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Congenital adrenal hyperplasia

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ABSTRACT: The congenital adrenal hyperplasias are a group of autosomal recessive disorders associated with impaired steroidogenesis. Several types of the congenital adrenal hyperplasias are associated with decreased cortisol production and excessive adrenal sex steroid secretion. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is the most common and prototypic example of this group of disorders. Herein, we review the clinical features, pathophysiology, molecular genetics, and treatment of 21-hydroxylase deficiency. There is also a brief discussion of other steroidogenic enzyme defects that are associated with clinical features due to excessive androgen secretion.

KEYWORDS: adrenal gland, congenital adrenal hyperplasia, hyperandrogenism, steroidogenesis

Introduction

The congenital adrenal hyperplasias (CAH) are a group of inherited disorders affecting both children and adults. These autosomal recessive disorders are due to "loss of function" mutations in genes encoding enzymes involved in adrenal steroid synthesis (1–3). In the virilizing forms of CAH, the mutations impair cortisol biosynthesis and lead to the accumulation of steroid intermediates proximal to the deficient enzyme (Table 1). The loss of negative feedback inhibition by cortisol leads to increased hypothalamic corticotrophin-releasing hormone (CRH) and pituitary adrenocorticotrophic hormone (ACTH) secretion. The specific enzyme defect governs the clinical and hormonal phenotype. These disorders demonstrate a phenotypic spectrum depending on the severity of the mutation which range from complete loss of function to partial enzyme activity.

In the virilizing forms of CAH, increased ACTH secretion leads to increased adrenal androgen synthesis and adrenal cortical hyperplasia. Thus, the clinical signs and symptoms reflect androgen excess in addition to glucocorticoid and mineralocorticoid deficiencies. In general, the severity of the enzyme deficiency dictates the magnitude of the glucocorticoid and mineralocorticoid deficiencies.

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Steroidogenesis

The human adrenal cortex consists of three zones, the zona glomerulosa which is primarily responsible for aldosterone production, the zona fasciculata which is primarily responsible for glucocorticoid production, and the zona reticularis which is primarily responsible for adrenal androgen production. Since steroid cells do not store large amounts of hormone, steroid hormone secretion reflects steroid hormone biosynthesis.

Through a series of enzymatic steps (FIG. 1), cortisol is synthesized from cholesterol (4,5). Cholesterol is taken up by the adrenal cortical cell and is converted to pregnenolone by the enzyme cholesterol desmolase (P450scc), encoded by *CYP11A1*, in the mitochondria. Import of cholesterol into the mitochondria is largely mediated by steroidogenic acute regulatory protein (StAR); this is the rate limiting step for steroidogenesis (6). The enzyme 17α-hydroxylase/17,20-lyase (P450c17) is the qualitative regulator of steroidogenesis. This single enzyme encoded by a single gene, *CYP17A1*, is capable of two activities. In the zona fasciculata, it performs 17α-hydroxylation to generate 17α-pregnenolone. The 17,20-lyase activity of P450c17 converts 17α-pregnenolone to dehydroepiandrosterone (DHEA).

Table 1. Clinical phenotypes and genetic loci for the different types of virilizing congenital adrenal hyperplasias

Features	Defective gene	Chromosomal localization	Incidence	Initial presentation	Ambiguous genitalia	Elevated metabolite	Other features
21-hydroxylase deficiency	*CYP21*	6p21.3	1 : 15,000	Acute adrenal insufficiency	Ambiguous genitalia in females	17-hydroxyprogesterone	Salt-wasting in classical CAH
11β-hydroxylase deficiency	*CYP11B1*	8q24.3	1 : 100,000	Acute adrenal insufficiency rare	Ambiguous genitalia in females	DOC, 11-deoxycortisol	Hypertension
3β-hydroxysteroid deficiency	*HSD3B2*	1p13.1	Rare	Acute adrenal insufficiency	Ambiguous genitalia in males and females	DHEA, $17\Delta^5$-pregnenolone	Infertility in adolescent and adult women
POR deficiency or Antley–Bixler syndrome	*POR*	7q11.2	Rare	Heterogeneous	Ambiguous genitalia in males and females	Mildly elevated 17-hydroxyprogesterone	Skeletal malformations
Placental aromatase deficiency	Aromatase	15q21.1	Rare	Virilization	Ambiguous genitalia in females	Androstenedione, testosterone	Maternal virilization

In the zona fasciculata, the enzyme 3β-hydroxysteroid dehydrogenase type 2 encoded by the gene, *HSD3B2*, converts 17α-pregnenolone to 17α-progesterone which is subsequently converted to 11-deoxycortisol by 21-hydroxylase (P450c21). The 21-hydroxylase enzyme is a cytochrome P450 protein encoded by *CYP21A2* and located in the endoplasmic reticulum. The last step of cortisol biosynthesis is mediated by 11β-hydroxylase (P450c11β) which converts 11-deoxycortisol to cortisol; this enzyme is encoded by *CYP11B1*.

Aldosterone biosynthesis is primarily regulated by serum sodium and serum potassium concentrations and the plasma renin-angiotensin system. For aldosterone biosynthesis, pregnenolone is converted to progesterone by HSD3B2 and subsequently to deoxycorticosterone by P450c21. The final steps of aldosterone biosynthesis are mediated by aldosterone synthase (P450c11AS) which is encoded by *CYP11B2*.

DHEA can be converted by HSD3B2 to androstenedione. In the adrenal cortex, DHEA can also undergo a sulfotransferase reaction to be converted to dehydroepiandrosterone sulfate (DHEAS). DHEA, androstenedione, and DHEAS are often called the "adrenal androgens." However, these hormones do not have a high affinity for the androgen receptor and must be converted to more potent androgens by other enzymes. Adrenarche refers to the increased secretion of these "adrenal androgens," which generally begins between 6–8 years of age. This process of adrenarche is limited to a few primate species. Adrenarche is not dependent on hypothalamic (gonadotropin-releasing hormone [GnRH]), pituitary (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]), or gonadal (testosterone or estradiol) hormone secretion (7,8).

Androstenedione is produced by both the zona reticularis of the adrenal cortex and the theca cells in the ovary. In the ovary, androstenedione serves as the precursor for estradiol biosynthesis in the granulosa cells. The steroidogenic compartments of the ovary are partitioned into two distinct compartments, theca cells and granulosa cells. The theca cells secrete androstenedione in response to LH stimulation and the granulosa cells synthesize estrogens in response to FSH.

