CONGENITAL ADRENAL HYPERPLASIA

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ABSTRACT

Congenital Adrenal Hyperplasia (CAH) is a term used to describe a group of genetically determined disorders of defective steroidogenesis that result in variable deficiency of the end products cortisol and/or aldosterone and their deleterious, including life-threatening, effects on metabolism and electrolytes with simultaneous diversion to the accumulation of androgens and their virilizing effects. Although we discuss the various enzymatic defects that are involved, we focus on the most common enzyme deficiency, 21-hydroxylase. Depending on the residual enzymatic activity governed by the genetic mutation, 21-hydroxylase deficiency CAH is classified as either classical (salt wasting or simple virilizing) or non-classical. In classical 21-hydroxylase deficiency CAH, there is an accumulation of 17-hydroxyprogesterone which is shunted into the intact androgen pathway and may lead to prenatally virilized external genitalia in females as early as 9 weeks of gestation. Inadequately treated patients may develop progressive penile or clitoral enlargement, premature adrenarche, precocious puberty, rapid linear growth accompanied by premature epiphysis maturation leading to compromised final adult height and impaired fertility. Moreover, inadequately treated salt loss increases the risk for adrenal crises. In contrast, over treatment with cortisol results in "Cushingoid" effects including retarded bone development. We describe the various defects, their manifestations and goals for therapy, and emerging newer therapies for CAH to both correct the deficiency in cortisol and aldosterone secretion while suppressing overproduction of ACTH.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) refers to a group of disorders that arise from defective steroidogenesis. The production of cortisol in the zona fasciculata of the adrenal cortex occurs in five major enzyme-mediated steps. CAH results from deficiency in any one of these enzymes. Impaired cortisol synthesis leads to chronic elevations of ACTH via the negative feedback system, causing overstimulation of the adrenal cortex and resulting in hyperplasia and over-secretion of the precursors to the enzymatic defect. The five forms of CAH are summarized in Table 1. Impaired enzyme function at each step of adrenal cortisol biosynthesis leads to a unique combination of elevated precursors and deficient products. The most common enzyme

deficiency that accounts for more than 90% of all CAH cases is 21-hydroxylase deficiency (1).

EPIDEMIOLOGY

Data from close to 6.5 million newborn screenings worldwide indicate that classical CAH occurs in about 1:13,000 to 1:15,000 live births (2). It is estimated that 75% of patients have the salt-wasting phenotype(1). Non-classical 21OHD CAH (NC21OHD) is more common. The incidence in the heterogeneous population of New York City is about 1 in 100, making NC21OHD the most frequent autosomal recessive disorder in humans. NC21OHD is particularly prevalent in certain populations, showing a high ethnic specificity. In the Ashkenazi Jewish population, 1 in 3 are carriers of the allele, and 1 in 27 are affected with the disorder (3-5).   CAH resulting from 11β-hydroxylase deficiency (11β-OHD) is the second most common cause of CAH, accounting for 5-8% of all cases (6). It occurs in about 1 of every 100,000 live births in the general population (7) and is more common in some populations of North African origin (8). In Moroccan Jews, for example, the disease incidence was initially estimated to be 1 in 5,000 live births (9); subsequently, it was shown to occur less frequently (10), but remains more common than in other populations. The other forms of CAH are considered rare diseases and their incidence is unknown in the general population.

PATHOPHYSIOLOGY

Adrenal steroidogenesis occurs in three major pathways: glucocorticoids, mineralocorticoids, and sex steroids as shown in Figure 1. The adrenal gland architecture suggests that the adrenal acts as three separate glands: zona glomerulosa, zona fasciculate, zona reticularis (11). The hypothalamic-pituitary-adrenal feedback system is mediated through the circulating level of plasma cortisol by negative feedback of cortisol on CRF and ACTH secretion. (Figure 2) Therefore, any CAH condition that results in a decrease in cortisol secretion leads to increased ACTH production, which in turn stimulates (1) excessive synthesis of adrenal products in those pathways unimpaired by the enzyme deficiency and (2) a build-up of precursor molecules in pathways blocked by the enzyme deficiency.
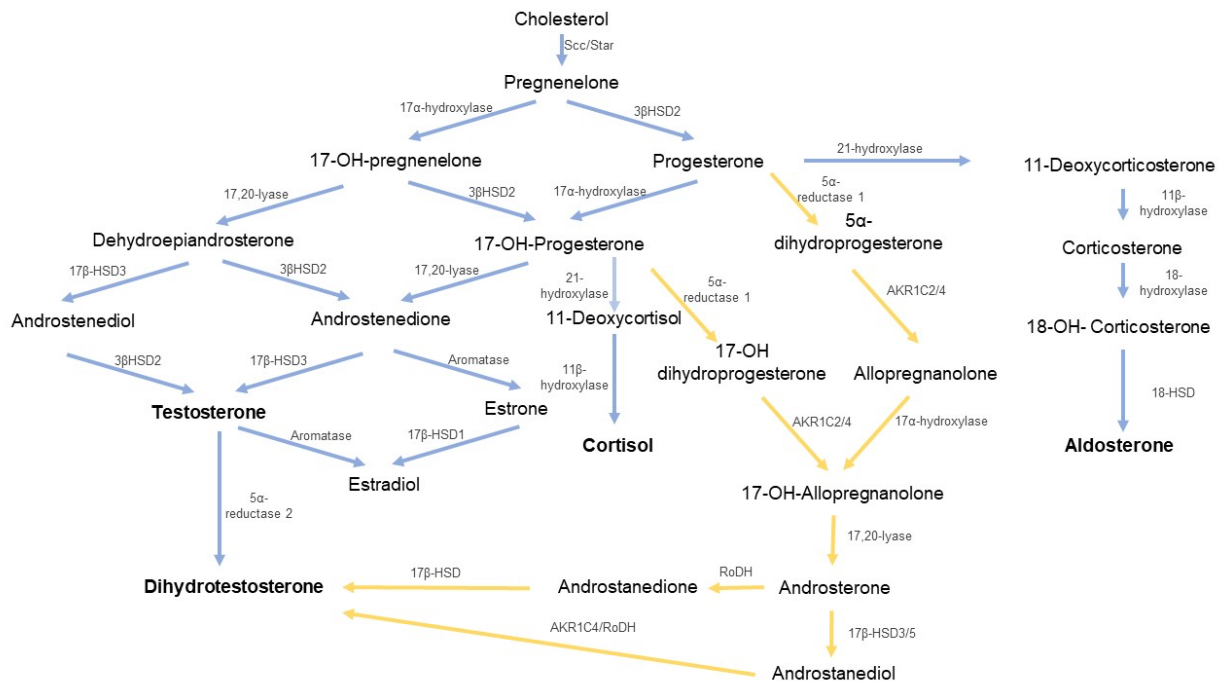
Figure 1. The classical and backdoor pathways of adrenal steroidogenesis: The classical pathway is highlighted in blue and the backdoor pathway is highlighted in orange. In the classical pathway, five enzymatic steps are necessary for cortisol production. In the first step of adrenal steroidogenesis, cholesterol enters mitochondria via a carrier protein called steroidogenic acute regulatory protein (StAR). ACTH stimulates cholesterol cleavage, the first and rate limiting step of adrenal steroidogenesis. The five enzymes required for cortisol production are cholesterol side chain cleavage enzyme (SCC), 17α-hydroxylase, 3β-hydroxysteroid dehydrogenase (3βHSD2), 21-hydroxylase, and 11β-hydroxylase. The backdoor pathway is an alternative pathway producing dihydrotestosterone. The enzymes include 5α-reductase 1, aldo keto reductases, retinol dehydrogenase RoDH, 17β-hydroxysteroid dehydrogenases, 17α-hydroxylase.
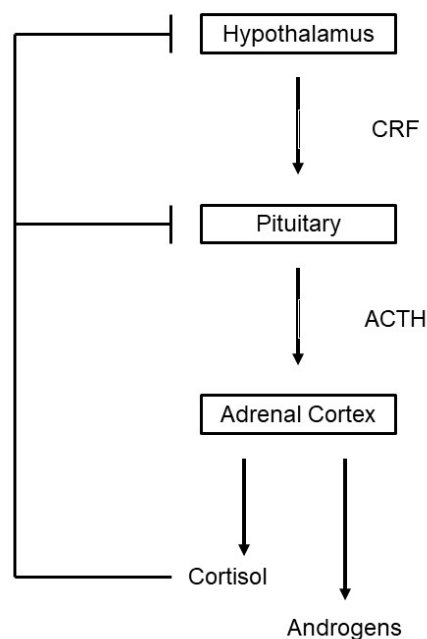
Figure 2. The hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin-releasing factor produced in the hypothalamus stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary gland. ACTH stimulates the production of cortisol and androgens in the adrenal cortex. There is negative feedback on the hypothalamus and pituitary gland by cortisol.

