulators involved in this axis include intra-islet insulin, glucose per se, GLP1, GIP, intra-islet autonomic nervous system, and possibly preserved insulin sensitivity to α-cell. Decrease in intra-islet insulin due to declining level of glucose acts as a stimulus to increase glucagon secretion (insulin switch-off signal). In addition, glucose can directly regulate glucagon secretion, and this effect is modulated by its effect on K_{ATP} channel present on α-cell membrane through its entry via GLUT1. GLP1 inhibits glucagon secretion, and this effect is possibly mediated through increase in intra-islet insulin or due to the direct inhibitory effect of GLP1 on α-cell or by promoting somatostatin secretion from δ-cell (which in turn inhibits α-cell). GIP stimulates glucagon secretion and plays a protective role during hypoglycemia. Intact intra-islet autonomic nervous system and preserved insulin sensitivity to α-cell also determine the glucagon response to glucose.

7. *What is entero-insular axis?*

Entero–insular axis or "incretin–β-cell axis" encompasses secretion of insulin triggered by release of peptides from enteroendocrine cells (incretins) in response to oral administration of nutrients. The concept of entero–insular axis evolved after the demonstration of greater insulin response to oral glucose load as compared to intravenous glucose load. This phenomenon was attributed to release of peptides from enteroendocrine cells in response to glucose. These peptides were named as incretins and include glucagon-like peptide 1 (GLP1) and glucose-dependent insulinotropic peptide (GIP). GLP1 is secreted from L-cells present in the distal ileum and colon, whereas GIP is from K-cells present in the duodenum and jejunum. Incretin-mediated insulin secretion contributes to 60–70 % of prandial insulin secretion. Both the incretins are rapidly degraded in circulation by an enzyme called dipeptidyl-peptidase 4 present in endothelial cells.

8. *How do islet cells cross-talk with each other?*

Circulating level of glucose is the key determinant for normal functioning of β-cell and α-cell. In addition, there is an intra-islet periportal circulation which facilitates cross-talk among different islet cells in a paracrine manner. In response to rising glucose level, increase in intra-islet insulin inhibits α-cell (insulin switch-on signal), whereas in response to hypoglycemia reduction in intra-islet insulin stimulates the α-cell to secrete glucagon (insulin switch-off signal). Somatostatin secreted from δ-cell has inhibitory effects on both α- and β-cells. During recovery from hypoglycemia, somatostatin prevents rebound

hyperglycemia (by inhibition of α-cells) and prevents recurrence of hypoglycemia (by inhibition of β-cells). When euglycemia is achieved, glucagon not only stimulates β-cell but also δ-cell which in turn results in inhibition of its own secretion by insulin as well as by somatostatin, respectively. However, the effect of insulin on regulation of δ-cell function is not well defined. Incretins play an important role in facilitating the cross-talk among islet cell as GLP1 stimulates β-cell and inhibits α-cell. Further, GIP stimulates α-cell in the presence of hypoglycemia. Therefore, the cross-talk among the islet cells is crucial to maintain euglycemia in a healthy individual. The cross-talk among the islet cells is depicted in the figure given below (Fig. 12.1).

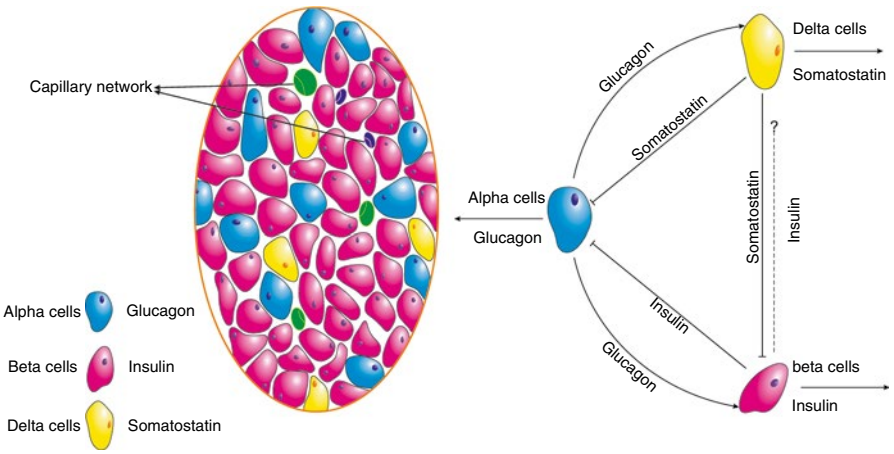

Fig 12.1 Depicting cross-talk among different islet cells in the regulation of glucose homeostasis

9. *What are the characteristic features of type 1 diabetes mellitus?*

Young age of onset, the presence of islet autoimmunity, and absolute insulin deficiency are the characteristic features of T1DM. Type 1 diabetes has trimodal distribution of age with first peak at 3–6 years, second at peripubertal age, and finally at 35–40 years of age. Absolute insulin deficiency is the hallmark feature of T1DM and manifests as severe osmotic symptoms, sarcopenia, and ketosis/ketoacidosis. Approximately, 30–40% of patients present with DKA, while the rest have hyperglycemia and asymptomatic ketonemia without acidosis. These patients require insulin since the diagnosis of diabetes. The presence of islet autoimmunity in these patients is a strong pointer toward immune-mediated β-cell destruction, and multiple islet autoantibodies (≥2) are usually present in these patients.

10. *Is there a phase of prediabetes in patients with type 1 diabetes?*

Evolution of T1DM occurs through series of stages that begins with the development of autoimmunity in genetically predisposed individuals.

During the initial stage, T-cell-mediated (CD-4 positive) β-cell injury results in insulitis. This stage is characterized by the appearance of multiple islet cell autoantibodies (ICAs). With progressive β-cell damage, there is a loss of first-phase insulin response followed by the development of glucose intolerance. This phase is referred as prediabetes phase of T1DM and is usually observed 1–2 years prior to the development of overt diabetes. Eventually, with further progression of β-cell destruction (>95 %), there is development of severe hyperglycemia and ketoacidosis. However, the prediabetes phase is usually not appreciated in patients with T1DM because of rapid destruction of β-cells.

11. *What is the islet antibody profile in patients with type 1 DM?*

The islet autoantibodies in patients with T1DM include anti-GAD-65 antibody, islet cell autoantibody (ICA), insulinoma-associated antigen 2 (IA-2) antibody, anti-insulin antibody (IAA), and anti-zinc transporter antibody (ZnT8). One or more of these autoantibodies are present in >95 % of newly diagnosed patients with T1DM, and the presence of ≥2 autoantibodies have a high predictive value for the diagnosis of T1DM. The characteristics of islet antibodies in patients with T1DM are summarized in the table given below.

	Age at diagnosis		**Duration of disease**	
Autoantibody	**<15 years (%)**	**>15 years (%)**	**At diagnosis (%)**	**At 10 years (%)**
ICA	80–85	60–80	85	10
Anti-GAD-65	65	70–80	80	50
IA-2	70–80	40–60	80	50
IAA	30–65	20–35	–	–
ZnT8	–	–	60–80	–

In those with onset of T1DM in childhood, ICA and IA-2 are the most prevalent autoantibodies, while anti-GAD-65 autoantibodies are the most common antibodies in adolescents and adults. Nevertheless, prevalence of autoantibody positivity progressively declines with advancing duration of disease. However, anti-GAD-65 antibody is positive in 50 % of patients even 10 years after the onset of disease.

12. *What is GAD-65?*

Glutamic acid decarboxylase (GAD) is an enzyme which catalyzes the decarboxylation of glutamate to gamma-aminobutyric acid (GABA). There are two isoforms of GAD: GAD65 expressed in the pancreas and GAD67 in the central nervous system. In pancreas, GAD-65 is selectively expressed in β-cells and mediates the synthesis of GABA which inhibits insulin and glucagon secretion in a paracrine manner.

13. *Is there a difference in clinical profile of children with T1DM who have parental history of T2DM?*

    Children with T1DM who have parental history of T2DM have late onset of disease (17.2 vs. 16.1 years), increased prevalence of metabolic syndrome (44 vs. 38 %), and higher HbA1c and triglycerides and require greater dose of insulin as compared to children with T1DM who do not have parental history of T2DM. This suggests that insulin resistance (familial) can modulate the phenotypic expression of T1DM in children who have parental history of T2DM.

14. *What is the risk of developing T1DM in an offspring of a couple with T1DM?*

    Only 10–15 % of patients with T1DM have a family history of T1DM. The risk of developing T1DM in an offspring is higher, if father has T1DM as compared to mother (4.6 % vs. 2 %); however, the risk is 10 % if both parents have T1DM. The risk is approximately 5 % if any sibling has T1DM. If a parent and one sibling have the disease, the risk of developing T1DM in the other sibling is 30 %. The concordance rate for occurrence of T1DM in identical twins is 30–50 %, while in dizygotic twins, it is 6 %.

15. *What are the treatment targets in patients with T1DM for children and adolescents?*

    The treatment targets in patients with T1DM for children and adolescents are summarized in the table given below.