Epidemiology

The most common type of virilizing CAH, accounting for 90–95% of cases, is 21-hydroxylase

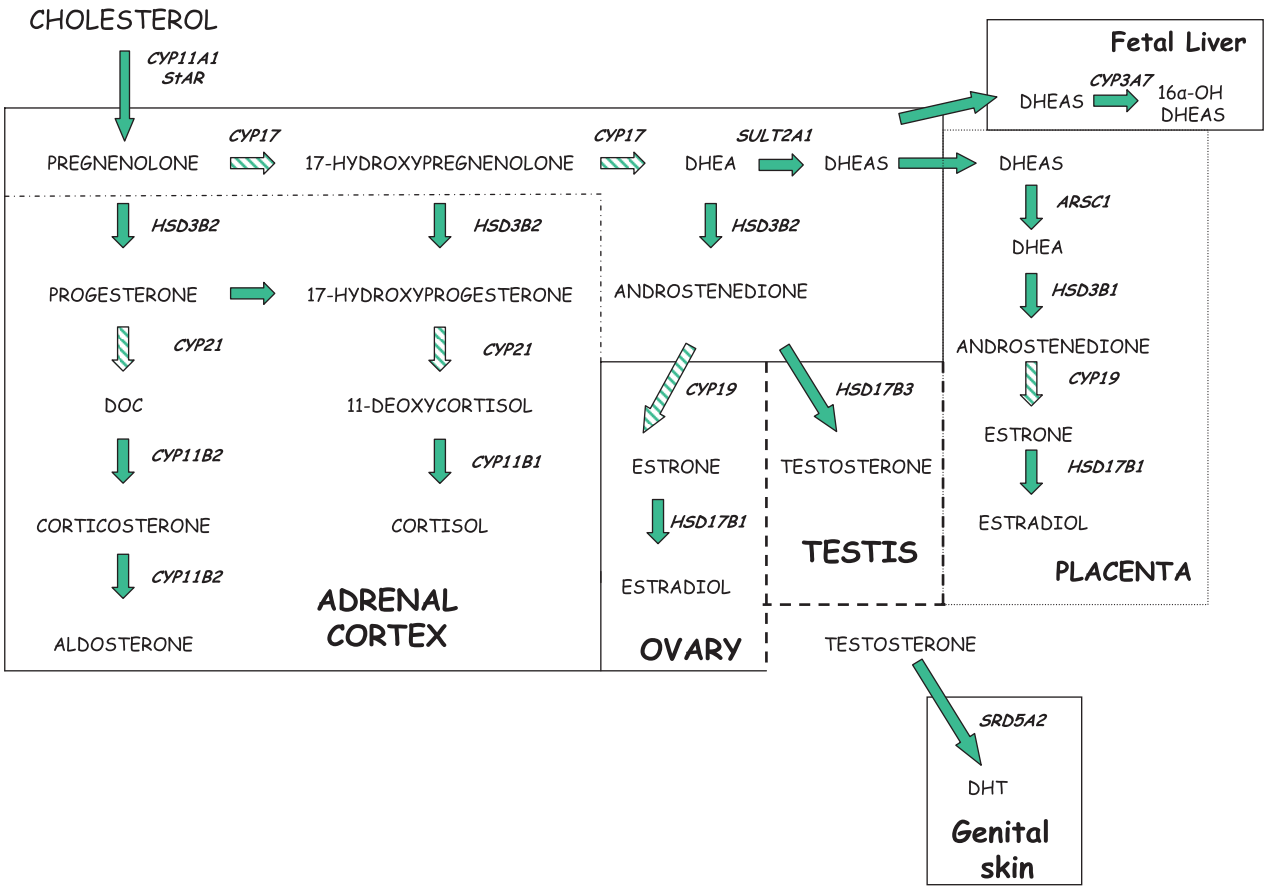

FIG. 1. Diagram of classical steroidogenic pathways. The substrates, products, and genes involved in adrenal, ovarian, testicular, and placental steroidogenesis are indicated. Substrates, products, and genes involved in gonadal steroidogenesis are indicated by dotted lines. Genes are 17 α-hydroxylase/17,20-lyase (*CYP17A1*), 3β-hydroxysteroid dehydrogenase (*HSD3B2*), 21-hydroxylase (*CYP21A2*), 11β-hydroxylase (*CYP11B1*), aldosterone synthase (*CYP11B2*), aromatase (*CYP19A1*), 17β-hydroxysteroid dehydrogenase type 1 (*HSD17B1*), 17β-hydroxysteroid dehydrogenase type 3 (*HSD17B3*), 5α-reductase type 2 (*SRD5A2*), sulfotransferase (*SULT2A1*), and steroid sulftase/arylsulfatase C (*ARSC1*). *CYP3A7* is a cytochrome P450 enzyme expressed in fetal liver where it catalyzes the 16α-hydroxylation of estrone (E1) and DHEA; its expression decreases postnatally. Steroidogenic enzymes that utilize P450 oxidoreductase, a flavoprotein encoded by P450-oxidoreductase (POR), to transfer electrons are indicated by hatched arrows [This figure was published in Witchel SF, Lee PA. Ambiguous genitalia. In Sperling MA, eds. *Pediatric Endocrinology*, 3rd edition. Copyright Elsevier 2008. Permission was granted by Elsevier].

deficiency which is due to mutations in the 21-hydroxylase (*CYP21A2*) gene located at chromosome 6p21.3 in the human leukocyte antigen (HLA) class III region (9–12). As noted above, P450c21 catalyzes the conversion of 17-hydroxyprogesterone to 11-deoxycortisol, a precursor of cortisol, and the conversion of progesterone to deoxycorticosterone, a precursor of aldosterone (FIG. 1). The continuum of CAH due to 21-hydroxylase deficiency is subclassified into salt-wasting and simple virilizing forms, collectively referred to as classical 21-hydroxylase deficiency, and the milder nonclassical or late onset form (13).

The reported incidence of classical 21-hydroxylase deficiency ranges from 1 in 5000 to 1 in 15,000 with variation between ethnic/racial backgrounds (14,15). For classical CAH, the carrier frequency is approximately one in 60 individuals. Prevalence is lower among African-Americans than Caucasians in the USA (16). The frequency of the nonclassic form is difficult to accurately determine due to problems of ascertainment. One study reported increased frequency among Hispanics, Yugoslavs, and Ashkenazi Jews (14). The frequencies of other types of virilizing CAH are less than 1 : 100,000 in most populations.