The clinical symptoms of the five different forms of CAH result from the specific hormones that are deficient and those that are produced in excess as outlined in Table 1. In the most common form 21OHD-CAH, the function of 21-hydroxylating cytochrome P450 is deficient, creating a block in the P450 cortisol production pathway. This leads to an accumulation of 17-hydroxyprogesterone (17-OHP), a precursor of the 21-hydroxylation step. Excess 17-OHP is then shunted into the intact androgen pathway, where the 17,20-lyase enzyme converts 17-OHP to Δ4-androstenedione, the major adrenal androgen. Mineralocorticoid (aldosterone) deficiency is a feature of the most severe form of the disease and hence named salt wasting CAH. The enzyme defect in the non-classical form of 21OHD CAH is mild and salt wasting does not occur. The analogy of all other enzyme deficiencies in terms of precursor retention and product deficiencies are shown in Table 1.

Table 1. Summary of the Clinical, Hormonal, and Genetic Features of Steroidogenic Defects (1)						
Condition	**Onset**	**Abnormality**	**Genitalia**	**Mineralocorticoid Effect**	**Typical Features**	**Gene**
Lipoid CAH	Congenital	StAR Protein	Female, with no sexual development	Salt wasting	All steroid products low	StAR 8p11.2
Lipoid	Congenital	P450scc	Female, with no	Salt wasting	All steroid	CYP11A

CAH			sexual development		products low	15q23-24
3β-HSD deficiency, classic	Congenital	3β-HSD	Females virilized, males under-virilized	Salt wasting	Elevated DHEA, 17-pregnenolone, low androstenedione, testosterone, elevated K, low Na, CO2	HSD3B2 1p13.1
3β-HSD deficiency, non-classic	Postnatal	3β-HSD	Normal genitalia with mild to moderate hyperandrogenism postnatally	None	Elevated DHEA, 17-pregnenolone, low androstenedione, testosterone	Absent or unknown
17α-OH deficiency	Congenital	P450c17	Variable sexual development	Hypokalemic low-renin hypertension	Normal or decreased androgens and estrogen, elevated DOC, corticosterone	CYP17 10q24.3
17,20-Lyase deficiency	Congenital	P450c17	Infantile female genitalia	None	Decreased androgens and estrogens	CYP17 10q24.3
Combined 17α-OH/17,20-lyase deficiency	Congenital	P450c17	Infantile female genitalia	Hypokalemic low-renin hypertension	Decreased androgens and estrogens	CYP17 10q24.3
Combined 17α-OH/17,20-lyase deficiency	Postnatal	P450c17	Infertility, Infantile female genitalia	None	Decreased follicular estradiol and increased progesterone	CYP17 10q24.3
Classic 21-OH deficiency, salt wasting	Congenital	P450c21	Females prenatally virilized, normal male genitalia, hyperpigmentation	Salt wasting	Elevated 17-OHP, DHEA, and androstenedione, elevated K, low Na, CO2	CYP21 6p21.3
Classic	Congenital	P450c21	Females	None	Elevated 17-	CYP21

21-OH deficiency, simple virilizing			prenatally virilized, normal male genitalia		OHP, DHEA, and androstenedione, normal electrolytes	6p21.3
Non-classic 21-OH deficiency	Postnatal	P450c21	Males and females with normal genitalia at birth, hyperandrogenism postnatally	None	Elevated 17-OHP, DHEA, and androstenedione on ACTH stimulation	CYP21 6p21.3
Classic CAH 11β-deficiency	Congenital	P450c11B1	Females virilized with atypical genitalia, males unchanged	Low-renin hypertension	Elevated DOC, 11-deoxycortisol (S); androgens, low K, elevated Na, CO2	CYP11B1 8q24.3
Non-classic CAH 11β-deficiency	Postnatal	P450c11B1	Males and females with normal genitalia at birth, hyperandrogenism postnatally	None	Elevated 11-deoxycortisol ± DOC, elevated androgens	CYP11B1 8q24.3
P450 Oxido-Reductase Deficiency	Congenital	POR	Females virilized with atypical genitalia, males under-virilized	None	Partial, combined and variable defects of P450c21, P450c17 and P450aro activity	POR 7q11.2

CAH, CONGENITAL ADRENAL HYPERPLASIA; DHEA, DEHYDROEPIANDROSTERONE; DOC, DEOXYCORTICOSTERONE; 3B-HSD, 3B-HYDROXYSTEROID DEHYDROGENASE; OH, HYDROXYLASE; 17-OHP, 17-HYDROXYPROGESTERONE. ADAPTED FROM: WAJNRAJCH MP AND NEW MI. CHAPTER 103: DEFECTS OF ADRENAL STEROIDOGENESIS. ENDOCRINOLOGY, ADULT AND PEDIATRIC. 6TH EDITION. 2010. PP 1897-1920. PERMISSION OBTAINED.

CLINICAL FEATURES

EXTERNAL GENITALIA

VIRILIZING FORMS OF CAH: CLASSICAL 21-HYROXYLASE DEFICIENCY AND 11B-HYDOXYLASE DEFICIENCY

Females with classical CAH owing to 21OHD and 11β-hydroxylase deficiency generally present at birth with virilization of their genitalia. Adrenocortical function begins at the 7th week of gestation (12); thus, a female fetus with classical CAH is exposed to adrenal androgens at the critical time of sexual differentiation (between 9 to 15 weeks gestational age). Androgens masculinize external female genitalia causing physical changes including: clitoral enlargement, fusion and scrotalization of the labial folds, and rostral migration of the urethral/vaginal perineal orifice, placing the phallus in the male position. The degree of genital virilization may range from mild clitoral enlargement alone to, in rare cases, a penile urethra (Prader V genitalia). Degrees of genital virilization are classified into five Prader stage (13) (see Figure 3).
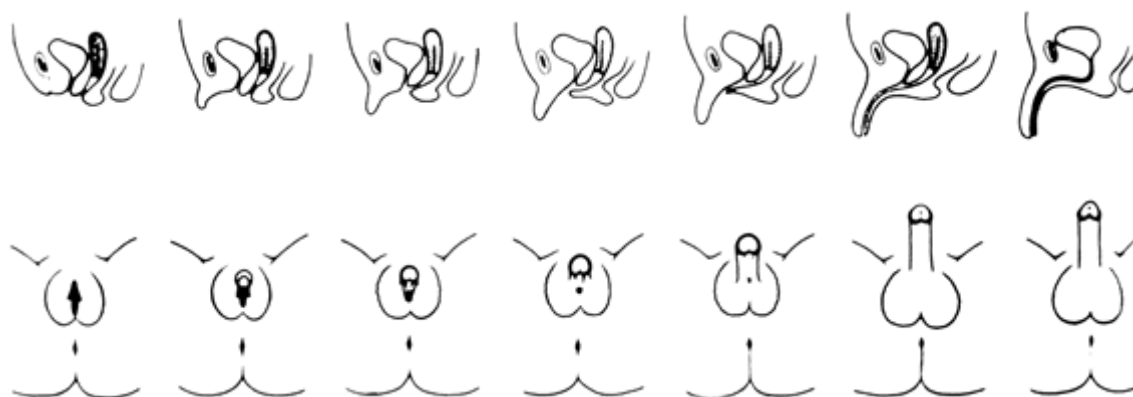

Figure 3. Different degrees of virilization according to the scale developed by Prader (15). Stage I: clitoromegaly without labial fusion Stage II: clitoromegaly and posterior labial fusion Stage III: greater degree of clitoromegaly, single perineal urogenital orifice, and almost complete labial fusion Stage IV: increasingly phallic clitoris, urethra-like urogenital sinus at base of clitoris, and complete labial fusion Stage V: penile clitoris, urethral meatus at tip of phallus, and scrotum-like labia (appear like males without palpable gonads).