Parameters	**Treatment targets**	
Blood glucose[a]	Pre-meals: 90–130 mg/dl	Bedtime: 90–150 mg/dl
HbA1C[b]	<7.5 %	
Blood pressure	<90th percentile for age, sex, and height	
Lipids	LDL-C <100 mg/dL	

[a]Postprandial blood glucose should be measured if there is a discrepancy between pre-meal glucose values and HbA1C

[b]HbA1c <7 % should be targeted if can be achieved without excessive risk of hypoglycemia. The treatment targets in young patients with T2DM are similar to T1DM in children and adolescents; however; the HbA1c target <7 % seems to be reasonable in young patients with T2DM

16. *What are the treatment modalities available for patients with T1DM?*

    Insulin is the mainstay of treatment for T1DM. Oral antidiabetic drugs have been used as an adjunct to insulin therapy with limited benefits. The treatment

modalities available for the management of T1DM are summarized in the table given below.

Modality	Remarks
Basal–bolus insulin therapy	Near physiological
	Able to achieve target HbA1c in 10–15 %
	Cumbersome
	High glycemic variability
Insulin pumps	Near physiological
	Expensive
	Only 20–30 % achieve target HbA1c
	Mechanical failure poses risk of DKA
	No difference in hypoglycemic events and weight gain as compared to basal–bolus
Metformin	Limited data
	Only in obese T1DM as an adjunct to insulin therapy
Pioglitazone	Not effective
α-glucosidase inhibitor	As an adjunct to insulin therapy
	Limited benefits
DPP4 inhibitor	Experimental as an adjunct to insulin therapy
	Reduces glycemic variability
GLP1 agonist	Experimental as an adjunct to insulin therapy
	Trend toward improvement in HbA1c in those with preserved C-peptide
SGLT 2 inhibitor	Limited and short-term data as an adjunct to insulin therapy
	Higher incidence of euglycemic DKA

17. *Why basal–bolus insulin regimen is preferred over fixed-dose premixed insulin in patients with T1DM?*

Premixed insulin consists of short-acting and intermediate-acting insulin in a fixed proportion, in order to deliver prandial and basal insulin together to minimize the number of injections, thereby providing convenience to the patients. Patients with T1DM are characterized by severe insulin deficiency, and administration of premixed insulin twice a day fails to mimic physiological insulin secretion as it does not adequately cover post-lunch and early morning hyperglycemia. In addition, the premixed insulin regimen is associated with higher glycemic excursions, lower patient's satisfaction, and poor quality of life score as compared to basal-bolus regimen even at the same level of HbA1c. Further, fixed-dose formulation does not allow the flexibility to adjust the dose of regular and NPH insulin independently. Therefore, in patients with T1DM, premixed insulin twice a day result in higher glycemic variability, frequent episodes

of hypoglycemia, and failure to achieve target HbA1c as compared to basal–bolus regimen. On the contrary, basal–bolus regimen mimics a near physiological insulin profile, and hence glycemic variability is less, and glycemic targets can be achieved more easily with better quality of life.

18. *Why is there failure to achieve glycemic targets despite intensive insulin therapy in patients with T1DM?*

Multiple daily injection or basal–bolus therapy helps to achieve glycemic targets only in 10–15 % of patients with T1DM, and even with the use of insulin pump therapy, only 30 % of individuals attain glycemic targets. Absolute insulin deficiency and intra-and interindividual variability in absorption of insulin are associated with wide swings in blood glucose levels which result in failure to achieve target HbA1c in these patients. In addition, concurrent comorbidities like gastroparesis, autonomic neuropathy, and celiac disease may also result in poor glycemic control due to mismatch between nutrient absorption and insulin action. Further, deterioration of glycemic control is often seen in adolescents due to increase in insulin resistance as a result of gonadal steroids-mediated GH–IGF1 surge.

19. *What are the determinants of intra-and interindividual variability in absorption of insulin?*

The major determinants of intra-and interindividual variability in insulin absorption include site of administration, type of insulin, and dose of insulin. The site of insulin administration determines the rate of absorption; however, it does not influence the extent of absorption. The abdomen is the preferred site as the rate of absorption is faster and less variable as compared to the thigh and arm. Other determinants of insulin absorption from injection site include subcutaneous blood and lymph flow and the first-pass catabolism (proteases in subcutaneous tissue). Regular insulin and rapid-acting insulin analogues do not have much difference in variability of absorption, whereas long-acting analogues have significant variability in absorption (NPH > glargine > detemir). Larger doses of insulin administered as a single injection have a greater variability in absorption as compared to smaller doses of insulin.

20. *Why are patients with T1DM predisposed to hypoglycemia?*

Patients with T1DM are predisposed to recurrent and severe hypoglycemia. The mechanisms for recurrent hypoglycemia include absolute insulin deficiency, impaired regulation of glucagon secretion, and autonomic failure. The first-line of defense to counteract hypoglycemia is suppression of insulin secretion, which is impaired in patients with T1DM due to absolute insulin deficiency. The second-line of defense against hypoglycemia is appropriate glucagon secretion. The key regulator of glucagon secretion

during hypoglycemia is reduction in intra-islet insulin which is a signal to α-cell to secrete glucagon; this is defective in patients with T1DM as a result of absolute insulin deficiency. In addition, autonomic neuropathy due to long-standing diabetes also impairs glucagon secretion and predisposes for neuroglycopenia.

21. *A 25-year-old man with T1DM who is on basal–bolus insulin regimen (lispro and glargine) had a glucose profile: FPG 200 mg/dl, post-breakfast 180 mg/dl, post-lunch 140 mg/dl, and post-dinner 130 mg/dl. He denies any episode of hypoglycemia. How to proceed further?*

Predominant abnormality in glucose profile of the index patient is fasting hyperglycemia. Fasting hyperglycemia may occur as a result of early morning hypoglycemia (Somogyi phenomenon) or hyperglycemia (dawn phenomenon). Therefore, 0300–0400h blood glucose estimation is recommended to differentiate between them. Fasting hyperglycemia due to Somogyi phenomenon requires reduction in insulin doses, whereas exaggerated dawn phenomenon needs an increase in insulin doses. The index patient had 0300h blood glucose of 60 mg/dl suggestive of Somogyi phenomenon as a cause for the fasting hyperglycemia; hence, the dose of glargine was reduced.

22. *When should treatment be initiated for hypertension in children and adolescents with T1DM?*

In children and adolescents with T1DM having systolic or diastolic BP consistently $\geq 90^{th}$ percentile for age, sex, and height, lifestyle modification including dietary intervention and exercise should be initiated. If target blood pressure is not achieved within 3–6 months, pharmacological intervention should be considered. If SBP or DBP is consistently $\geq 95^{th}$ percentile for age, sex, and height, pharmacological treatment along with lifestyle modification should be initiated. ACE inhibitors are the drug of choice for hypertension in patients with T1DM; however, if not tolerated, ARBs should be considered.

23. *When to add statin in children and adolescents with T1DM?*

Lipid profile should be measured in children and adolescents with T1DM ≥2 years of age after optimum blood glucose control. If LDL-C is <100 mg/dl, then five yearly monitoring is recommended. In children between 2 and 10 years of age having LDL-C>100 mg/dl, medical nutrition therapy (MNT) aimed at restricting saturated fat and dietary cholesterol intake (7 % of total calories and 200 mg/day, respectively) is indicated. Statin therapy is recommended in children older than 10 years (paucity of data <10 years), if LDL-C is >160 mg/dl or >130 mg/dl with one or more

cardiovascular disease (CVD) risk factors, after optimal glycemic control and MNT. Lipid profile should be monitored yearly thereafter. However, the treatment recommendations for hypertriglyceridemia in children and adolescents with T1DM are not defined. The treatment strategies for dyslipidemia in children and adolescents with T1DM are summarized in the table given below.

Age	LDL-C	Therapy
2–10 years	>100 mg/dl	MNT
>10 years	100–130 mg/dl	MNT
	130–160 mg/dl with ≥1 CVD risk factor	MNT + statin
	>160 mg/dl	MNT + statin

24. *When to screen for microvascular complications in patients with T1DM?*

The children and adolescents with T1DM of ≥5 years of duration should undergo annual screening for albuminuria by estimation of albumin to creatinine ratio (ACR) in a random spot urine sample. The presence of an elevated ACR >30 mg/g should be reconfirmed with repeat testing twice within 6 months duration (three samples). Treatment with ACE inhibitors or ARBs should be considered if two out of three tests are abnormal. Annual screening for retinopathy is recommended by dilated fundus examination in children and adolescents with T1DM who have duration of diabetes ≥3 years and either older than 10 years or entered into puberty (whichever is earlier). Annual comprehensive foot examination is recommended at the onset of puberty or at age ≥10 years, whichever is earlier, once the duration of diabetes is ≥5 years.

25. *When should a child of 9 years of age with T1DM of 5 years of duration with no pubertal development be screened for diabetic retinopathy?*

Annual screening for diabetic retinopathy is recommended at the onset of puberty or at age ≥10 years, whichever is earlier, once the duration of diabetes is ≥3 years. The index child has duration of diabetes of 5 years but does not have any pubertal sign; therefore, he should be screened at the age of 10 years.