21-hydroxylase deficiency

Genetics

CAH due to 21-hydroxylase deficiency is associated with mutations in the *CYP21A2* gene. This

gene is located at chromosome 6p21 in close proximity to a nonfunctional pseudogene (*CYP21P* also known as *CYP21A1*). The de novo mutation rate is low; most disease-causing mutations represent gene conversion events between the functional gene and the pseudogene. Most affected individuals are compound heterozygotes bearing different *CYP21A2* mutations on each allele. In general, phenotype correlates with molecular genotype (17,18). The phenotype generally reflects the residual enzyme activity of the mildest mutation (19). To date, over 100 mutations have been reported.

Since inheritance of 21-hydroxylase deficiency is autosomal recessive, the recurrence risk is 25% for future pregnancies of the biological parents of the proband. In one study involving women with late-onset CAH, the prevalence of 21-OH-deficiency among live-born children was 2.5% which was higher than the 0.2% calculated prevalence. The suggested explanation for the higher than expected prevalence was the tendency for affected individuals to marry within their own ethnic background; some ethnic groups are enriched for *CYP21A2* variants (20).

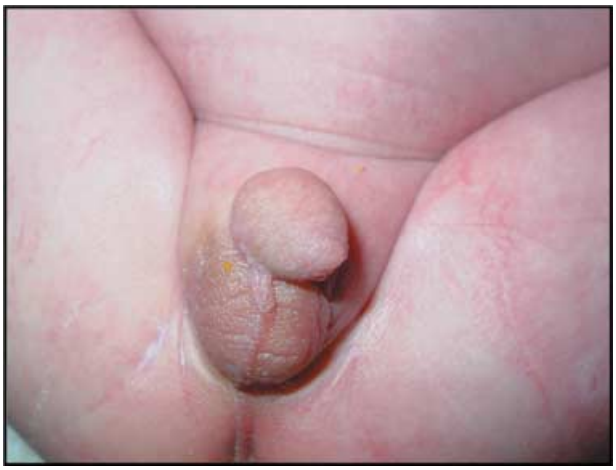

FIG. 2. Virilized female with congenital adrenal hyperplasia. This photograph illustrates fused rugated labioscrotal folds in a 46,XX infant with 21-hydroxylase deficiency. No gonads are palpable and the phallus is enlarged.

Clinical features

Ambiguous genitalia. Due to their exposure to androgens from approximately the sixth week of gestation, infant girls with classical 21-hydroxylase deficiency may have genital ambiguity. Typical external genital physical findings include clitoromegaly, partially fused and rugated labia majora, and a common urogenital sinus in place of a separate urethra and vagina (FIG. 2). The extent of virilization in these 46,XX infants can range from a nearly male appearance of the external genitalia with an enlarged phallus to minimal clitoromegaly. For 46,XX female infants, a normal uterus is present and can be identified on ultrasound evaluation. The ovaries may be too small to be visualized by sonography. Despite excessive antenatal androgen exposure, ovarian position is normal and internal wolffian structures regress. External genital development is normal in affected boys apart from hyperpigmentation of the external genitalia.

Affected female infants are generally identified on physical examination because of their genital ambiguity, but extensive virilization can occasionally lead to gender misassignment at birth. Genital ambiguity in the newborn period is typical for most affected females with either classical salt-losing or simple virilizing forms of CAH. The external genitalia are typically normal for females affected by nonclassical CAH. In the absence of newborn screening programs, infants with classical salt-losing CAH present within the first few weeks of life with hypotension, failure to thrive, hyponatremia, and hyperkalemia. The "cut-off" levels for most newborn screening programs do not identify children with nonclassical CAH.

Salt-wasting. Approximately 75% of patients with classic 21-hydroxylase deficiency have severely impaired 21-hydroxylation of progesterone and, thus, cannot adequately synthesize aldosterone. Aldosterone regulates sodium and potassium homeostasis. The excessive renal sodium excretion in untreated salt-losing patients results in hypovolemia, hyperreninemia, hyponatremia, and hyperkalemia especially in infancy. Cortisol deficiency in these patients contributes to poor cardiac function, poor vascular response to catecholamines, decreased glomerular filtration rate, and increased secretion of antidiuretic hormone. Thus, cortisol and aldosterone deficiency together cause hyponatremic dehydration and shock in untreated and inadequately treated patients.

Premature pubarche. Premature pubarche is defined as the appearance of sexual hair or apocrine odor before 8 years of age in girls and 9 years in boys. Again, in the absence of screening programs, males and females with simple virilizing CAH can present with premature pubarche, genital

enlargement, acne, adult-type apocrine odor, tall stature, and advanced skeletal maturation. Examination of the external genitalia may reveal clitoral enlargement in girls and phallic enlargement with prepubertal testes in boys. These children typically secrete sufficient amounts of aldosterone such that they are not overt salt-losers. Review of growth curves often reveals accelerated linear growth velocity. In addition to these physical manifestations, skeletal maturation as assessed by a bone age X-ray (X-ray of the left hand in children ≥ 2 years and hemi-skeleton in children < 2 years of age) may be advanced.

Hirsutism. Hirsutism represents the relative sensitivity of the hair follicle to androgen exposure and is a common clinical manifestation of hyperandrogenemia. Hirsutism must be differentiated from hypertrichosis, the generalized excessive growth of androgen-independent hair related to familial factors, metabolic disorders (e.g., thyroid disturbances, anorexia nervosa), or medication (e.g., phenytoin, minoxidil, diazoxide, glucocorticoids, cyclosporine). Hirsutism is defined as excessive growth of coarse terminal hairs in androgen-dependent areas in a female. These areas include the mustache area, chin, neck, upper and lower abdomen, upper and lower back, and inner aspect of the thighs (21,22). In these body regions, androgens influence hair growth such that vellus hairs become longer, darker, and curlier and are considered to be terminal hairs. Testosterone and DHT act through androgen receptors in the dermal papilla to increase hair follicle size. Skin has the capacity to synthesize sex steroids de novo from cholesterol as well as to interconvert specific steroids (23).

The modified Ferriman–Gallwey score provides a semisubjective method to assess the magnitude of hair growth in nine androgen-dependent areas such as the mustache area, chin, upper chest, abdomen, and back (24). Although a Ferriman–Gallwey score of 8 or more is usually considered to indicate hirsutism, variation among ethnic groups occurs. The Ferriman–Gallwey scoring system loses value following cosmetic treatments. Virilization and masculinization are terms used to describe the presence of more severe symptoms of androgen excess. Specifically, these terms refer to the presence of clitoromegaly, masculine body habitus, male-pattern hair loss, and voice changes.