Internal Genitalia

In contrast to the virilization of the external genitalia, internal female genitalia, the uterus, fallopian tubes and ovaries, develop normally. Females with CAH do not produce anti-Müllerian hormone (AMH), which is produced by the testicular Sertoli cells. Internal female structures are Müllerian derivatives and are not androgen responsive. Therefore, the affected female born with virilized external genitalia but normal female internal genitalia has the possibility of normal fertility (1). Surgical correction of the external genitalia (genitoplasty) may be considered in severely virilized females. This is further discussed in a later section, Treatments.

Postnatal Effects and Growth

Deficient postnatal treatment in boys and girls results in continued exposure to excessive androgens, causing progressive penile or clitoral enlargement, the development of premature pubic hair (pubarche),

axillary hair, acne, and impaired fertility. Advanced somatic and epiphyseal development occurs with rapid growth during childhood. This rapid linear growth is usually accompanied by premature epiphyseal maturation and closure, resulting in a final adult height that is below that expected from parental heights (on average -1.1 to -1.5 SD below the mid-parental target height) (14). This is on average 10 cm below the mid-parental height (15). On the other hand, poor growth can occur in patients with 21OHD as a result of excess glucocorticoid treatment. Short stature occurs even in patients with good hormonal adrenal control. A study of growth hormone therapy alone or in combination with a GnRH analog and aromatase inhibitors in CAH patients with compromised height prediction showed improvement in short- and long-term growth to reduce the height deficit (15). Aromatase inhibitor as a sole adjunct treatment reduces bone age advancement without adverse effects on bone mineral density or visceral adipose tissue (16).

Puberty

In the majority of patients treated adequately from early life, the onset of puberty in both girls and boys with classical 21OHD occurs at the expected chronological age. A careful study showed that the mean ages at onset of puberty in both males and females were somewhat younger than the general population, but did not differ significantly among the three forms of 21OHD (17).

In those who are inadequately treated, advanced epiphyseal development can lead to central precocious puberty. In those with advanced bone maturation at the initial presentation, such as in simple virilizing males, the exposure to elevated androgens followed by the suddenly decreased androgen levels after initiation of glucocorticoid treatment may cause an early activation of the hypothalamic-pituitary-gonadal axis. Studies suggest that excess adrenal androgens (aromatized to estrogens) inhibit the pubertal pattern of gonadotropin secretion by the hypothalamic-pituitary axis. This inhibition, via a negative feedback effect, can be reversed by glucocorticoid treatment (17, 18).

Following the onset of puberty, in a majority of successfully treated patients, the milestones of further development of secondary sex characteristics in general appear to be normal (17). In adolescents and adults, signs of hyperandrogenism may include male-pattern alopecia (temporal balding) and acne. Female patients may develop hirsutism and menstrual irregularities. Although the expected age of menarche may be delayed in females with classical CAH (18), when adequately treated many have regular menses after menarche (19, 20). Menstrual irregularity and secondary amenorrhea with or without hirsutism occur in a subset of post-menarchal females, especially those in poor hormonal control. Primary amenorrhea or delayed menarche may occur if a female with classical CAH is untreated, inadequately treated, or over treated with glucocorticoids (20). In addition, women with CAH may develop polycystic ovarian syndrome (PCOS), likely as a complication of poorly controlled CAH (21, 22).

Gender Role Behavior and Cognition

Prenatal androgen exposure in females affected with the classic forms of 21OHD CAH not only has a masculinizing effect on the development of the external genitalia, but also on childhood behavior. Both physical and behavioral masculinization were related to genotype, indicating that behavioral masculinization in childhood is a consequence of prenatal androgen exposure. Further, changes in childhood play behavior is correlated with reduced female gender satisfaction. Prenatal androgen exposure is related to a decrease in self-reported femininity in dose response manner in adulthood (23). Affected adult females are more likely to have gender dysphoria, and experience less heterosexual interest

and reduced satisfaction with the assignment to the female sex (24). In contrast to females, males affected with CAH do not show a general alteration in childhood play behavior, core gender identity and sexual orientation (24). The rates of bisexual and homosexual orientation were increased in women with all forms of 21OHD CAH. They were found to correlate with the degree of prenatal androgenization (24). Of interest, bisexual/homosexual orientation was correlated with global measures of masculinization of nonsexual behavior and predicted independently by the degree of both prenatal androgenization and masculinization of childhood behavior (25). Among 46,XX CAH patients raised as girls, reported rates of gender dysphoria varied from 6.3% to 27.2%(26). Most expressed gender dysphoria in late adolescence and adulthood. Cultural views may determine serious biases in reports of gender dysphoria coming from countries with disproportional societal advantages for males and/or religious restrictions. Male gender raised patients were approximately 10% of CAH cohorts, mostly from underdeveloped countries, with a high proportion of late diagnoses (26).With regards to cognitive abilities, such as visuospatial/motor ability and handedness, the effect of prenatal androgen exposure continues to be elucidated. A study found males and females with CAH scored higher than their siblings of the same sex in measures of visual special processing suggesting that androgens affect spatial ability (27). The effect of CAH on intelligence is controversial. One study showed no evidence of intellectual deficit in either females or males with CAH. Intelligence was not significantly associated with disease characteristics (28).

Fertility

Difficulty with fertility in females with CAH may arise for various reasons, including anovulation, secondary polycystic ovarian syndrome, irregular menses, non-suppressible serum progesterone levels, or an inadequate introitus. Fertility is reduced

in salt-wasting 21OHD (29). In a retrospective survey of fertility rates in a large group of females with classical CAH, simple virilizers were shown to be more likely to become pregnant and carry the pregnancy to term than salt-wasters. Adequate glucocorticoid therapy is an important variable with respect to fertility outcome. The development of PCOS in CAH patients is not uncommon and may be related to both prenatal and postnatal excess androgen exposure, which can affect the hypothalamic-pituitary-gonadal axis. An inadequate vaginal introitus can affect up to a third of classical CAH adult females. Since vaginal dilation is needed to maintain good patency, vaginoplasty is delayed until sexual intercourse is regular or when the patient can assume responsibility for vaginal dilatation (30).

Males with CAH, particularly if poorly treated, may have reduced sperm counts and low testosterone as a result of small testes due to suppression of gonadotropins and sometimes intra-testicular adrenal rests(31, 32). All of these complications may result in diminished fertility. In male patients with classical CAH, several long-term studies indicate that those who have been adequately treated undergo normal pubertal development, have normal testicular function, and normal spermatogenesis and fertility (32, 33). However, small testes and aspermia can occur in patients as a result of inadequately controlled disease (34, 35). Testicular adrenal rest tumor can lead to end stage damage of testicular parenchyma, most probably as a result of longstanding obstruction of the seminiferous tubules (36). In contrast, some investigators have reported normal testicular maturation as well as normal spermatogenesis and fertility in patients who had never received glucocorticoid treatment (37).

Studies demonstrate that post pubertal males with inadequately treated CAH are at a very high risk to develop testicular adrenal rest tumors (TARTs) In one study, almost all these patients were found to have

adenomatous adrenal rests within the testicular tissue, as indicated by the presence of specific 11β-hydroxylated steroids in the blood from gonadal veins (38). These tumors have been reported to be ACTH dependent and to regress following adequate steroid therapy (39-43). These testicular adrenal rests are more frequent in males with salt-wasting CAH and are associated with an increased risk of infertility(30, 44) . Regular testicular examination and periodic testicular ultrasonography are recommended for early detection of testicular lesions. If present, dexamethasone treatment can be considered to suppress TARTs.

Salt-Wasting 21-Hydroxylase Deficiency

When the deficiency of 21-hydroxylase is severe, adrenal aldosterone secretion is not sufficient for sodium reabsorption by the distal renal tubules, and individuals suffer from salt wasting in addition to cortisol deficiency and androgen excess. Infants with renal salt wasting have poor feeding, weight loss, failure to thrive, vomiting, dehydration, hypotension, hyponatremia, and hyperkalemic metabolic acidosis progressing to adrenal crisis (azotemia, vascular collapse, shock, and death). Adrenal crisis can occur as early as age one to four weeks. The salt wasting is presumed to result from inadequate secretion of salt-retaining steroids, primarily aldosterone. In addition, hormonal precursors of the 21-OH enzyme may act as antagonists to mineralocorticoid action in the sodium-conserving mechanism of the immature newborn renal tubule (45-47).