26. *What is the effect of puberty on diabetic retinopathy?*

Onset and progression of puberty is associated with development and worsening of diabetic retinopathy. The postulated mechanisms include peripubertal GH–IGF1 surge, rising level of gonadal steroids, and alterations in sex hormone-binding globulin (SHBG). GH–IGF1 surge is associated with deleterious effect on vessel wall, which include vascular cell proliferation and

angiogenesis. Gonadal steroids, testosterone, lead to rise in blood pressure, worsening of dyslipidemia, and increase in vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1). Estrogen also increases the expression of vascular endothelial growth factor (VEGF) and thereby promotes angiogenesis. Decline in SHBG during peripubertal period in boys results in increase in free testosterone which further worsens diabetic retinopathy.

27. *Why is there worsening of diabetic retinopathy after intensive insulin therapy?*

Intensive insulin therapy is associated with initial worsening of diabetic retinopathy followed by slow progression of the disease. The initial worsening of diabetic retinopathy after initiation of intensive insulin therapy has been attributed to increased breakdown of blood–retinal barrier (consequent to upregulation of VEGF and HIF 1α), retinal ischemia due to loss of glucose-mediated vasodilatation, and increased neovascularisation as a result of elevated IGF1 consequent to hyperinsulinemia. Further, increased IGF1 induces VEGF expression which also contributes to angiogenesis. Therefore, periodic fundus examination should be performed after initiation of intensive insulin therapy.

28. *What is limited joint mobility?*

Limited joint mobility is a manifestation of long-standing uncontrolled severe hyperglycemia and is seen in patients with both T1DM and T2DM. Clinically, it is appreciated by either "prayer sign" or "table sign." This occurs as a result of abnormal cross-linking of collagen fibers due to accumulation of advanced glycosylation end products. Limited joint mobility correlates with diabetic microvascular complications particularly diabetic retinopathy (Fig. 12.2).

Fig 12.2 Limited joint mobility in a patient with type 1 diabetes illustrated as "prayer sign"

29. *How to define diabetic ketoacidosis?*

The diagnostic criteria for diabetic ketoacidosis (DKA) include blood glucose >250 mg/dl, ketonemia/ketonuria [plasma β-(OH) butyrate >3 mmol/L, plasma acetone/acetoacetate positive in >1:2 dilution and/or urine ketones ≥3+], and blood pH <7.3 with serum bicarbonate <15 mEq/L. The presence of ketoacidosis in a diabetic patient with blood glucose <250 mg/dl is termed as euglycemic diabetic ketoacidosis. The common causes of euglycemic DKA include pregnancy, starvation, alcohol intake, and suboptimal treatment with insulin. Recently, the use of SGLT2 inhibitors has been shown to be associated with euglycemic DKA in both patients with T1 and T2DM.

30. *What are the biochemical parameters to be monitored in a patient with DKA?*

The biochemical parameters to be monitored in a patient with DKA include blood glucose, arterial pH, serum anion gap, and potassium. Hourly monitoring of blood glucose is recommended for initial 24h to titrate the rate of insulin infusion. When blood glucose level is reduced to <200 mg/dl, 5 % dextrose infusion should be added to prevent hypoglycemia, and the dose of insulin infusion is to be reduced. Arterial pH and serum anion gap should be monitored every four to six hours, and with effective treatment, arterial pH increases and serum anion gap progressively decreases. Serum K^+ should be monitored at baseline and every four to six hours. The presence of hypokalemia at presentation suggests severe depletion of body stores of potassium, and hypokalemia may worsen after insulin infusion therapy. Monitoring of plasma β-(OH) butyrate may be useful, but estimation of urine ketones by Rothera's test or Ketostix may not be rewarding. Failure to respond to therapy suggests inadequate fluid replacement, suboptimal insulin therapy, occult infection, or other causes of metabolic acidosis (lactic acidosis or uremia).

31. *A patient with T1DM who presented with DKA was optimally managed with insulin infusion and fluids with normalization of arterial pH and anion gap; however, ketonuria is persisting. How to interpret?*

Ketone bodies (acetone, acetoacetate, and β-hydroxybutyrate), being lipophilic, accumulate in adipose tissue. The slow release of ketone bodies into circulation from adipose tissue explains the persistence of ketonuria even after recovery from DKA. Further, in patients of DKA who present with severe dehydration or shock, acetoacetate is converted to β-hydroxybutyrate, and after recovery from DKA, β-hydroxybutyrate is rapidly converted to acetoacetate and acetone and excreted in urine. Therefore, during recovery phase of DKA, serum β-hydroxybutyrate level progressively declines, whereas urinary ketones (acetone, acetoacetate) persist or may even increase.

32. *A 16-year-old boy who is a known patient of T1DM presented with altered sensorium. On examination, he had mild dehydration and BP was 110/70 mmHg. His blood glucose was 280 mg/dl, arterial pH 7.28, bicarbonate 12 mEq/L, and urine ketone 3+. Serum sodium was 132 mEq/L, potassium 4 mEq/L, and urea 40 mg/dl. How to proceed further?*

The index patient presented with DKA and altered sensorium. Patients with DKA usually do not present with altered sensorium unless accompanied with marked dehydration, dyselectrolytemia, or severe acidosis. DKA-related altered sensorium is usually associated with hyperosmolality (serum osmolality >320 mOsm/Kg), which results in marked cerebral intracellular dehydration. In the index case, calculated serum osmolality was 295 mOsm/Kg; there was no electrolyte abnormality and acidosis was mild. Therefore, alternative causes for altered sensorium should be actively sought in the index patient including meningitis, cortical vein thrombosis, stroke, and rhinocerebral mucormycosis. In addition, rapid reduction in blood glucose can also lead to altered sensorium in a patient recovering from DKA due to cerebral edema as a result of osmotic disequilibrium. The index patient was evaluated and was found to have concurrent pyogenic meningitis.

33. *How do SGLT2 inhibitors induce DKA?*

Few cases of euglycemic DKA have been reported with the use of SGLT2 inhibitors in patients with both T1DM and T2DM particularly during stress. Decrease in blood glucose due to glucosuria as a result of SGLT2 inhibitors use led to deceptive decrease in insulin doses and consequent development of DKA in these patients during the period of stress (a state of heightened insulin resistance). This is because of relatively lower portal concentration of insulin is required to suppress hepatic glucose output (fasting hyperglycemia) as compared to inhibition of ketosis. In addition, hyperglucagonemia associated with the use of SGLT2 inhibitors also favors ketogenesis. Further, hypovolemia due to water and Na^+ loss resulting from SGLT2 inhibition lead to increased counterregulatory hormones secretion (cortisol and epinephrine), and consequent increased lipolysis and ketogenesis.

34. *What are the infections specific to diabetes?*

Patients with diabetes are predisposed for certain infections which include emphysematous pyelonephritis, emphysematous cholecystitis, malignant otitis externa, rhino–orbito–cerebral mucormycosis, and liver abscess. The increased risk for these infections in patient with diabetes is due to glucotoxicity-mediated lazy leukocyte syndrome and impaired humoral and cellular immunity (Fig. 12.3).

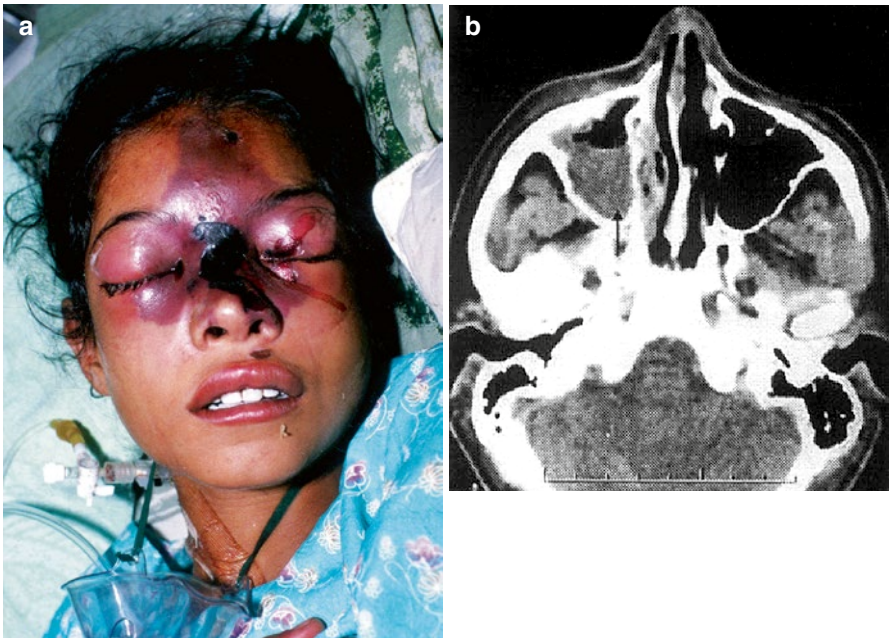

Fig 12.3 (**a**) A patient of T1DM having bilateral ptosis, periorbital cellulitis, and black necrotic patch over the nose due to rhino–orbital mucormycosis, (**b**) CT scan of the paranasal sinuses showing isodense lesion in right ethmoid gallery and maxillary sinus (*black arrow*) in the same patient

35. *What is the most common age of presentation of T2DM in young individuals?*

    Increased prevalence of childhood obesity as a result of sedentary lifestyle and consumption of calorie-dense food predisposes for the early development of diabetes. Approximately 90% of young individuals with T2DM present between 10 and 18 years of age. During puberty, gonadal steroids-mediated GH–IGF1 surge and increase in adipose tissue mass result in worsening of insulin resistance and consequent development of hyperglycemia in genetically predisposed individuals.