For women, hirsutism can be a presenting symptom of nonclassical CAH, polycystic ovary syndrome (PCOS), Cushing's syndrome, or androgen-secreting tumors. Among 270 consecutive Spanish women referred for evaluation of hirsutism, irregular menses, acne, or androgenic alopecia, six women (2.2%) were diagnosed with nonclassical CAH on the basis of elevated ACTH-stimulated 17-hydroxyprogesterone (17-OHP) levels and molecular genotype analysis (25). The reported prevalences for nonclassical CAH range from 0.6% to 10% (26–28).

Irregular menses and reproductive function. Hyperandrogenism is associated with chronic anovulation, amenorrhea, and infertility. The amenorrhea may be primary or secondary. Fertility correlates with severity of the disorder, with infertility being more common among women with the classical salt-losing form and least common among women with the late-onset form. Ultrasonography may demonstrate ovarian morphology reminiscent of PCOS. PCO morphology may be present among women with undertreated classical CAH and may be present in 40% of women with late-onset CAH (29). One factor contributing to the chronic anovulation observed in women with hyperandrogenism is that the androgen excess impairs hypothalamic sensitivity to progesterone resulting in a persistently rapid GnRH pulse frequency which favors LH hypersecretion (30). The LH hypersecretion may contribute to a vicious cycle in which excessive ovarian androgen secretion intensifies the consequences of the excessive adrenal androgen production. Failed implantation due to elevated progesterone concentrations and unsatisfactory sexual intercourse due to small vaginal introitus also contribute to reproductive issues (31). Another potential cause of the reproductive dysfunction is that the excessive androgen exposure in utero induces prenatal programming of the hypothalamus which predisposes to LH hypersecretion (32). Data supporting this mechanism are derived from studies involving nonhuman primates in which prenatal androgen treatment was associated with postnatal hyperandrogenism, oligo-ovulation, and polycystic ovaries (33).

Acne. Acne can occur among patients with hyperandrogenism and may be the primary clinical manifestation of CAH. Severe cystic acne refractory to oral antibiotics and retinoic acid has been attributed to nonclassical 21-OH deficiency. Additionally, male-pattern baldness in young women with this disorder has been noted as the sole presenting symptom. Severe androgenic alopecia in association with marked virilization has also been reported in older women.

Adrenal medulla. The inner portion of the adrenal is the medulla which secretes epinephrine. In addition to promoting maturation of the developing chromaffin cells during gestation, glucocorticoids stimulate expression of phenylethanoloamine-N-methyltransferase (PNMT) (34). This enzyme converts norepinephrine to epinephrine. Merke et al. reported that plasma epinephrine and metanephrine concentrations were lower among individuals with CAH compared to control subjects and that magnitude of the hormonal deficiency correlated with the severity of adrenocortical dysfunction (35). Free metanephrine concentrations as a measure of adrenomedullary function also correlated with molecular genotype; lower metanephrine concentrations predicted more severe mutation (36). Histological examination of adrenal glands from three individuals with CAH showed cortical hyperplasia, poorly defined zones, and extensive intermingling of cortical and chromaffin cells (35). Thus, cortisol deficiency in CAH results in abnormal development of the adrenal medulla and epinephrine deficiency. This impaired development and function of adrenal medulla likely contribute to the development of hypoglycemia during acute adrenal crises.

Diagnosis

Classical CAH in infants and children

The diagnosis of CAH is entertained among infants with ambiguous genitalia and nonpalpable gonads. Thus, female infants with the classical forms of 21-hydroxylase deficiency, salt-losing, and simple virilizing are generally ascertained by their ambiguous external genital differentiation. During the initial physical examination of a child with ambiguous external genital development, the symmetry of the external genitalia, the presence and location of palpable gonads, genital skin pigmentation, and the presence of additional anomalies should be noted (FIG. 2). A rectal examination may be helpful to assess for a midline uterine cervix. The extent of virilization can be documented by noting the configuration, stretched dorsal length, and diameter of the phallus, including the glans penis. Additional important observations include the location of the urethral opening, the number of perineal orifices, the degree of genital fold (labiourethral) fusion, and the extent of fusion of the genital swellings (labioscrotal folds). Since the labioscrotal folds fuse from posterior to anterior, the appearance of the labioscrotal folds may vary from posterior labial fusion, a partially fused hemiscrota, or a completely fused scrotum with the fusion extending to the midline urethral opening. Observation of urination may be needed to locate the position of the urethra. It may be helpful to visualize the force, diameter, and direction of the urinary stream.

Laboratory studies to confirm the diagnosis of 21-hydroxylase deficiency in an infant with ambiguous genitalia include karyotype, abdominal/pelvic ultrasound to identify the uterus, and serum 17-OHP determination. Serum electrolytes and plasma renin activity (PRA) are important to assess for mineralocorticoid deficiency. In classical 21-hydroxylase deficiency, the random 17-OHP concentrations are quite elevated with values higher than 5000 ng/dL, and often much higher among affected infants (37). Androstenedione and progesterone concentrations are also typically elevated. Some children, the so-called simple virilizers, who present after the neonatal period with premature pubarche and phallic enlargement/clitoromegaly may also have sufficiently elevated random 17-OHP concentrations to be diagnostic of CAH. Plasma renin activity or direct renin concentrations may be elevated in the so-called simple virilizers, indicating that they are compensating for subclinical salt loss.

It is important to remember that the external genitalia appearance reflects prenatal androgen exposure and sensitivity. The differential diagnosis of ambiguous genitalia is extensive and includes maternal hyperandrogenism as well as disorders affecting male and female sexual differentiation and genital development. Thus, additional studies to determine the etiology of the disorder of sexual differentiation may need to be performed and should be tailored according to the findings on clinical examination. In addition to those already mentioned, additional laboratory studies may include fluorescence in-situ hybridization (FISH) analysis with X- and Y-specific DNA probes and serum testosterone, LH, FSH, 17-hydroxypregnenolone, androstenedione, dihydrotestosterone, anti-Mullerian hormone, and electrolytes determinations to delineate the specific type of disorder of sexual differentiation.

CAH in older children. The differential diagnosis of the child with premature development of pubic hair includes premature adrenarche, congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, and inherited glucocorticoid resistance. In addition to the premature development of pubic hair, additional features of CAH include

tall stature, accelerated growth velocity, apocrine body odor, phallic enlargement/clitoromegaly, and advanced skeletal maturation. Rapid progression of signs and symptoms suggests the possibility of an androgen-secreting tumor or Cushing's syndrome. Steroid hormone concentrations, especially DHEAS, are often extremely elevated with tumors. When Cushing's syndrome is a possibility, studies to confirm hypercortisolism and loss of diurnal variation are necessary and should precede diagnostic imaging studies.