Affected males who are not detected in a newborn screening program are at high risk for a salt-wasting adrenal crisis because their normal male genitalia do not alert medical professionals to their condition. They may be discharged from the hospital after birth without diagnosis and experience a salt-wasting crisis at home. On the other hand, salt wasting females are born with atypical genitalia that trigger the diagnostic process and appropriate treatment. It is important to

recognize that the extent of genital virilization may not differ among the two forms of classical CAH, the simple virilizing and the salt-wasting form. Thus, even a mildly virilized newborn with 21OHD should be observed carefully for signs of a potentially life-threatening crisis within the first few weeks of life. The difference between salt-wasting and simple virilizing form of 21OHD is the quantitative difference in activity of the 21-hydrozylase enzyme, which results from specific mutations. In vitro expression studies show that as little as 1% of 21-hydroxylase activity is sufficient to synthesize enough aldosterone to prevent significant salt wasting (48). It has been observed that an aldosterone biosynthetic defect apparent in infancy may ameliorate with age (49, 50).A spontaneous partial recovery of aldosterone biosynthesis in an adult patient with a homozygous deletion of the *CYP21A2* gene who had documented severe salt wasting in infancy has been reported (51). Therefore, it is desirable to follow the sodium and mineralocorticoid requirements carefully by measuring plasma renin activity (PRA) in patients who have been diagnosed in the neonatal period as salt wasters.

Simple-Virilizing 21-Hydroxylase Deficiency

The salient features of classical simple virilizing 21OHD are prenatal virilization and progressive postnatal masculinization with rapid somatic growth and advanced epiphyseal maturation leading to early epiphyseal closure and likely short stature. There is usually no evidence of mineralocorticoid deficiency in this disorder.

Diagnosis at birth of a female with simple virilizing CAH is usually made immediately because of the apparent genital ambiguity. Since the external genitalia are not affected in newborn males, hyperpigmentation and an enlarged phallus may be the only clues suggesting increased ACTH secretion and cortisol deficiency. Diagnosis at birth in males thus rests on prenatal or newborn screening. If a

female is not treated with glucocorticoid replacement therapy early post-natally, her genitalia may continue to virilize due to continued excess adrenal androgens, and pseudo precocious puberty may occur. In patients with salt-wasting 21OHD, signs of hyperandrogenism in children affected with CAH include early onset of facial, axillary, and pubic hair, adult body odor, and rapid somatic growth and bone age advancement, leading to short stature in adulthood. The same issues as discussed above related to puberty, fertility, behavior and cognition apply to patients with simple-virilizing 21OHD (1).

Non-Classical 21-Hydroxylase Deficiency

Non-classical 21OHD (NC-21OHD), previously known as late-onset 21OHD, is much more common than the classical form, with an incidence as high as 1:27 in Ashkenazi Jews (3). Individuals with the non-classical

(NC) form of 21OHD have only mild to moderate enzyme deficiency and present postnatally, eventually developing signs of hyperandrogenism. Females with NC-CAH do not have virilized genitalia at birth.

NC-CAH may present at any age after birth with a variety of hyperandrogenic symptoms. This form of CAH results from a mild deficiency of the 21-hydroxylase enzyme. Table 2 summarizes the main clinical characteristics of all forms of 21OHD CAH. While serum cortisol concentration is typically low in patients with the classic form of the disease, it is usually normal in patients with NC 21OHD. Similar to classical CAH, NC-CAH may cause premature development of pubic hair, advanced bone age and accelerated linear growth velocity in both males and females. Severe cystic acne has also been attributed to NC-CAH (52, 53).

Table 2. Clinical Features in Individuals with Classic and Non-Classic 21-Hydroxylase Deficiency in the Untreated Form		
Feature	**21-OH Deficiency**	
	Classic	**Non-Classic**
Prenatal virilization	Females only	Absent
Postnatal virilization (hyperandrogenism)	Females and Males	Typical
Salt wasting	~75% of all individuals	Absent

Women may present with a variety of symptoms of androgen excess which may be highly variable and organ-specific, including hirsutism, temporal baldness, acne and infertility. Menarche in females may be normal or delayed, and secondary amenorrhea is a frequent occurrence. Further masculinization may include hirsutism, male habitus, deepening of the voice, or male-pattern alopecia (temporal recession). Polycystic ovarian syndrome may also be seen as a secondary complication in these patients. Possible reasons for the development of PCOS include reprogramming of the hypothalamic-

pituitary-gonadal axis from prenatal exposure to androgens, or chronic levels of excess adrenal androgens that disrupt gonadotropin release and have direct effects on the ovary, ultimately leading to the formation of cysts. Because of the overlap of hyperandrogenic symptoms, it is important to consider NC 21OHD in a patient diagnosed with PCOS (54, 55).

In adult males, early balding, acne, or impaired fertility may prompt the diagnosis of NC-CAH. A highly reliable constellation of physical signs of adrenal androgen excess is the presence of pubic hair,

enlarged phallus, and relatively small testes. Males may have small testes compared to the phallus, which results from suppression of the hypothalamic-pituitary-gonadal axis from adrenal androgens. They may also develop TARTs, which can cause infertility, although some untreated men have been fertile(31, 33). Signs of NC-CAH in adult males may be limited to short stature, oligo-zoospermia and impaired fertility.

A subset of individuals with NC-21OHD are completely asymptomatic when detected (usually as part of a family study or evaluation for infertility), but it is thought, based on longitudinal follow-up of such patients, that symptoms of hyperandrogenism may wax and wane with time. The presence of 21OHD can also be discovered during the evaluation of an incidental adrenal mass (56). One study showed that an increased incidence of adrenal incidentalomas has been found, which was reported as high as 82% in patients with 21OHD and up to 45% in subjects heterozygous for 21OHD mutations. This probably arises from hyperplastic tissue areas and does not require surgical intervention (57). Overall, however, CAH is an uncommon cause of incidentalomas, accounting for less than 1% in one series (58, 59).

OTHER FORMS OF CONGENITAL ADRENAL HYPERPLASIA

11-B HYDROXYLASE DEFICIENCY

Virilization and low renin hypertension are the prominent clinical features of 11β hydroxylase deficiency (11β-OHD) (60). The virilizing signs and symptoms of this disorder are similar to or more severe than classical 21OHD. Despite failure of aldosterone production, overproduction of deoxycorticosterone (DOC), *in vivo* a less potent mineralocorticoid, causes salt retention and hypertension. Elevated blood pressure is usually not identified until later in childhood or in adolescence, although its appearance in an infant 3 months of age

has been documented (61). In addition, hypertension correlates variably with biochemical values, and clinical signs of mineralocorticoid excess and the degree of virilization are not well correlated. Some severely virilized females are normotensive, whereas mildly virilized patients may experience severe hypertension leading to fatal vascular accidents (62, 63). Complications of long-standing uncontrolled hypertension, including cardiomyopathy, retinal vein occlusion and blindness have been reported in 11β-OHD patients (64, 65). Potassium depletion develops concomitantly with sodium retention, but hypokalemia is variable. Renin production is suppressed secondary to mineralocorticoid-induced sodium retention and volume expansion.

A mild non-classical form of 11β-OHD CAH has been reported. Unlike the common non-classical form of 21OHD, this form is very rare. Non-classical 11β-OHD has been diagnosed in normotensive children with mild virilization or precocious pubarche (6) and in adults with signs of hyper-androgen effect (66) as well as a woman with infertility (67) (69). Despite a hormonal profile consistent with 11β-OHD, mutations in the *CYP11B1* gene may not always be present (66). The best biochemical marker of 11β-OHD is elevated serum 11-deoxycortisol concentration.

3-β Hydroxysteroid Dehydrogenase Deficiency

There are two forms of the 3 β -hydroxysteroid dehydrogenase enzyme (3 β -HSD): type I and type II. Type II 3 β -HSD enzyme is expressed in the adrenal cortex and gonads and is responsible for conversion of Δ5 (delta 5) to Δ4 (delta 4) steroids (1). This enzyme is essential for the formation of progesterone, which is the precursor for aldosterone, and 17-OHP, which is the precursor for cortisol in the adrenal cortex as well as for androstenedione, testosterone, and estrogen in the adrenal cortex and gonads (68, 69). Therefore, deficiency of 3ß-HSD in the classic form of 3ß-HSD deficiency CAH results in insufficient cortisol

synthesis, salt-wasting in the most severe form, and virilization of external genitalia in females due to androgen effect from the peripheral conversion of circulating Δ5 precursors to active Δ4 steroids. Simultaneous type II 3ß-HSD deficiency in the gonads results in incomplete virilization of the external genitalia in males. Thus, genital ambiguity can result in both sexes (70).