36. *An 18-year-old boy was incidentally detected to have diabetes. He was obese (BMI-32 Kg/m^2) and had grade 4 acanthosis nigricans. His father has T2DM. Biochemistry revealed fasting plasma glucose of 190 mg/dl, postprandial glucose 220 mg/dl, and HbA1c 8.8%. How to manage further?*

    The presence of features of insulin resistance (obesity and acanthosis nigricans), family history of T2DM, incidental detection, and peripubertal onset of the disease in the index case point to a diagnosis of T2DM in the young. He

should be carefully examined for other features of insulin resistance (double chin, skin tags, and central obesity), hypertension, and xanthelasmas. He should also be evaluated for dyslipidemia and microvascular complications. Female patients should be looked for features of PCOS. The index patient was advised to follow lifestyle modification and was initiated on metformin 1 g twice a day after meals. At 3 months of follow-up, he lost 5 Kg weight and his HbA1c was reduced to 6.8 % (Fig. 12.4).

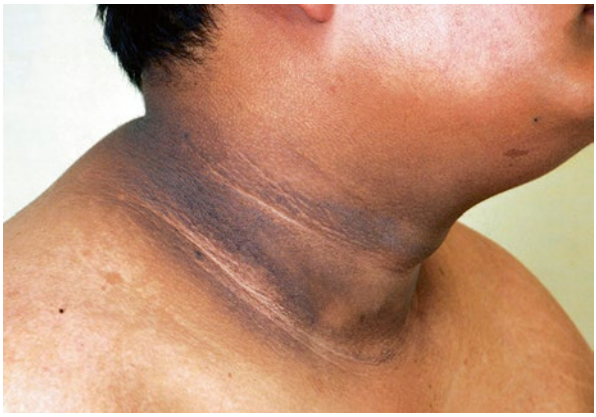

Fig 12.4 Grade IV acanthosis nigricans in an adolescent with T2DM

37. *What are the monogenic forms of diabetes?*

Monogenic disorders are characterized by mutation of a single gene. The monogenic forms of diabetes are rare and contribute only 1–2 % of individuals with diabetes. Monogenic forms of diabetes could be due to defects in β-cell function (MODY, neonatal diabetes, mitochondrial diabetes, and mutant insulin syndromes) or defects in insulin action (congenital lipodystrophic diabetes, type A insulin resistance syndrome, leprechaunism, and Rabson–Mendenhall syndrome).

38. *What is maturity onset diabetes of the young?*

Maturity onset diabetes of the young (MODY) includes a heterogeneous group of monogenic disorders and is clinically characterized by the early onset of diabetes (<25 years of age), vertical transmission of disease in three generations (autosomal dominant mode of inheritance) with at least one family member with onset of disease <25 years of age, and not requiring insulin for at least initial 5 years. Patients with MODY have a primary defect in β-cell function and are usually nonobese and are negative for islet autoantibodies.

39. *What are the various subtypes of MODY?*

Various subtypes of MODY are classified on the basis of mutations in genes implicated in the development of the pancreas, insulin synthesis, and secretion. The different subtypes of MODY are summarized in the table given below.

Types	Gene	Function
MODY 1	Hepatocyte nuclear factor-4α (HNF 4A)	Transcription factor
MODY 2	Glucokinase (GCK)	Glycolytic enzyme
MODY 3	Hepatocyte nuclear factor-1α (HNF 1A)	Transcription factor
MODY 4	Insulin promoter factor 1/pancreas–duodenum homeobox protein 1 (IPF1/PDX1)	Transcription factor
MODY 5	Hepatocyte nuclear factor-1β (HNF 1 B)	Transcription factor
MODY 6	Neurogenic differentiation 1 (NEUROD1)	Transcription factor
MODY 7	Kruppel-like factor 11 (KLF11)	Transcription factor
MODY 8	Carboxyl ester lipase (CEL)	Lipase
MODY 9	Paired-box gene 4 (PAX4)	Transcription factor
MODY 10	Insulin (INS)	Insulin
MODY 11	Tyrosine kinase, B-lymphocyte specific (BLK)	Transcription factor

40. *What is the most common form of MODY?*

Among the various subtypes of MODY, type 3 is the most common form. MODY 3 is due to inactivating mutations in HNF 1A gene, which result in reduced β-cell mass and impaired insulin secretion. During adolescence and early adulthood, these individuals have normal fasting plasma glucose, but have hyperglycemia during oral glucose tolerance test. However, patients with MODY 3 show progressive decline in β-cell function and have a high prevalence of microvascular complications. The characteristic features of MODY 3 include low renal threshold for glucose (renal glycosuria), increased proinsulin to insulin ratio, and good therapeutic response to sulfonylureas. Renal glycosuria in these patients is possibly related to decrease in SGLT 2 expression, which is also regulated by HNF 1A gene.

41. *How to differentiate MODY from type 2 diabetes?*

As both MODY and type 2 diabetes can present in young individuals with non-ketotic hyperglycemia, it is important to differentiate between them as these

disorders have different etiology, treatment strategy, associated comorbidities, and prognosis. The important differentiating features between the two disorders are summarized in the table given below.

Parameters	MODY	T2DM in young
Inheritance	Monogenic (autosomal dominant)	Polygenic
Family history	Present in ≥3 generation	Present
Penetrance	80–95 %	Variable (10–40 %)
Pathophysiology	β-cell dysfunction	Insulin resistance and β-cell dysfunction
Phenotype	Nonobese	Commonly obese
Features of insulin resistance	Absent	Commonly present
Treatment	Sulfonylureas	Metformin

42. *Why does glucokinase gene mutation lead to MODY in some and neonatal diabetes in others?*

Glucokinase is highly expressed in pancreatic β-cells and hepatocytes. It converts glucose to glucose–6–phosphate by the transfer of phosphate from ATP, the rate-limiting step in glucose metabolism. Therefore, glucokinase is a key enzyme which regulates the rate of entry of glucose into the glycolytic pathway and its subsequent metabolism in β-cell. Heterozygous mutations of glucokinase gene result in partial deficiency of this enzyme and manifests as MODY 2, whereas homozygous mutations of this gene result in permanent neonatal diabetes mellitus.

43. *What are the characteristics of MODY 2?*

In contrast to most other forms of MODY where onset of hyperglycemia occurs during or after adolescence, MODY 2 is characterized by the presence of hyperglycemia since birth. In addition, these individuals have mildly elevated fasting plasma glucose and minimal rise in glucose after OGTT (Δ rise in glucose <54 mg/dl). The most affected individuals are asymptomatic and are detected during screening (e.g., during pregnancy). Microvascular complications are rare in individuals with MODY 2 and majority can be controlled on diet alone.

44. *How do hepatocyte nuclear transcription factors regulate insulin secretion and glucose metabolism?*

Hepatocyte nuclear transcription factors are expressed not only in the liver but also in the pancreatic β-cells and urogenital tissues. Hepatocyte nuclear transcription factors include HNF-1α, HNF-1β, and HNF-4α. These proteins regulate tissue-specific gene expression and thereby determine growth and development, as well as facilitate metabolic signaling in these organs. During embryogenesis, these transcription factors act in concert to promote islet development and regulate the expression of insulin gene, and genes-encoding proteins which are linked to insulin secretion.

45. *What are the characteristics of MODY 1?*

The clinical features of MODY 1 are similar to that of MODY 3 with onset of diabetes during or after adolescence and progressive decline in β-cell function. Similar to patients with MODY 3, approximately one-third of patients with MODY 1 also require insulin for optimal glycemic control. The characteristic features of MODY 1 are low serum triglyceride, apolipoproteins AII and CIII, and lipoprotein (a). These lipid abnormalities occur as a result of decreased HNF 4α which is expressed in hepatocytes and are involved in regulation of lipoprotein synthesis in the liver. In addition, both micro- and macrovascular complications are frequently present in patients with MODY 1.