For patients with CAH, random 17-OHP and androstenedione concentrations may be mildly elevated, but may not be diagnostic for CAH. To establish the diagnosis, children suspected of having CAH may require provocative ACTH stimulation tests (Cortrosyn, 0.25 mg) to elicit an elevated ACTH-stimulated 17-OHP concentration. Since CAH, classical and nonclassical, is an autosomal recessive disorder, *CYP21A2* mutations are likely to be identified on both alleles. Through studies comparing ACTH-stimulated 17-OHP responses and *CYP21A2* molecular genotypes, mutations on both alleles are likely when the ACTH-stimulated 17-OHP value exceeds 1500 ng/dL (38,39).

CAH in adolescent and adult women. Development of the clinical features associated with nonclassical CAH occurs during the peripubertal and young adult years. Affected patients do not manifest symptoms due to glucocorticoid or mineralocorticoid deficiency. Rather, their signs and symptoms are due to excessive adrenal androgen secretion. Typical clinical features include hirsutism, irregular menses, infertility, androgenic alopecia, and acne. This results in an ascertainment bias such that affected females are identified more commonly than affected males. Affected males are generally identified through family studies.

Among adolescent and adult females, the differential diagnosis also includes polycystic ovary syndrome, Cushing's syndrome, androgen-secreting tumors, and hyperprolactinemia (FIG. 3). Some women with nonclassical CAH develop clitoromegaly. Women with PCOS may have insulin resistance, obesity, and acanthosis nigricans. However, there are no clinical features that consistently distinguish women with nonclassical CAH from those with PCOS.

Random 17-OHP, androstenedione, and DHEAS concentrations do not discriminate nonclassical CAH from PCOS. Unstimulated 17-OHP screening determinations should be obtained in the morning during the follicular phase of the menstrual cycle. Levels obtained during the luteal phase of the menstrual cycle may be difficult to accurately interpret because the corpus luteum secretes progesterone and 17-OHP. Pre-treatment with dexamethasone may suppress the morning 17-OHP rise (29). Escobar-Morreale and colleagues suggest that, in adult women, an unstimulated morning 17-OHP value greater than 170 ng/dL may be a sensitive screening tool for nonclassical CAH. However, they caution that the cut-off level needs to be established for each laboratory (25). Provocative ACTH stimulation testing with Cortrosyn (0.25 mg) may be necessary to establish or exclude the diagnosis of nonclassical CAH. A blood sample for progesterone, 17-OHP, 17-hydroxypregnenolone, DHEA, androstenedione, and cortisol should be obtained prior to the Cortrosyn stimulation and either 30 or 60 minutes after the Cortrosyn.

Laboratory techniques. Laboratory techniques used to measure 17-OHP include radioimmunoassays (RIA), enzyme-linked immunosorbent assays (EIA), and time-resolved fluoroimmunoassays (FIA). A limitation of these methods is the overestimation of 17-OHP concentrations secondary to insufficient antibody specificity. The presence of cross-reacting steroids of fetal adrenal origin may hinder the interpretation of 17-OHP concentrations among preterm and term infants. Recent technical developments involve mass spectrometry (MS) linked to gas chromatography (GS) or liquid chromatography (LC). Detection of 17-OHP from dried whole blood spots using LC-tandem-MS has been reported (40).

Treatment

Upon confirmation of the diagnosis based on clinical and laboratory findings, hormone replacement therapy can be instituted. The goal of treatment is to provide sufficient glucocorticoid and, if necessary, mineralocorticoid to prevent excessive adrenal androgen secretion and excessive salt loss while allowing for normal linear growth velocity. Undertreatment is associated with progressive virilization, increased risks for acute adrenal crises, and an accelerated tempo of bone maturation. Overtreatment leads to short stature and Cushingnoid features. Nevertheless, despite adequate glucocorticoid therapy, GnRH-dependent precocious puberty, testicular adrenal rest tumors, or postpubertal ovarian hyperandrogenism can complicate treatment of CAH.

Glucocorticoid therapy to provide the equivalent of approximately 7–18 mg/m^2/day of hydrocortisone can be started and adjusted as necessary to

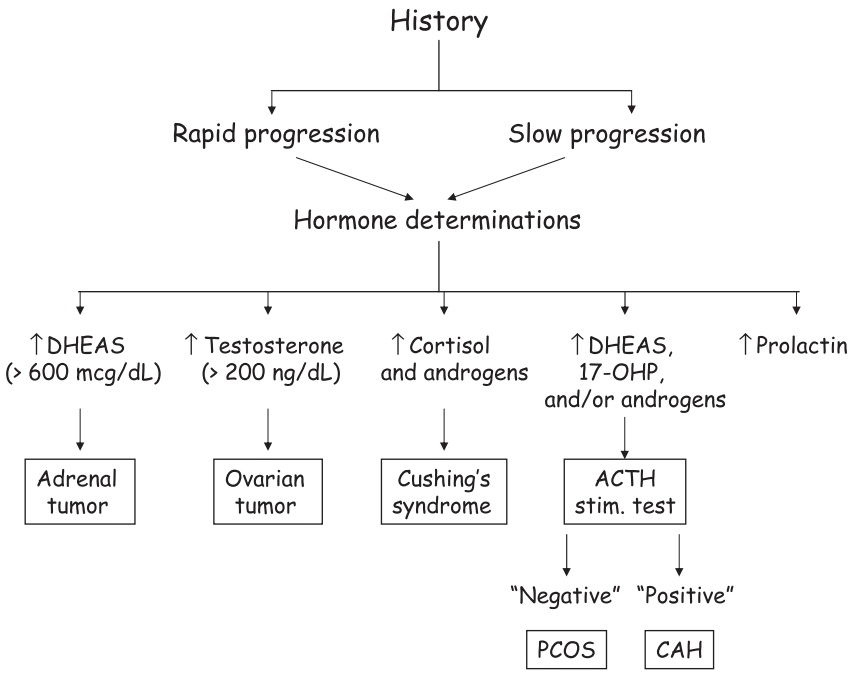

FIG. 3. Algorithm for an initial approach to the adolescent or young adult woman with hirsutism. Key: PCOS, polycystic ovary syndrome; CAH, congenital adrenal hyperplasia.

maintain androgen concentrations within the normal range for age. The total dose is generally divided into three doses per day. Some suggest the use of a reverse diurnal dosing such that the highest dose is administered at night whereas other suggest that the highest dose should be administered in the morning (41). The overall goal of therapy is normal growth and development. Androstenedione concentrations should be within the normal range for age and stage of pubertal development. Oversuppression of 17-OHP concentrations is associated with Cushingnoid features. During childhood, hydrocortisone is often considered to be the preferred glucocorticoid because longer-acting glucocorticoids such as prednisone and dexamethasone may interfere with linear growth velocity.