17 α -Hydroxylase/17,20 Lyase Deficiency

Steroid 17 α-hydroxylase/17,20 lyase deficiency accounts for approximately 1% of all CAH cases and affects steroid synthesis in both the adrenals and gonads(71). Patients have impaired cortisol synthesis, leading to ACTH over secretion, which increases serum levels of deoxycorticosterone and especially corticosterone, resulting in low renin hypertension, hypokalemia, and metabolic alkalosis. Affected females are born with normal external genitalia, however affected males are born with under-virilized genitalia due to their deficient gonadal testosterone production. 17 α-Hydroxylase/17,20 lyase deficiency is often recognized at puberty in female patients who fail to develop secondary sex characteristics (72).

Congenital Lipoid Adrenal Hyperplasia

Congenital lipoid adrenal hyperplasia is an extremely rare and severe form of CAH which is caused by mutations in the steroidogenic acute regulatory protein (StAR). Both the adrenal glands and the gonads exhibit a severe defect in the conversion of cholesterol to pregnenolone (73, 74). More specifically, StAR mediates the acute steroidogenic response by moving cholesterol from the outer to inner mitochondrial membrane (the rate-limiting step of steroidogenesis), and when this does not occur, cholesterol and cholesterol esters accumulate (75). In the most severe form, males with congenital lipoid hyperplasia are born with female-appearing external genitalia. Females have a normal genital phenotype at birth but remain

sexually infantile without treatment. Salt wasting occurs in both males and females. If not detected and treated, the severe form of lipoid CAH is usually fatal (76). Several cases have been reported that demonstrate that lipoid CAH has a spectrum of clinical presentation, with varying degrees of genital ambiguity (including normal male genitalia in a 46, XY male) and adrenal insufficiency. Mutations in the StAR protein have been reported that retain partial protein function, leading to variable phenotype (77).

The cholesterol side-chain cleavage enzyme (P450scc) is a very slow enzyme located on the inner mitochondrial membrane and catalyzes the conversion of cholesterol to pregnenolone in the first and rate-limiting step in the production of corticosteroids (78). The CYP11A1 gene encoding P450cc lies on chromosome 15q23-24. Mutations in this gene are rare with approximately 20 patients reported (77-79).

Cytochrome P450 OxidoReductase Deficiency

Cytochrome P450 oxido-reductase (POR) deficiency is another rare form of CAH that is caused by a mutation on 7q11.2(80-82) P450 oxidoreductase is an important cofactor for electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) to several enzymes of steroidogenesis including 21-hydroxylase and 17α-hydroxylase/17,20-lyase. The various constellations of partial enzymatic deficiency of 21-hydroxylase, 17α-hydroxylase/17,20-lyase, and aromatase in the developing fetus account for the broad range of genital anomalies seen in both sexes (80). Female newborns may have severe virilization, and males may have under-virilized genitalia due to combined partial 21-hydroxylase and 17α-hydroxylase/17,20-lyase deficiencies. Maternal virilization and low maternal estriol levels are common findings during pregnancies with affected male and female fetuses. Maternal gestational virilization is

likely due to the P450 oxidoreductase effect on placental P450 aromatase (82). Many affected patients also have Antley-Bixler syndrome (type 2) characterized by craniosynostosis, radio-humeral or radioulnar synostosis, arachnodactyly and bowing of the femur (82).

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GENETICS

In general, all forms of CAH are transmitted in an autosomal recessive mode of inheritance as a monogenic disorder. However, there have been reports of cases where none or only one mutation in the responsible gene was identified, including in cases of 21 OHD CAH (83, 84), deficiency of the cholesterol side chain cleavage enzyme (85) and POR deficiency (80). The genes responsible for each form of CAH are shown in **Table 1**.

21-HYDROXYLASE DEFICIENCY

The gene encoding the enzyme 21-hydroxylase, *CYP21A2*, is a microsomal cytochrome P450 located on the short arm of chromosome 6 (86) in the human lymphocyte antigen (HLA) complex (87). *CYP21A2* and its homologue, the pseudogene *CYP21P*, alternate with two genes called C4B and C4A (87, 88) that encode the two isoforms of the fourth component (C4) of serum complement (89). *CYP21A2* and *CYP21P*, which each contain 10 exons, share 98% sequence homology in exons and approximately 96% sequence homology in introns (90, 91).

More than 140 mutations have been described including point mutations, small deletions, small insertions, and complex rearrangements of the gene (92). The most common mutations appear to be the result of either of two types of meiotic recombination events between *CYP21* and *CYP21P*: 1) misalignment and unequal crossing over, resulting in large-scale DNA deletions, and 2) apparent gene conversion events that result in the transfer to *CYP21A2* of smaller-scale deleterious mutations present in the *CYP21P* pseudogene (1, 93).

Both classical and non-classical 21-hydroxylase deficiency are inherited in a recessive manner as allelic variants of the CYP21A2 gene. Classical 21-hydroxylase deficiency tends to result from the presence of two severely affected alleles and non-classical 21-hydroxylase deficiency tends to result from the presence of either two mild 21-hydroxylase deficiency alleles or one severe and one mild allele (compound heterozygote). It is important to note, however, that the 10 most common mutations observed in *CYP21A2* cause variable phenotype effects and are not always concordant with genotype. One study demonstrated that the genotype-phenotype concordance was as high as 90.5% for salt-wasting CAH, 85.1% for simple-virilizing CAH, and 97.8% for non-classical CAH (94). In a study of 1,507 subjects with CAH by New et al, a direct genotype–phenotype correlation was noted in less than 50% of the genotypes studied. However, in the salt wasting and non-classical forms of 21OHD CAH, a phenotype was strongly correlated to a genotype (95). Moreover, it was shown in 2008 that CAG repeats in the androgen receptor have a great influence on variability in virilization of external genitalia of CAH women (96).
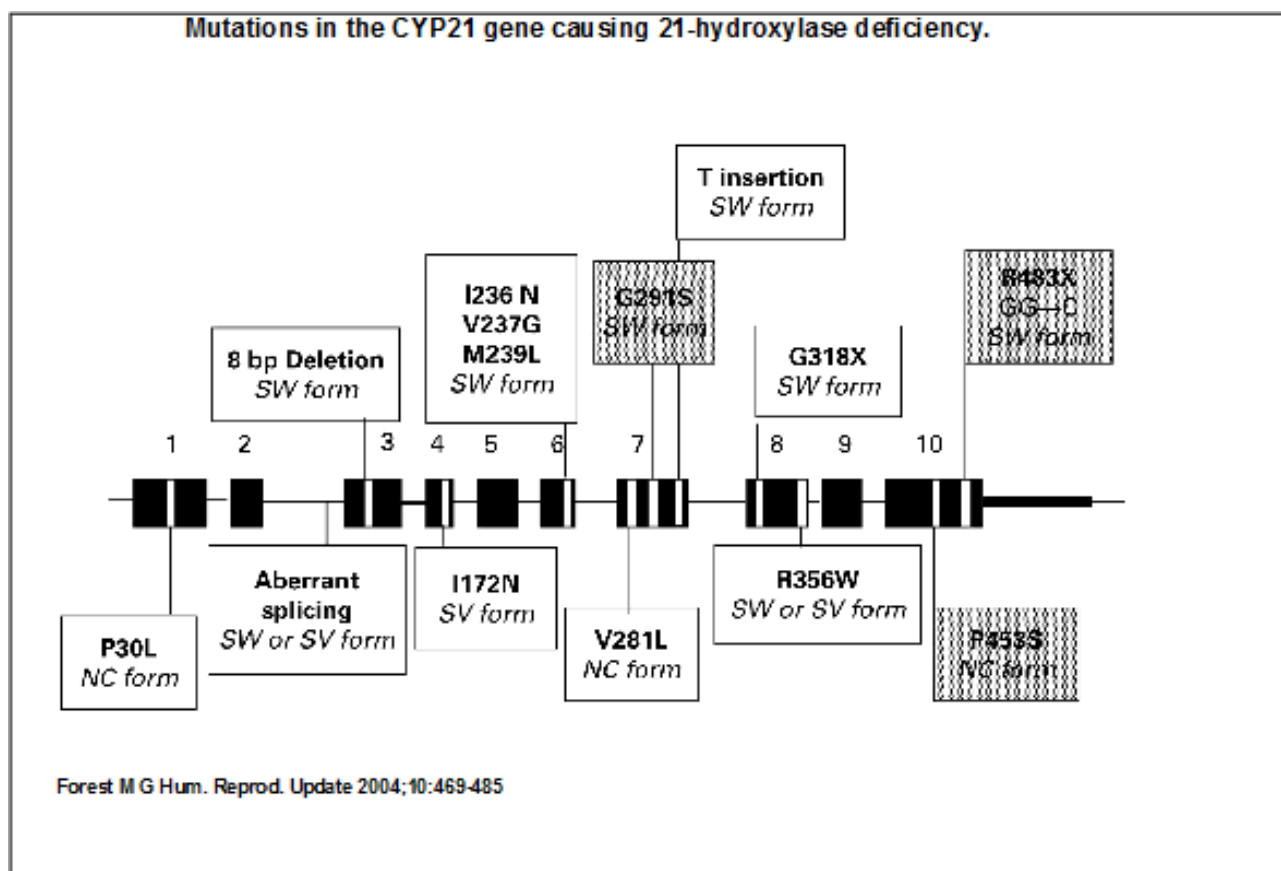
Figure 4. Common mutations in *CYP21A2* gene and their related phenotypes. The numbers indicated exons of the gene. From: Forest MG. Recent advances in the diagnosis and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency (97).