46. *What are the characteristics of MODY 5?*

MODY 5 is a result of mutation of gene-encoding HNF-1β and is characteristically associated with developmental abnormalities of urogenital tract like renal cysts, vaginal aplasia, and rudimentary/bicornuate uterus. These abnormalities are related to the expression of HNF-1β in urogenital epithelial tissues. In addition, MODY 5 is commonly associated with nondiabetic renal failure (75 %) due to hypoplastic glomerulocystic kidney disease, oligomeganephronia, or renal dysplasia. Diabetes is prevalent in approximately 60 % of individuals and occurs at an early age. MODY 5 is unique in that it is the only form of MODY where insulin resistance has been reported along with β-cell dysfunction.

47. *What is the role of NEUROD1 in MODY 6?*

NEUROD1 is a transcription factor which is involved in the regulation of growth and development of islet and neural tissues (cerebellum, hippocampus, and inner ear). Homozygous inactivating mutations of genes encoding NEUROD1 result in permanent neonatal diabetes and cerebellar hypoplasia, whereas heterozygous mutations result in MODY 6 with subtle or no neurological abnormalities in these individuals.

48. *How to treat patients with MODY?*

The basic pathophysiological defect in patients with MODY is β-cell dysfunction (rather than insulin resistance), and some subtypes of MODY are extremely sensitive to sulfonylureas. Therefore, the treatment of choice in patients with MODY is sulfonylureas. As insulin sensitivity is normal, insulin sensitizers have no role in patients with MODY. Patients with MODY 2 have mild hyperglycemia and usually respond to lifestyle modification, while those with MODY 1 and 3 require sulfonylureas for glycemic control; however, one-third of patients with MODY 1 and 3 may require insulin. In addition, glinides have also been used in patients with MODY 3 with favorable results. Patients with MODY 10 require insulin as they have insulin gene defect. There are anecdotal reports of the use of DPP4 inhibitor/GLP1 receptor agonists in the management of MODY 3 (HNF-1α) with limited benefits.

49. *What is latent autoimmune diabetes of adults?*

Latent autoimmune diabetes of adults (LADA) is also known as type 1.5 diabetes as it shares features of both T1DM and T2DM. The onset of diabetes after 30 years of age, non-requirement of insulin for at least 6 months after the diagnosis, and evidence of islet cell autoimmunity are the characteristic features of LADA. However, some individuals may present between 25 and 35 years of age as shown in the landmark United Kingdom Prospective Diabetes Study (UKPDS). These individuals are often diagnosed to have type 2 diabetes and started on oral antidiabetic drugs; however, most of these individual will require insulin within a few years.

50. *What are the differentiating features between T1DM and LADA?*

The differentiating features between T1DM and LADA are summarized in the table given below.

Parameters	**T1DM**	**LADA**
Age of onset	Childhood	Young adults (>30 years)
Presentation with DKA	Common	Rare
Insulin dependence	Since diagnosis	Usually after 6 months of diagnosis
HLA association	HLA DR 3, DR 4 (increased expression of destructive genotype)	HLA DQ A1, B1 (decreased expression of protective genotype)
Islet autoimmunity	Multiple autoantibodies (≥2) present at diagnosis	Usually single autoantibody positive (GAD65 or ICA)[a]

[a]*GAD65* glutamic acid decarboxylase, *ICA* islet cell autoantibody

51. *Is there any difference in antibody profile of patients with LADA and T1DM?*

The presence of multiple (≥2) islet autoantibodies (ICA, GAD65, IA-2, IAA, and ZnT8) at diagnosis is usually a feature of T1DM, while patients with LADA commonly have single autoantibody (ICA or GAD65) at diagnosis. Approximately 90 % of individuals with T1DM who are ICA positive, are also positive for anti-GAD 65 antibody, whereas only <20 % of individuals with LADA who have ICA positivity have anti-GAD 65 antibody. In addition, IA-2 and IAA are more commonly present in patients with T1DM than in LADA.

52. *Why is β-cell destruction tardy in LADA?*

The immunological mechanisms implicated in β-cell destruction in patients with LADA and T1DM are not well understood. However, certain differences in islet antigenicity and T-cell response to islet proteins (of molecular weight 65–90 and 21–38 kDa) may explain the slow destruction of β-cells in patients with LADA as compared to T1DM.

53. *What are the predictors of β-cell failure in LADA?*

The presence of multiple islet autoantibodies and higher antibody titer predict the progression of β-cell failure in patients with LADA. Patients with the presence of ≥2 antibodies (ICAs, GADAs, IA-2As, or IAA) at diagnosis have severe impairment in β-cell function over 5 years, whereas those who are only ICA or GAD65 positive at diagnosis have decline in β-cell function by 12 years. However, those who have only IA-2A positivity at diagnosis do not have decline in β-cell function over 12 years. Further, patients who are ICA negative at diagnosis and develop ICA later have a progressive decline in fasting plasma C-peptide, thereafter. ICA positivity has a positive predictive value (PPV) of 74 %, whereas GAD65 and/or IA-2As positivity have PPV of 47 % to predict β-cell failure. Further, high GAD65 titers (41.4 U/ml) and/or IA-2As index (>2.7) predict a complete β-cell failure, whereas low GAD65 titer predicts slowly progressive β-cell failure.

54. *How to differentiate LADA from type 2 DM?*

Age of onset of diabetes >30 years, lean body habitus (BMI<25 Kg/m^2), acute onset of osmotic symptoms (e.g., polydypsia/polyuria/weight loss), the presence of autoimmune disorders in the patient or family, the absence of family history of T2DM, high fasting plasma glucose, and early failure to oral antidiabetic drugs should raise a suspicion of LADA. On the contrary, patients with

T2DM are usually obese, asymptomatic, or mildly symptomatic and may have a family history of T2DM. Further, the presence of features of insulin resistance (e.g., acanthosis nigricans, skin tags, double chin) and a good response to oral antidiabetic drugs also support the diagnosis of T2DM.

55. *What are the treatment strategies for patients with LADA?*

LADA is defined by lack of insulin requirement at the onset of diabetes; however, many individuals will require insulin for adequate glycemic control within few years. Insulin therapy should be offered to patients with LADA at the onset of disease to preserve endogenous β-cell function by providing β-cell rest and suppression of insulitis through immunomodulation. Exogenous insulin therapy results in decreased expression of β-cell autoantigens and may activate T_{reg} cells and inhibit autoreactive T cells, thereby delaying the ongoing immunoinflammatory destruction of β-cells. Preservation of residual β-cell function helps to prevent wide swings in blood glucose and decrease the risk of hypoglycemia. Sulfonylureas are to be avoided as these drugs enhance the expression of autoantigens in β-cells and hasten the immunoinflammatory process. Metformin can be used in some patients who have features of insulin resistance, particularly in obese individuals.

56. *What is "fibrocalculous pancreatic diabetes"?*

"Fibrocalculous pancreatic diabetes" (FCPD) is characterized by abdominal pain, exocrine pancreatic insufficiency, diabetes mellitus (non-ketotic), and pancreatic calcification in the absence of alcoholism and gall stone disease. FCPD has been described almost exclusively from developing countries of tropical world. In the current classification of diabetes, FCPD is not considered as a separate entity and is classified under secondary diabetes (Fig. 12.5).

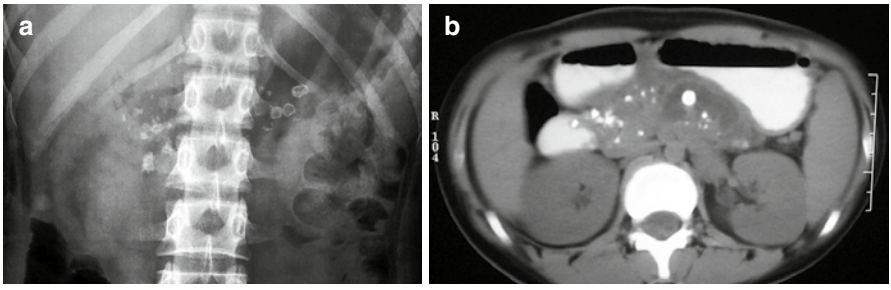

Fig 12.5 (**a**) X-ray of abdomen showing pancreatic calcification and (**b**) CT abdomen showing hyperdense lesions in the pancreas suggestive of pancreatic calcification in a patient with FCPD

57. *What is the etiopathogenesis of FCPD?*

The etiopathogenesis of FCPD remains elusive; however, various theories have been proposed. FCPD has been reported predominantly from developing countries where malnutrition is widespread. However, the cause and effect relationship between the two is not established. Besides malnutrition, consumption of cassava (*Manioc esculenta*) as a staple food in some part of the tropics was shown to be associated with chronic pancreatitis and FCPD. The alkaloids linamarin and lotaustralin present in cassava produce cyanide compounds which are detoxified by sulfur-containing amino acids. These amino acids are deficient in individuals with malnutrition; therefore, accumulation of cyanogens result in chronic pancreatitis. In addition, oxidative stress and genetic factors (SPINK 1) have also been incriminated. Increased secretion of a putative peptide termed as pancreatic stone protein has also been suggested for the development of pancreatic calcification.

58. *What are the clinical manifestations of FCPD?*

The classic triad of FCPD comprises of abdominal pain, steatorrhea, and diabetes. Hyperglycemia is usually severe but is not accompanied with ketosis. Microvascular complications are common; however, macrovascular complications are rare. This dichotomy is possibly due to lack of hypertension and atherogenic lipid profile.