Older adolescents and young adults may like the convenience of fewer daily doses and use prednisone (5.0–7.5 mg divided into two daily doses) or dexamethasone (0.25–0.5 mg daily). The growth suppressive potencies of prednisone and dexamethasone are greater than their anti-inflammatory potencies (42). It is also important to remember that the mineralocorticoid activity of these glucocorticoids varies with prednisolone having less mineralocorticoid activity than hydrocortisone and dexamethasone having no mineralocorticoid activity. Antiandrogens such as spironolactone may be helpful to treat the hirsutism. For some women with nonclassical CAH who are not desiring pregnancy, treatment with oral contraceptives may be adequate.

For children with overt or subtle salt loss, mineralocorticoid replacement with 9α-fludrocortisone (0.1–0.2 mg per day) can be used. Infants with classical salt-losing CAH may require higher mineralocorticoid doses due to their decreased salt intake and relative pseudohypoaldosteronism.

During acute stress such as fever, trauma, and surgery, patients with CAH require increased glucocorticoid "stress" dosing. In most instances, tripling the usual hydrocortisone dose is sufficient. Families should have injectable hydrocortisone readily available for situations in which oral medications are not tolerated such as intercurrent gastrointestinal illnesses. All affected individuals should wear a Medic-Alert identification badge to alert emergency health-care providers to their disorder.

Additional considerations

Newborn screening

Newborn screening is performed in many countries. Dried whole blood spots collected on filter paper are assayed for 17-OHP concentrations.

Due to confounding factors for specificity and sensitivity, newborn screening generally detects infants with classical salt-losing and simple virilizing forms of CAH (43).

Linear growth

Linear growth is affected by congenital adrenal hyperplasia, even with close therapeutic monitoring. Both undertreatment and overtreatment put patients at risk for short stature, the former causing premature epiphyseal closure induced by high levels of sex steroids and the latter resulting in glucocorticoid-induced inhibition of the growth axis.

Precocious puberty

Precocious puberty due to increased hypothalamic GnRH secretion may occur in some affected children (44). In this situation, the hypothalamic GnRH pulse generator prematurely resumes pulsatile GnRH secretion leading to increased LH and FSH secretion resulting in increased gonadal steroid production. In these situations, the precocious puberty is considered to be secondary to the virilizing disorder, but the mechanism through which the GnRH pulse generator is prematurely activated is unclear.

Bone mineral density

Osteoporosis is a concern for individuals with CAH due to the need for chronic glucocorticoid therapy. Glucocorticoids influence bone metabolism by suppressing osteoblast activity, promoting increased bone resorption by osteoclasts, and interfering with calcium absorption from the gastrointestinal tract (45).

Bone mineral density outcome in prior reports has been inconsistent because of differing glucocorticoid doses, potential compliance issues, and subject heterogeneity. Chakhtoura et al. reported that total cumulative glucocorticoid dose and total average glucocorticoid dose were associated with lower lumbar and femoral T-scores; increased body mass index (BMI) appeared to have a protective effect on bone mineral density (46). Among Swedish women with CAH, the frequency of osteopenia and osteoporosis was increased irrespective of age group, clinical phenotype, molecular genotype, and height compared to healthy age-matched control women (47).

Since dual X-ray absorptiometry (DXA) is based on a two-dimensional technique, interpretation of areal bone mineral density assessed by DXA scan can be confounded by bone width and height. Thus, DXA can underestimate bone mineral density in shorter individuals (48). It has been suggested that maintaining vitamin D sufficiency should be a goal for individuals with CAH (49).

Gonadal rest tumors

During early gestation, cells destined to become the steroid-producing cells of the adrenal cortex and gonads differentiate from neighboring regions of the coelomic epithelium. Subsequently, some adrenal precursor cells migrate and descend into the scrotum with the testes. In most instances, these adrenal cells regress during early infancy (50). However, these cells seem to persist in boys with congenital adrenal hyperplasia, to maintain ACTH responsiveness, and can develop into testicular tumors in boys with CAH. Colocalization of these aberrant adrenal cells with ovaries is extremely uncommon (51).

The testicular tumors are more common in the boys who are undertreated or poorly compliant. These tumors tend to be benign, bilateral, and are believed to arise from these aberrant ACTH-responsive adrenal cells. Due to their location in the mediastinum testis, obstruction of the seminiferous tubules leading to gonadal dysfunction and infertility can occur. Histologic features of the tumors are typical for steroid-producing tissue. Expression studies detected adrenal-specific mRNA in such testicular tumors confirming the adrenal-like properties of the tissue (52). Increased glucocorticoid doses generally cause the tumors to shrink, but not all tumors respond (53). A recent report indicated that testis-sparing surgical excision of adrenal rest tumors failed to restore gonadal function (54).

Psychosocial considerations and quality of life

Gender role develops as a result of society's expectations concerning behavior. Though prenatal factors such as hormones and environmental exposures are hypothesized to influence gender role, the specific details regarding how prenatal androgen exposure affects gender identity of girls with virilizing CAH are uncertain (55,56). During childhood, prenatal androgen exposure is reported to influence gender-related behavior and cognitive function. For example, most girls with CAH have been reported to exhibit increased behavior more typical of boys during childhood in terms of toy preferences, rough

play, and aggressiveness. However, the impact upon degrees of femininity and masculinity and cognitive function (spatial and verbal abilities) and handedness does not appear to persist into adult life (57,58). Most women are heterosexual, and their sexual identity is almost invariably female. Available data for 46,XX women with congenital adrenal hyperplasia are inconsistent regarding self-esteem, behavioral self-image, body image, sexual preferences, and psychological adjustment (59–63). The impact of environmental influences cannot be distinguished from the effects of the prenatal hormonal exposures.

The issue of fertility is inextricably related to psychosocial adjustment. Women with classical CAH may experience pain with vaginal penetration (64). As surgical, medical, and psychological treatments have improved, more women with 21-hydroxylase deficiency have successfully completed pregnancies and given birth, most by cesarean section. About 80% of women with simple virilizing disease and approximately 60% of those with the severe salt-wasting form are fertile (65).