DIAGNOSIS

HORMONAL DIAGNOSIS

Potential diagnosis of CAH must be suspected in infants born with atypical genitalia. The physician is obliged to make the diagnosis as quickly as possible to initiate therapy. The diagnosis and rational decision of sex assignment must rely on the determination of genetic sex, the hormonal determination of the specific deficient enzyme, and an assessment of the patient's potential for future sexual activity and fertility. Physicians are urged to recognize the physical characteristics of CAH in newborns (e.g., atypical genitalia) and to refer such cases to appropriate clinics

for full endocrine evaluation. As indicated in Table 1, each form of CAH has its own unique hormonal profile, consisting of elevated levels of precursors and elevated or diminished levels of adrenal steroid products. Traditionally, laboratories measured urinary excretion of adrenal hormones or their urinary metabolites (e.g., 17-ketosteroids). However, collection of 24-hour urine excretion is difficult, particularly in neonates. (98) Therefore, simple and reliable immunoassays are utilized now for measuring circulating serum levels of adrenal steroids (99). Alternatively, a non-invasive random urine collection in the first days of life for steroid hormone metabolites and precursor/product ratio assessments can be measured simultaneously. It can be used
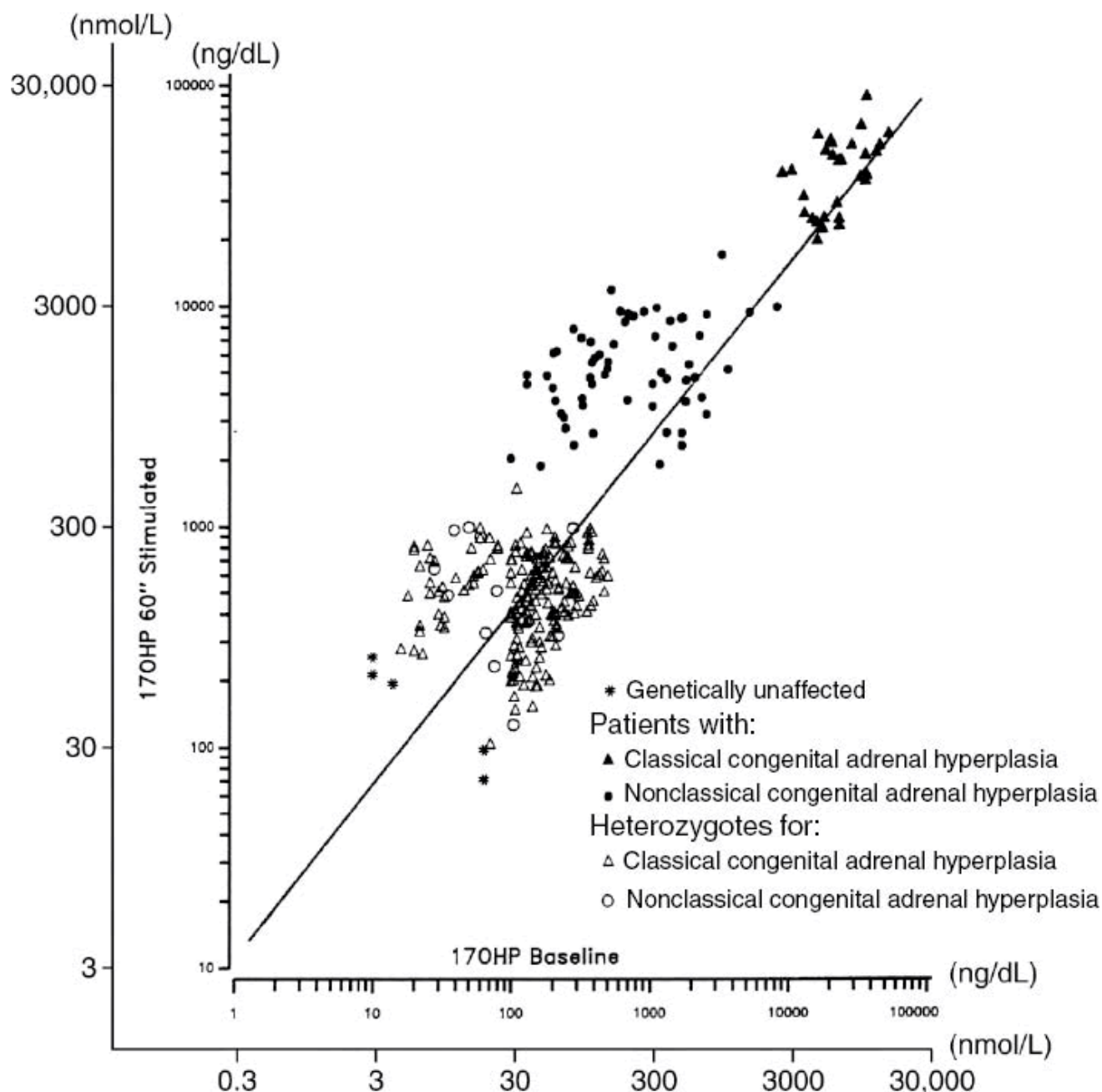
independently or in conjunction with serum steroid assays to increase accuracy and confidence in making the diagnosis and distinguishing the separate enzymatic forms of the disorder (100, 101).

Diagnosis of the 21OHD CAH can also be confirmed biochemically by a hormonal evaluation in blood or serum. In a randomly timed blood sample, a very high concentration of 17-hydroxyprogesterone (17-OHP), the precursor of the defective enzyme, is diagnostic of classical 21OHD. Such testing is the basis of the newborn-screening program developed to identify classically affected patients who are at risk for salt wasting crisis (102). Only 20μl blood, obtained by heel prick and blotted on microfilter paper, is used for this purpose to provide a reliable diagnostic measurement of 17-OHP. The simplicity of the test and the ease of transporting microfilter paper specimens by mail have facilitated the implementation of CAH newborn screening programs worldwide. As of 2009, all 50 states in the United States screen for CAH. Now, more than 35 countries worldwide have also established newborn screening programs. (103) False-positive results are, however, common with premature infants (104). Appropriate references based on weight and gestational age are therefore in place in many screening programs (105). The majority of screening programs use a single screening test without retesting of questionable 17-OHP concentrations. To improve efficacy, a small number of programs perform a second screening test of the initial sample to re-evaluate borderline cases identified by the first screening. Current immunoassay methods used in newborn screening programs yield a high false positive rate. To decrease this high rate, liquid-chromatography- tandem mass spectrometry measuring different hormones (17-OHP, Δ4-androstenedione, and cortisol) has been suggested as a second-tier method of analyzing positive results (106).