59. *Why is ketosis uncommon in FCPD?*

Diabetic ketosis is uncommon in patients with FCPD and has been reported in <15 % of patients. Despite severe hyperglycemia, ketosis is less common because of the presence of residual β-cell function, loss of α-cell function (decreased glucagon), reduced availability of non-esterified fatty acids due to lack of subcutaneous fat, and carnitine deficiency associated with malnutrition.

60. *What are the causes of pancreatic calcification?*

Pancreatic calcification is seen in patients with chronic alcoholic pancreatitis, primary hyperparathyroidism, FCPD, kwashiorkor, cystic fibrosis, hereditary pancreatitis, and pancreatic tumor. Large intraductal calcification is characteristic of FCPD, while small intraductal and parenchymal calcification is a feature of chronic alcoholic pancreatitis.

61. *How to manage a patient with FCPD?*

Patients with FCPD require management for hyperglycemia, exocrine pancreatic insufficiency, and chronic abdominal pain. For glycemic control, majority

(85 %) of patients require insulin therapy. Incretin-based therapies should be avoided in these individuals. Pancreatic enzyme supplements are recommended in patients with steatorrhea, and fat soluble vitamins should be adequately replenished. For pain abdomen, analgesics (non-opioid/opioid) may be used. If pain is unbearable or nonresponsive to medical management, surgical intervention should be considered.

62. *What is ketosis-prone diabetes?*

Ketosis-prone diabetes refers to a heterogeneous group of disorders with propensity to develop diabetic ketosis/ketoacidosis either at onset or during the course of disease. The classification of ketosis-prone diabetes (KPD) based on the presence or absence of autoimmunity (A + or A-) and β-cell function (β+ or β-) is summarized in the table given below.

Types of KPD	Autoimmunity	β-cell function
Type 1A (A +β−)	+	−
Type 1B (A −β−)	−	−
Type 2A (A +β+)	+	+
Type 2B (A − +)	−	+

This classification was based on the characteristics of adult patients admitted with DKA and followed up thereafter. However, this classification adds confusion to the existing nomenclature of diabetes and has limited utility in clinical practice.

63. *What is Flatbush diabetes?*

Flatbush diabetes was described in African–American young adults residing in an area named Flatbush in New York City, USA. These individuals presented with DKA, required insulin for a short time, and subsequently were able to discontinue insulin therapy, while maintaining euglycemia (with or without OHA) for several months to years. The majority of patients were overweight/obese (67 %) and had strong family history of diabetes (88 %). Evidence of islet autoimmunity was conspicuously absent, and β-cell function was relatively preserved in these patients.

64. *Why do patients with Flatbush diabetes present with DKA despite preserved β-cell function?*

The key determinant of DKA despite preserved β-cell function in patients with Flatbush diabetes remains elusive. The mechanisms proposed include severe oxidative stress, and glucotoxicity-mediated β-cell dysfunction and insulin resistance. The high prevalence of G6PD deficiency in these patients

has also been implicated in worsening of β-cell function due to failure of β-cell to combat oxidative stress. With intensive insulin therapy, glucotoxicity and oxidative stress are ameliorated which result in restoration of β-cell function and consequent insulin independence in majority of these patients during follow-up.

65. *What is the natural history of Flatbush diabetes?*

After resolution of DKA, approximately 50 % of the patients are able to discontinue insulin therapy within 6 months; however, 80 % of these patients require oral antidiabetic drugs (sulfonylurea or metformin) and the rest 20 % could maintain euglycemia only on lifestyle modification at 1 year of follow-up. Recurrence of DKA is rare (2 %) in these patients.

66. *What is neonatal diabetes mellitus?*

The onset of diabetes mellitus before 6 months of age is termed as neonatal diabetes (NDM). However, 5–10 % of children with NDM may present between 6 and 12 months of age. Neonatal diabetes is classified as transient NDM (TNDM), permanent NDM (PNDM), or syndromic NDM. Forty-five percent of infants with NDM have transient diabetes, 45 % have permanent NDM, and 10 % have syndromic NDM. NDM is an uncommon disorder with prevalence of 1 case per 300,000–500,000 live births.

67. *Why is T1DM uncommon before 6 month of age?*

T1DM is uncommon before the age of 6 months possibly because immune system is immature to elicit an immune response against environmental antigens. However, infants with IPEX syndrome (immune dysfunction, polyendocrinopathy, enteropathy, X-linked syndrome), a form of neonatal diabetes, can present with immune-mediated diabetes within 6 months of age.

68. *What is transient neonatal diabetes mellitus?*

Transient neonatal diabetes mellitus (TNDM) usually manifests within first several days or weeks of life and resolves by 12 weeks of life. Children with TNDM present with failure to thrive, increased thirst, and frequent urination. Although the blood glucose levels are very high, ketonemia is extremely rare. Half of the infants with TNDM may have relapse of diabetes during adolescence and early adulthood.

69. *What are the causes of transient neonatal diabetes mellitus?*

Defective growth and development of β-cell during embryogenesis and in fetal life results in decreased insulin secretion and consequently hyperglyce-

mia. Overexpression of genes (*ZAC* and *HYMAI*) at 6q24 locus is the most common cause of TNDM. Normally, genes at 6q24 locus which are inherited from the mother undergo imprinting (silencing), whereas paternal alleles remain active and are responsible for growth and development of β-cell during intrauterine life. However, duplication of paternal allele or loss of DNA methylation of maternal allele results in two copies of genes in active form, which result in inhibition of β-cell growth and development. Other mutations responsible for TNDM include hepatocyte nuclear factor-1β (HNF1β), *KCNJ11*, and *ABCC8*. The latter two mutations contribute to approximately 25 % of cases of TNDM. The resolution of diabetes in these children is possibly attributed to partial defect in metabolic signaling pathway involved in glucose-mediated insulin secretion and progressive maturation of "glucose-β-cell axis" with increasing age.

70. *What are the causes of permanent neonatal diabetes mellitus?*

Permanent neonatal diabetes mellitus (PNDM) is characterized by onset of hyperglycemia early in life without an intervening period of remission. PNDM is due to defect in insulin secretion and/or impaired β-cell growth and development. The most common cause of PNDM is heterozygous mutations in ATP-sensitive K^+ channel (K_{ATP}). KCNJ11 gene mutations account for 30 % of patients with PNDM, whereas ABCC8 and insulin gene mutation contribute 10 % each to PNDM. Further, homozygous mutations in *PDX1/IPF-1* and glucokinase (*GCK*) result in PNDM, while heterozygous mutations of these genes lead to MODY.

71. *How does mutations in ATP-sensitive K^+ channel (K ATP) cause neonatal diabetes?*

The ATP-sensitive potassium channel (K_{ATP}) in pancreatic β-cells is formed by four Kir6.2 subunits and four SUR1 subunits. Kir6.2 is encoded by *KCNJ11* gene and SUR1 by *ABCC8* gene. The Kir6.2 subunits form the central pore and are surrounded by SUR1 subunits. The K_{ATP} channel is a link between glucose metabolism and insulin secretion. Entry of glucose into the β-cell generates ATP through glycolysis and increases ATP/ADP ratio, which results in closure of K_{ATP} channel (preventing K^+ efflux) leading to depolarization of β-cell membrane. This result in opening up of voltage-dependent calcium channel and allow the entry of calcium from extracellular fluid into β-cell, thereby initiating the process of insulin release by exocytosis. Activating mutations of *KCNJ11* or *ABCC8* reduces the sensitivity of K_{ATP} channel to ATP. This results in persistently opened up K_{ATP} channel, and consequent inhibition of downstream cascade of events involved in insulin secretion, thereby leading to decrease in insulin secretion and neonatal diabetes.

72. *How to treat neonatal diabetes mellitus?*

Insulin should be used for initial metabolic control in infants with NDM, and as very small doses of insulin are required in these infants, therapy with insulin pump may be preferred. Once glycemic control is attained, majority of patients with *KCNJ11* (90 %) and *ABCC8* mutations (85 %) can be switched to sulfonylureas (e.g., glibenclamide). Sulfonylureas bind to SUR1 subunits at β-cell membrane and result in the closure of K_{ATP} channel through ATP-independent mechanism, thereby promoting insulin secretion. Glibenclamide is initiated at a dose of 0.05 mg/Kg/day (in divided doses) and can be titrated, if required. Sulfonylurea therapy is associated with better glycemic control with lower risk of hypoglycemia as compared to insulin in infants with NDM. This is attributed to sulfonylurea-mediated improvement in β-cell sensitivity to incretins.

73. *What is lipodystrophic diabetes?*

Lipodystrophic diabetes is a group of metabolic disorders characterized by generalized or partial wasting/loss of adipose tissue mass, severe insulin resistance, hyperglycemia, hypertriglyceridemia, and hepatic steatosis. Previously, this entity was referred as lipoatrophic diabetes; however, this nomenclature was reframed to lipodystrophic diabetes later, as it refers to the presence of either lipoatrophy and/or lipohypertrophy in these individuals. Patients with generalized lipodystrophy have global loss of fat mass, as opposed to patients with partial lipodystrophy who have loss of adipose tissue mass in upper half of the body with accumulation of fat in lower half of the body.