Compared with affected women, affected men have fewer problems with reproductive function, specifically gonadal function. Most men have normal sperm counts and are able to father children. However, as noted above, testicular rest tumors can impair gonadal function. Another cause of infertility is increased adrenal androgen secretion which can suppress hypothalamic-pituitary-gonadal function.

Prenatal treatment

The use of prenatal treatment with dexamethasone to decrease virilization of the affected female fetus remains an unresolved controversy (3,31). To be effective, dexamethasone therapy needs to be started as soon as the pregnancy is confirmed. However, long-term prospective follow-up data are lacking regarding the safety of high dose glucocorticoid exposure during early gestation. Since only affected females truly benefit, 7/8 potential outcomes (unaffected and/or male fetuses) are needlessly exposed to dexamethasone.

Other forms of virilizing congenital adrenal hyperplasias

3β-hydroxysteroid dehydrogenase/isomerase deficiency

Congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency is associated with genital ambiguity of both male and female infants. This disorder is due to mutations of the 3β-hydroxysteroid dehydrogenase type 2 *(HSD3B2)* gene; its protein product is expressed in the adrenal cortex and gonads where it converts the Δ^5 steroids, pregnenolone, 17-hydroxypregnenolone, and DHEA, to the respective Δ^4 steroids, progesterone, 17-OHP, and androstenedione. Loss of cortisol-negative feedback inhibition leads to increased DHEA synthesis in 46,XX fetuses with peripheral conversion of DHEA to more potent androgens and subsequent virilization of the external genitalia. Loss of function *HSD3B2* mutations interfere with testosterone biosynthesis leading to undervirilization of the external genitalia in affected 46,XY fetuses. Acute adrenal insufficiency can occur in the neonatal period because complete loss-of-function mutations impair biosynthesis of mineralocorticoids, glucocorticoids, and sex steroids. Typical presentations for the nonsalt-losing forms include premature pubarche in children and hirsutism, chronic anovulation, oligo-amenorrhea, and infertility in adolescent and adult women.

Confirmatory laboratory findings include elevated 17-hydroxypregnenolone concentrations, elevated ratios of Δ^5 to Δ^4 steroids, i.e., 17-hydroxypregnenolone to 17-hydroxyprogesterone, and elevated ratios of 17-hydroxypregnenolone to cortisol. To ascertain for 3β-hydroxysteroid dehydrogenase deficiency using Cortrosyn (0.25 mg) testing, two blood samples for progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, DHEA, androstenedione, and cortisol should be obtained. The first blood sample should be obtained immediately prior to administration of the Cortrosyn. The second blood sample can be obtained either 30 or 60 minutes after the Cortrosyn (66,67). Women with PCOS may have slightly elevated Δ^5 steroid, 17-hydroxypregnenolone, and DHEA, concentrations. However, stimulated 17-hydroxypregenolone concentrations are generally greater than 10,000 ng/dL among women with CAH associated with *HSD3B2* mutations.

11β-hydroxylase deficiency

Congenital adrenal hyperplasia due to 11β-hydroxylase deficiency accounts for < 8% of cases of CAH. Clinical features include glucocorticoid deficiency, excessive adrenal androgen secretion, hypertension, and hypokalemia. This disorder, associated with loss of function mutations of the 11β-hydroxylase (*CYP11B1*) gene, disrupts the final

step of cortisol biosynthesis. Affected female infants may present with ambiguous genitalia; children may present with premature pubarche. Adolescent and adult women typically present with features due to hyperandrogenism. Phenotypic heterogeneity for the magnitude of virilization and hypertension occurs. The typical laboratory finding is an elevated serum 11-deoxycortisol concentration. Serum 17-OHP, androstenedione, and testosterone concentrations may be mildly elevated. PRA concentrations are low or suppressed. The hypertension and hypokalemia are attributed to the accumulation of cortisol precursors such as 11-deoxycortisol and deoxycorticosterone. Whereas the incidence of 11β-hydroxylase deficiency has been estimated to be 1 : 100,000 among Caucasians, the incidence among Israeli Jews of Moroccan origin is reported to be as high as 1 : 7000 (68,69). ACTH stimulation tests may be necessary to establish the diagnosis of 11β-hydroxylase deficiency; 11-deoxycortisol concentrations should be determined on both the pre- and post-Cortrosyn blood samples.

P450-oxidoreductase deficiency or Antley–Bixler syndrome: *POR* deficiency

This form of CAH is due to loss of function mutations in the P450-oxidoreductase (*POR*) gene which is located at chromosome 7q11.2. The protein product of the *POR* gene serves as the electron donor for three steroidogenic enzymes: (i) 17α-hydroxylase/17,20-lyase (P450c17); (ii) 21-hydroxylase (P450c21); and (iii) aromatase (P450aro). Genital ambiguity of both males and females may occur with virilization of affected female infants and undervirilization of affected males. Investigation of these infants suggests the existence of an alternative pathway for androgen biosynthesis limited to the fetus (FIG. 4). In this pathway, 17-OHP is sequentially converted to 5α-pregnane-3α17α-diol-20-one and then to androstenedione (70).

In addition to abnormalities of the external genitalia, affected individuals manifest skeletal malformations such as craniosynostosis, midface hypoplasia, low set ears, choanal atresia or stenosis, and radiohumeral and/or radio-ulnar synostosis. Some mothers virilize during pregnancy (70).

Postnatally, androgen concentrations are not elevated and progressive virilization generally does not occur. Basal and ACTH-stimulated steroid profiles are variable because POR deficiency affects multiple steroidogenic enzymes; serum 17-OHP concentrations tend to be elevated. Urinary analysis of steroid excretion using gas chromatography and mass spectroscopy can provide hormonal confirmation of the diagnosis. Cortisol deficiency may occur; affected individuals may benefit from glucocorticoid replacement therapy (71).

Placental aromatase deficiency

The enzyme aromatase, P450aro, is expressed in ovaries, placenta, and extragonadal tissues including muscle, liver, adipose tissue, brain, and hair follicles where it converts androgens to estrogens. During gestation the human fetal adrenal secretes DHEAS which provides substrate to the placenta for estrogen production. Loss of function mutations in the aromatase gene (*CYP19A1*) decreases placental estrogen production and leads to accumulation of androgenic precursors. Clinical features are progressive maternal virilization which may include hirsutism and clitoromegaly. Affected female infants may have virilized external genitalia. Decreased placental aromatase deficiency impairs placental conversion of DHEAS to estrogens, resulting in elevated androgen concentrations (72,73).