The gold standard for hormonal diagnosis is the corticotropin stimulation test (250 μg cosyntropin intravenously), measuring levels of 17-OHP and Δ4-androstenedione at baseline and 60 min. These values can then be plotted in the published nomogram (Figure 5) to ascertain disease severity (107). It is important to note that the corticotropin stimulation test should not be performed during the initial 24 hours of life as samples from this period are typically elevated in all infants and may yield false-positive results. Testing is typically performed between 48 to 72 hours of life in newborns suspected of classical CAH and glucocorticoid treatment is initiated while awaiting results. In newborns who are hemodynamically unstable and adrenal crisis is suspected, stimulation testing may delay life-saving treatment. Screening measurements of 17-OHP, cortisol, and adrenal androgen along with ACTH can be obtained before emergent glucocorticoid administration. The corticotropin stimulation test is crucial in establishing hormonal diagnosis of non-classical form of the disease since early-morning values of 17-OHP may not be sufficiently elevated to allow accurate diagnosis.

17OHP NOMOGRAM FOR THE DIAGNOSIS OF STEROID 21 – HYDROXYLASE DEFICIENCY 60 MINUTE CORTROSYN STIMULATION TEST

* Genetically unaffected

Patients with:
- ▲ Classical congenital adrenal hyperplasia
- ● Nonclassical congenital adrenal hyperplasia

Heterozygotes for:
- △ Classical congenital adrenal hyperplasia
- ○ Nonclassical congenital adrenal hyperplasia

Figure 5. Nomogram relating baseline to ACTH-stimulated serum concentrations of 17-hydroxyprogesterone (17-OHP). The scales are logarithmic. A regression line for all data points is shown. Data points cluster as shown into three nonoverlapping groups: classic and non-classic forms

of 21-hydroxylase deficiency are readily distinguished from each other and from those that are heterozygotes and unaffected. Distinguishing unaffected from heterozygotes is difficult. (107) Adapted from: New MI, Lorenzen F, Lerner AJ, et al. 1983 Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab 57:320-6. Permission obtained.

Prenatal Diagnosis of 21OHD

A number of approaches to prenatal identification of affected fetuses have been used. In 1965, Jeffcoate et al first reported a successful prenatal diagnosis of 21OHD, based on elevated levels of 17-ketosteroids and pregnanetriol in the amniotic fluid (108). The hormonal diagnostic test for 21OHD is amniotic fluid 17-OHP. Hormonal diagnosis is rarely used and considered only when molecular diagnosis is unavailable.

Advances in genotyping of the *CYP21A2* gene have made molecular genetic studies of extracted fetal DNA the ideal method to diagnose 21OHD CAH in the fetus. Approximately 95% to 98% of the mutations causing 21OHD have been identified through a combination of molecular genetic techniques to study large gene rearrangement and arrays of point mutations (109, 110). Of the currently available methods for prenatal diagnosis of CAH, chorionic villus sampling (CVS), rather than amniocentesis, with molecular genotyping is the preferred diagnostic method in use. CVS is performed between the 9th and 11th week of gestation, while amniocentesis is usually performed in the second trimester. The timing of prenatal diagnosis is particularly important when deciding to treat the fetus at risk for CAH with dexamethasone prenatally to prevent virilization of the genitalia (see Prenatal Treatment below). As we only wish to treat affected females until term and only 1/8 of the fetuses will be affected and 1/2 will be males, 7 out of 8 fetuses do not require treatment. Thus, amniocentesis, which is performed later in gestation, results in treatment of unaffected fetuses for a longer period of time than CVS. However, amniocentesis can be used as a reliable alternative method of prenatal diagnosis when CVS in unavailable. In such instances, the supernatant is used for hormonal measurement and the cells are cultured to obtain a genotype through DNA analysis. The supernatant hormone measurements distinguish affected status from unaffected status only in SW patients. Nonetheless, pitfalls do occur in a small percentage of the patients undergoing prenatal diagnosis utilizing genetic diagnosis, such as undetectable mutations (111), allele drop outs (112), or maternal DNA contamination. Commercially available genetic testing utilizing short range PCR to detect common mutations may miss rare de novo mutations and thus the ACTH stimulation test remains vital to the evaluation. Determination of satellite markers may increase the accuracy of molecular genetic analysis (113).

Non-Invasive Prenatal Diagnosis of CAH

Virilization of the genitalia in a female fetus affected with CAH owing to 21OHD and 11B-OHD can be treated prenatally with dexamethasone administered to the mother (see Prenatal Treatment below). Because CAH is an autosomal recessive disorder, the risk is 1/4 of the fetus being affected with the disease and 1/8 of the fetus being a female with atypical genitalia. Therefore, 7 out of 8 pregnancies will receive unnecessary treatment until the sex and the affection status of the fetus are known. Treatment with dexamethasone must begin before the 9th week of gestation, yet chorionic villous sampling can only be done at the 9-11th week, with karyotype and DNA results available 2-3 weeks later. Non-invasive prenatal diagnosis would eliminate unnecessary treatment and invasive procedures such as CVS and amniocentesis. Dennis Lo et al. in 1997 discovered the presence of fetal DNA in the maternal circulation

(113). Fetal DNA has been extracted and enriched with high accuracy and yield in fetal Rh factor identification (114), aneuploidy and monogenic disorders such as thalassemia and cystic fibrosis (115). Identification of the SRY sequence in maternal blood, performed in multiple academic centers and now available in commercial laboratories, has also achieved excellent accuracy in several studies (116, 117). In non-invasive prenatal diagnosis of CAH, by extracting fetal DNA from the maternal blood as early as 4-5 weeks gestation, the SRY sequence can be identified to determine sex (118). If the fetal genetic sex is deduced to be female (SRY sequence not identified), DNA analysis on extracted fetal DNA can be used to determine CAH affected status. Targeted massive parallel sequencing of cell-free fetal DNA in maternal plasma was used for the noninvasive prenatal diagnosis of CAH due to 21OHD(119). In the fourteen expectant families studied, each with a previous child affected with classical CAH (proband) and parents with at least one mutant CYP21A2 gene, the fetal CAH affection status was correctly deduced using this method from maternal plasma drawn as early as 5 weeks and 6 days (119).

Preimplantation Diagnosis

Preimplantation genetic diagnosis (PGD) identifies genetic abnormalities in preimplantation embryos prior to embryo transfer, so only unaffected embryos established from IVF are transferred. The procedure has been utilized in many monogenic recessive disorders such as cystic fibrosis, hemoglobinopathies, spinal muscular atrophy and Tay Sach's disease. PGD is being used for a growing number of genetic diseases (120). There is only one report of PGD utilized in a family whose offspring is at risk for CAH (121), however we know from experience that families are seeking PGD with greater frequency. It would be desirable to have further studies of preimplantation diagnosis in CAH families.

Prenatal Treatment

In 21OHD, prenatal treatment with dexamethasone was introduced in France in 1978 (122) and in the United States in 1986 (123). Institution of therapy before the 9th week of gestation, prior to the onset of adrenal androgen secretion, effectively suppresses excessive adrenal androgen production and prevents virilization of external female genitalia. Dexamethasone is used because it binds minimally to cortisol binding globulin (CBG) in the maternal blood, and unlike hydrocortisone, it escapes inactivation by the placental 11-dehydrogenase enzyme. Thus, dexamethasone crosses the placenta from the mother to the fetus and suppresses ACTH secretion with longer half-life compared to other synthetic steroids (123).