74. *How does lipodystrophy cause diabetes?*

Both excess and deficient adipose tissue mass are associated with insulin resistance and hyperglycemia. Decreased fat mass in patients with lipodystrophic diabetes results in a state of leptin deficiency, which in turn leads to decreased hepatic and peripheral insulin sensitivity via central mechanism through arcuate nucleus of hypothalamus. In addition, increased circulating free fatty acids as a result of lack of deposition as triglyceride at eutopic site (adipocytes) lead to lipotoxicity. Decreased secretion of insulin-sensitizing adipocytokines (adiponectin) due to paucity of adipocytes further contributes to insulin resistance.

75. *How to classify lipodystrophic syndromes?*

Lipodystrophic syndromes are classified into congenital and acquired lipodystrophic syndrome. Congenital lipodystrophy is a result of mutations in genes responsible for adipocyte differentiation and growth and development, whereas acquired lipodystrophy is usually drug-induced. The various types of lipodystrophies are summarised in the table given below.

Type of lipodystrophy		Remarks
Congenital	*Generalized*	
	Berardinelli–Seip syndrome	*AGPAT2* and *BSCL2* mutation Some variants may be associated with short stature, mental retardation, and cardiomyopathy
	Partial	
	Familial partial lipodystrophy	LMNA, PPARG, and AKT2 mutation Loss of adipose tissue from extremities
	Werner's syndrome	WRN mutation Short stature and feature of progeria
Acquired	*Generalized*	
	Lawrence syndrome	Triggered by panniculitis or autoimmunity
	Partial	
	Barraquer–Simons syndrome	Lipoatrophy in upper half of the body and lipohypertrophy in lower half of the body Possibly autoimmune mediated or viral infection
	Drugs	Protease inhibitors rhGH therapy Insulin therapy Pegvisomant

76. *What is congenital generalized lipodystrophy?*

Congenital generalized lipodystrophy (CGL) also called as Berardinelli–Seip syndrome is an autosomal recessive disorder characterized by markedly reduced subcutaneous adipose tissue. Children with CGL have voracious appetite, accelerated linear growth, acromegaloid facies, protuberant abdomen, and severe acanthosis nigricans (Fig. 12.6). Affected females commonly present with features of hyperandrogenism. The metabolic abnormalities associated with lipodystrophy include severe insulin resistance, diabetes mellitus, hypertriglyceridemia, and low HDL-C. Diabetes associated with CGL usually manifest during peripubertal period and is refractory to therapy with insulin and other antidiabetic drugs. There are two variants of CGL, type1 and type2. The differences between these two subtypes are summarized in the table given below.

Parameters	Type1 CGL	Type2 CGL
Mutations	*AGPAT2* gene	*BSCL2* gene
Metabolically active fat (subcutaneous, intra-abdominal, intrathoracic, intermuscular, and bone marrow)	Absent	Absent
Mechanical fat (scalp, orbit, palm, and sole)	Preserved	Absent
Cardiomyopathy	Present	More common
Mental retardation	Present	More common
Focal lytic lesions in long bone	More common	Present

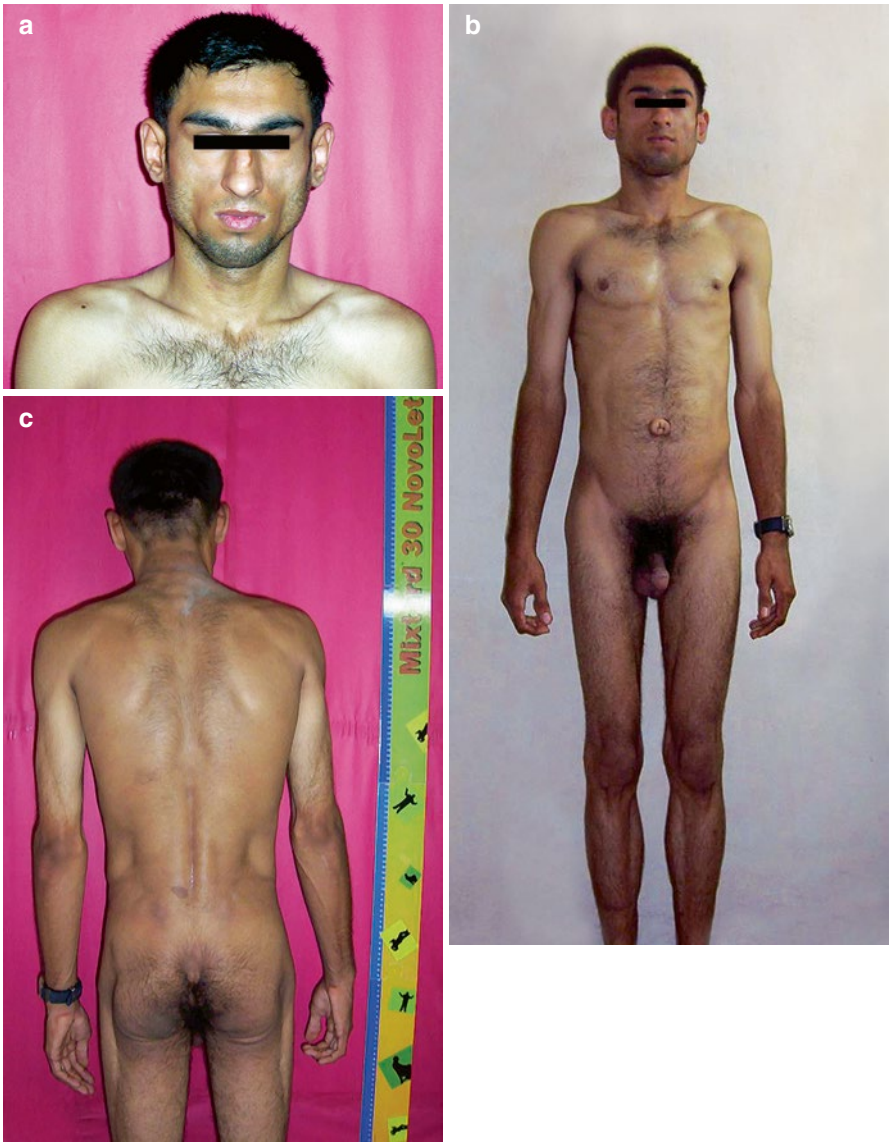

Fig 12.6 An adolescent with congenital generalized lipodystrophy

77. *What are the treatment strategies for lipodystrophic diabetes?*

Recombinant leptin therapy is the most effective treatment for lipodystrophic diabetes as it targets leptin deficiency, the key pathophysiological defect in these disorders. The use of recombinant leptin therapy not only improves meta-

bolic abnormalities but also decreases hepatic steatosis. However, due to non-availability of recombinant leptin and expenses incurred, insulin remains the mainstay of therapy for the management of hyperglycemia. Metformin and pioglitazone have been shown to be partially effective in improving metabolic abnormalities; however, their effect on redistribution of peripheral body fat may be detrimental. In addition, the use of fibrates is indicated for the management of hypertriglyceridemia. Statins have also been used to improve cardiovascular outcome through their pleiotropic effects.

78. *What is insulin therapy-related lipodystrophy?*

Insulin therapy-related lipodystrophy includes hypertrophy or atrophy of adipose tissue at injection site. Lipohypertrophy is more common than lipoatrophy and occurs with all insulin preparations including analogues. However, lipoatrophy was common with the use of animal-derived insulin and is rare with recombinant human insulin. Lipohypertrophy is a result of activation of local lipoprotein lipase (lipogenesis) by insulin, whereas lipoatrophy occurs as a result of release of cytokines (TNF-α) at local site in response to type III hypersensitivity reaction (Arthus reaction, as insulin acts as a hapten). Insulin therapy-related lipodystrophy leads to variability in absorption of insulin and consequently worsening of glycemic control. Therefore, the injection site should be examined periodically in patients who are receiving insulin. Management of insulin lipohypertrophy includes change in the site of insulin administration and, rarely, surgical excision. Lipoatrophy responds to change in injection site and sometimes to administration of dexamethasone along with insulin at the site of atrophy (Fig. 12.7).