Conclusions

Much has been learned about the pathophysiology and molecular genetics of the steroidogenic disorders associated with signs and symptoms of androgen excess. Some women with hirsutism have nonclassical congenital adrenal hyperplasia due to mild defects in adrenal steroidogenesis. It may be difficult to distinguish women with PCOS from those with nonclassical CAH on the basis of history and physical examination alone. One important clue is family history because mothers of women with PCOS may also have signs and symptoms of PCOS. Mothers of children with CAH tend to have fewer signs and symptoms of androgen excess. Measurements of basal steroid levels may not helpful in differentiating among the causes of increased androgen production in such patients and may even be misleading. Hence, ACTH stimulation tests may be necessary to establish or exclude the diagnosis of CAH. Accurate diagnosis is important to guide therapeutic interventions and will inform regarding the genetics and recurrence risks for a family. Treatment for women with CAH differs from those for women with PCOS. Women with CAH, particularly those trying to conceive, may benefit

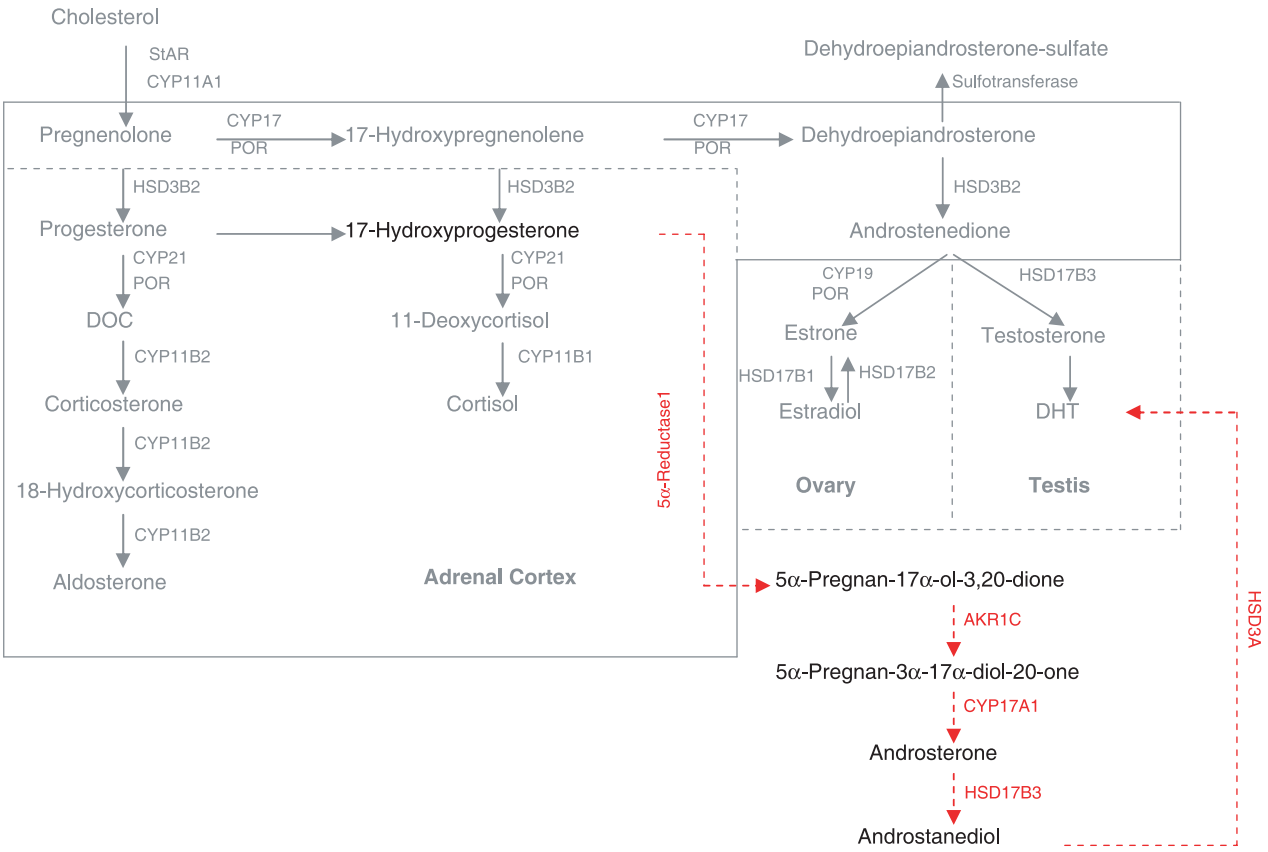

FIG. 4. Alternate steroidogenic pathways of adrenal cortex. This diagram illustrates the alternative pathway (in red) for prenatal androgen biosynthesis. The human P450c17 enzyme appears to prefer 5α-pregnane-3α17α-diol-20-one (5-pdiolone) to 17-hydroxypregnenolone as a substrate. Decreased P450-oxidoreductase (POR) activity impairs 17,20-lyase activity and leads to accumulation of 17-hydroxyprogesterone (17-OHP) which is subsequently directed towards this alternative pathway. Genes are steroidogenic acute regulatory protein (*StAR*); P450 side-chain cleavage enzyme (*CYP11A1*); 3β-hydroxysteroid dehydrogenase type 2 (*HSD3B2*); 17α-hydroxylase/17,20-lyase (*CYP17A1*); 21-hydroxylase (*CYP21A2*); 11β-hydroxylase (*CYP11B1*); aldosterone synthase (*CYP11B2*); 3α-hydroxysteroid dehydrogenase (*AKR1C*); 17β-hydroxysteroid dehydrogenase type 1 (*HSD17B1*); 17β-hydroxysteroid dehydrogenase type 2 (*HSD17B2*); 17β-hydroxysteroid dehydrogenase type 3 (*HSD17B3*); P450-oxidoreductase (POR); and P450 aromatase (*CYP19A1*).

from glucocorticoid replacement therapy. Oral contraceptives and spironolactone may be beneficial adjunctive treatments. Glucocorticoid treatment is generally not indicated for women with PCOS. Oral contraceptives and/or metformin are generally prescribed for women with PCOS. Accurate diagnosis is important to guide therapeutic interventions, e.g. cortisol vs. oral contraceptives vs. metformin. Accurate diagnosis is also important to predict recurrence risks for a family because CAH is an autosomal recessive disorder whereas PCOS is a multi-factorial polygenic disorder.

Future goals to enhance the care of patients with nonclassical CAH include optimizing hormone replacement therapies, better tools for monitoring adequacy of therapy, and facilitating the transition from pediatric to adult care.

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