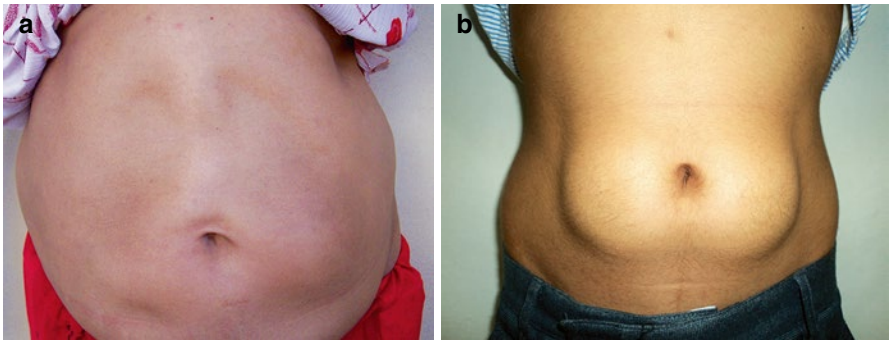

Fig 12.7 (**a**) Lipoatrophy and lipohypertrophy in a patient on insulin therapy, (**b**) lipohypertrophy in another patient with T1DM

79. *What is insulin resistance?*

Insulin resistance is defined as subnormal biological response to optimal concentration of insulin. In clinical practice, glucose lowering effect is often used to define the biological response to insulin. However, the optimal concentration of insulin required to produce normal biological response is variable as it depends on age, gender, ethnicity, adipose tissue mass, and physical activity of an individual. The clinical markers of insulin resistance include central obesity, acanthosis nigricans, skin tags and double chin, and polycystic ovarian disease in women (Fig. 12.8).

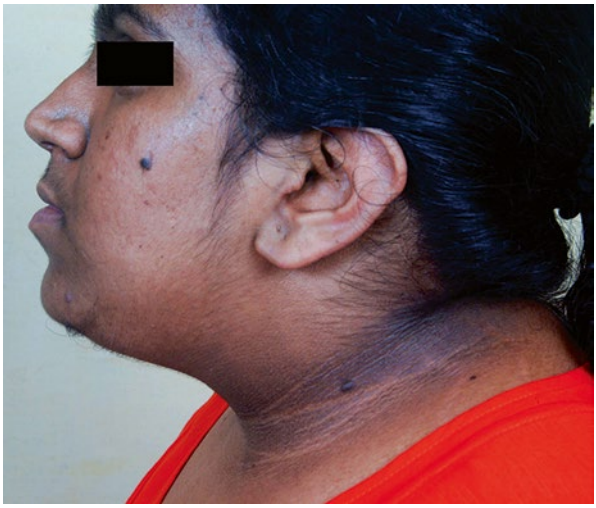

Fig 12.8 A young girl with hyperandrogenism, insulin resistance, and acanthosis nigricans (HAIRAN syndrome)

80. *What are the causes of insulin resistance?*

Insulin resistance may be classified as inherited or acquired. The genetic causes of insulin resistance primarily include disorders associated with insulin receptor mutation (leprechaunism, Rabson–Mendenhall syndrome, and type A insulin resistance) or post-receptor signaling defect (lipodystrophies). The acquired causes of insulin resistance include obesity, sedentary lifestyle, T2DM, endocrine disorders (Cushing's syndrome, pheochromocytoma, PCOS), type B insulin resistance (anti-insulin receptor antibody), and drugs (e.g., glucocorticoids).

81. *What are the differentiating features between type A and type B insulin resistance?*

Type A insulin resistance usually affects nonobese young women and is characterized by the presence of acanthosis nigricans, features of androgen excess, and hyperinsulinemia. Defect in insulin receptor or post-receptor signaling pathway is the key abnormality in type A insulin resistance. Type B insulin resistance is characterized by the presence of hirsutism, acanthosis nigricans, and concurrent autoimmune disorders (e.g., SLE, scleroderma) in older women. The presence of anti-insulin receptor antibody is the characteristic abnormality in type B insulin resistance. The differentiating features between these two disorders are summarized in the table given below.

Parameters	**Type A insulin resistance**	**Type B insulin resistance**
Female: male	19:1	6:1
Age of onset	Infancy or childhood	Middle-aged or older women
Acanthosis nigricans	Grade IV, diffuse	Grade II–III
Concurrent disorders	–	Autoimmune disorders
Biochemistry	Hyperglycemia Hyperinsulinemia Hyperandrogenemia	Hyperglycemia Hyperinsulinemia Hyperandrogenemia ANA positivity
Treatment	Insulin	Steroids

82. *What is leprechaunism?*

Leprechaunism, also known as Donohue syndrome, is a disorder characterized by severe insulin resistance. Affected newborns manifest characteristic elfin facies, prenatal growth failure, severe acanthosis nigricans, features of androgen excess (hirsutism, clitoromegaly in girls and macropenis in boys), diffuse lipoatrophy, muscular hypotrophy, mental retardation, fasting hypoglycemia with postprandial hyperglycemia, and marked hyperinsulinemia (>1,000 pmol/L). Very high level of insulin which acts through the IGF1 receptor is thought to be the cause of fasting hypoglycemia. Serum GH-IGF1 levels may be low due to feedback inhibition by high levels of insulin acting through IGF1 receptor at the hypothalamus. The disorder is inherited as an autosomal recessive trait and is due to homozygous or compound heterozygous mutations in insulin receptor gene which is located on chromosome 19. IGF1 therapy has been shown to be effective in some of these patients. However, these infants usually succumb to intercurrent infection within few months of their life (Fig. 12.9).

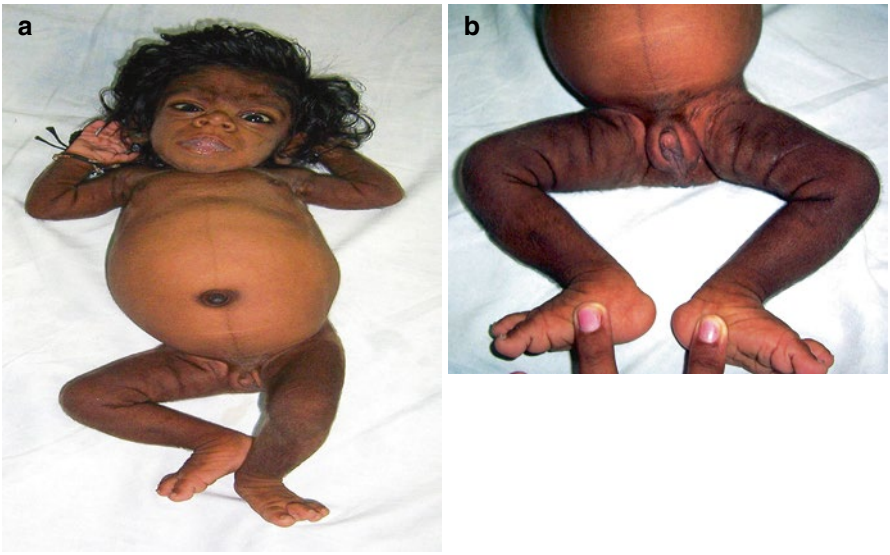

Fig 12.9 (**a**) Typical elfin facies, severe and diffuse acanthosis nigricans in an infant with leprechaunism, (**b**) clitoromegaly in the same patient as a manifestation of hyperinsulinemia-mediated androgen excess

83. *What is Rabson–Mendenhall syndrome?*

Rabson–Mendenhall syndrome is a disorder characterized by severe insulin resistance and manifests as characteristics facies (prominent widely spaced eyes, broad nose, and large, low-set ears), growth retardation, severe acanthosis nigricans, protuberant abdomen, thick nails, early dentition with crowding of teeth, features of androgen excess (hirsutism, clitoromegaly in girls and macropenis in boys), pineal hyperplasia, and fasting hypoglycemia and postprandial hyperglycemia. The disorder is intermediate in severity between Donohue syndrome and type A insulin resistance. It is inherited as an autosomal recessive trait and is due to homozygous mutations in insulin receptor gene. Insulin, insulin sensitizers, and IGF1 has been used with limited success. These children usually survive till second decade of life.

84. *When to suspect mitochondrial diabetes?*

Mitochondrial diabetes should be suspected in a young individual with diabetes (onset before 30 years) and sensorineural deafness. In addition, the family history of matrilinear transmission of diabetes should also raise a suspicion of mitochondrial diabetes. Matrilinear transmission refers to pattern of inheritance wherein a female transmits the disease to all her children, while the affected males fail to transmit the disease (as sperm sheds the mitochondria before

penetration into ovum). Patients with diabetes, having any of the following features like encephalomyopathy, hypertrophic cardiomyopathy, external ophthalmoplegia, optic atrophy, or stroke-like episodes should also be suspected to have mitochondrial diabetes. Mitochondrial disorders result in defective oxidative phosphorylation in β-cell, and hence impaired ATP generation and glucose-induced insulin secretion. These individuals are often initially misdiagnosed as type 1 (although islet cell autoantibodies are negative) or type 2 diabetes.

85. *What is DIDMOAD syndrome?*

The term DIDMOAD refers to diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (Wolfram syndrome). The earliest abnormality in affected individuals is diabetes mellitus (median age 6 years), followed by optic atrophy (median age 11 years), diabetes insipidus (median age 14 years), and deafness (median age 16 years). DIDMOAD syndrome is inherited as an autosomal recessive disorder and is due to mutations of *Wolframin* gene, which regulates calcium homeostasis in endoplasmic reticulum. Other manifestations include urogenital tract atonia and neurological features like ataxia, peripheral neuropathy, and psychiatric manifestations.

Further Readings

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