When dexamethasone administration begins as early as the 8th week of gestation, the treatment is blind to the disease status and sex of the fetus. If the fetus is later determined to be a male upon karyotype or an unaffected female upon DNA analysis, treatment is discontinued. Otherwise, treatment is continued to term. A simplified algorithm of management of potentially affected pregnancies is shown in Figure 6. The optimal dosage is 20 µg/kg/day of dexamethasone per maternal pre-pregnancy body weight, in three divided daily doses (124). It is recommended to start the treatment as soon as pregnancy is confirmed, and no later than 9 weeks after the last menstrual period (125, 126). The mother's blood pressure, weight, glycosuria, HbA1C, symptoms of edema, striae and other possible adverse effects of dexamethasone treatment should be carefully observed throughout pregnancy (127) . Prenatal dexamethasone treatment remains controversial and should be administered under an IRB approved research protocol(104).
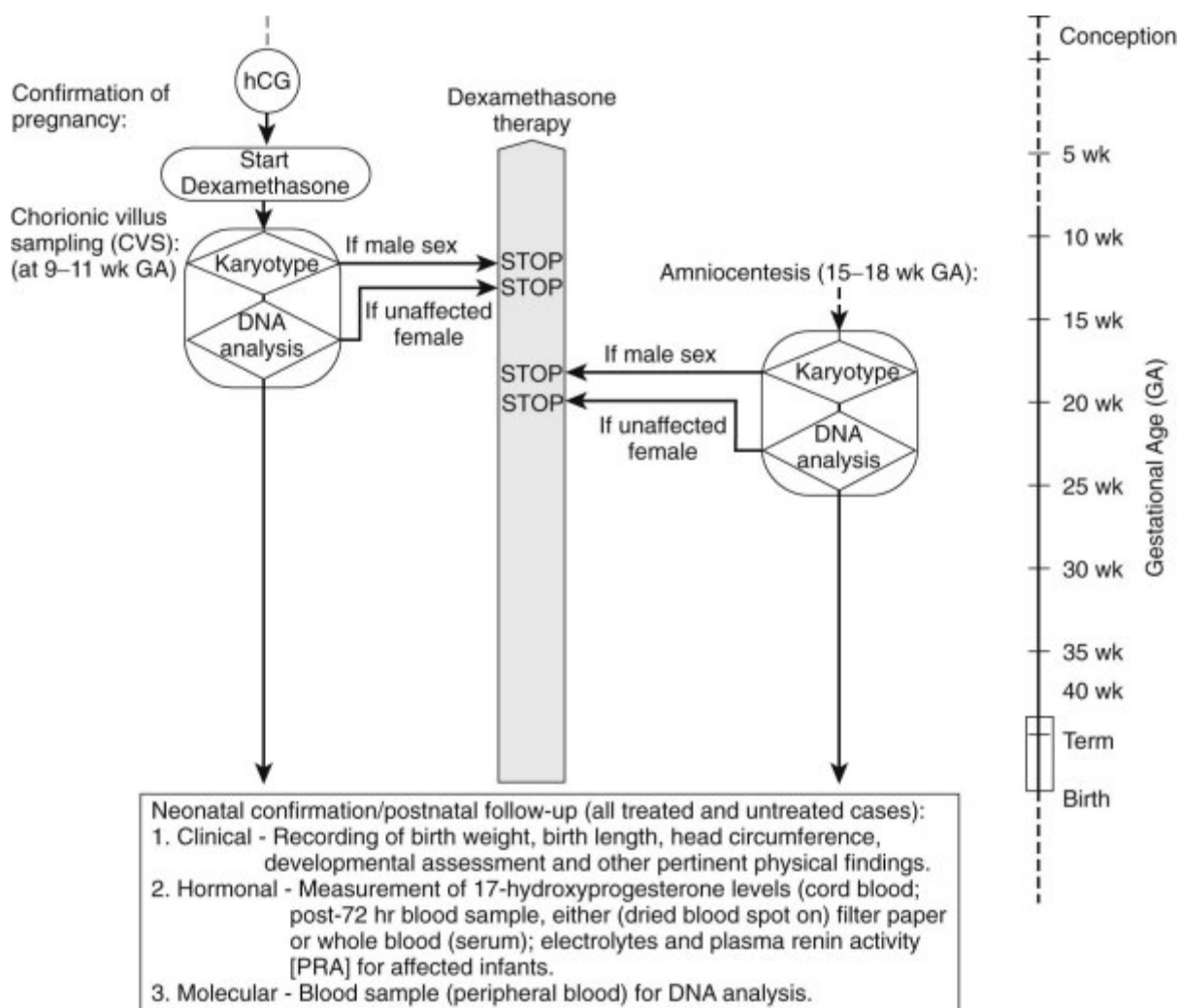
Figure 6. Algorithm of treatment, diagnosis and decision-making for prenatal treatment of fetuses at risk for 21-hydroxylase deficiency congenital adrenal hyperplasia. Mercado AB, Wilson RC, Cheng KC, Wei JQ, New MI 1995 Extensive personal experience: Prenatal treatment and diagnosis of congenital adrenal hyperplasia owing to steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 80:2014-2020. (125) Permission obtained.

Outcome of Prenatal Treatment of 21OHD

Prenatal treatment of 21OHD has proven to be successful in significantly reducing genital virilization in affected females. Our group has performed prenatal diagnosis in over 685 pregnancies at risk for 21OHD, and 59 affected female fetuses have been treated to term (128). Treatment was highly effective in preventing genital ambiguity when the mother was compliant until term. In all the female fetuses treated to term, the degree of virilization was on average 1.69, as measured using the Prader scoring system (Figure 3). Late treatment as well as no treatment resulted in much greater virilization, and the average Prader score was 3.73 for those female fetuses not treated. 124 and unpublished data Not only does prenatal treatment effectively minimize the degree of female genital virilization in the patients, it also lessens the

high-level androgen exposure of the brain during differentiation. The latter is thought to cause a higher tendency to gender ambiguity in some females with CAH (129, 130). Genital virilization in female newborns with classical 21OHD CAH has potential adverse psychosocial implications that may be alleviated by prenatal treatment (124).

Prenatal dexamethasone treatment has continued to be a subject of controversy (104). Some uncertainties and concerns have been expressed about the long-term safety of prenatal diagnosis and treatment (131, 132). Concerns have been raised in regards to the glucocorticoid effects on the fetal brain, which arise from studies of other conditions rather than direct studies on prenatal treatment of 21OHD CAH. These include studies whereby much higher doses of dexamethasone were given to the human subjects at the later part of pregnancy (133) or to animals (134, 135) and therefore hold little relevance to using dexamethasone prenatally in CAH. In a small-sample study of children prenatally treated with dexamethasone, Lajic and her colleagues found no effects on intelligence, handedness, memory encoding, or long-term memory, but short-term treated CAH-unaffected children had significantly poorer performance than controls on a test of verbal working memory. These patients also had lower questionnaire scores in self-perceived scholastic competence and social anxiety (136). However, parents described these children as more sociable than controls, without significant difference in psychopathology, school performance, adaptive functioning or behavioral problems (137). A larger multi-center study (US and France) indicated no adverse cognitive effects of short-term prenatal DEX exposure, including no adverse influence on verbal working memory; a small sample of dexamethasone treated girls affected with CAH showed lower scores on two of eight neuropsychological tests, however given the variability of cognitive findings in dexamethasone unexposed CAH-affected patients, this result cannot be linked to

dexamethasone with certainty (138). This indicates that further studies are needed.

Compelling data from large cohorts of pregnancies with prenatal diagnosis and treatment of 21OHD CAH prove its efficacy and safety (126, 139). All of the mothers who received prenatal treatment (partial or full-term) stated that they would take dexamethasone again for a future pregnancy (140). Rare adverse events have been reported in treated children, but no harmful effects have been documented that can be clearly attributed to the treatment (141). Another long-term follow-up study in Scandinavia showed that 44 children who were variably treated prenatally demonstrated normal prenatal and postnatal growth compared to matched controls. Further, there was no observed increase in fetal abnormalities or fetal death (142). Although some abnormalities in postnatal growth and behavior were observed among dexamethasone exposed offspring, none could logically be explained by the present knowledge of teratogenic effects of glucocorticoids.

Published studies of almost 600 pregnancies, 80 of which were prenatally treated until term and 27 who were male and received dexamethasone for a short period of time, the newborns in the dexamethasone treated group did not differ in weight, length or head circumference from untreated, unaffected siblings. No significant or enduring side effects were noted in either the mothers or the fetuses. Greater weight gain in treated versus untreated mothers did occur, as well as the presence of striae and edema. Excessive weight gain was lost after birth. No differences were found regarding gestational diabetes or hypertension (127). No cases have been reported of cleft palate, placental degeneration or fetal death, which have been observed in the rodent model of *in utero* exposure to high-dose glucocorticoids (143). One explanation for the safety of human versus rodent is that glucocorticoid receptor-ligand systems in human differ from that of rodents (144). A comprehensive long-term

outcome study looking at 149 male and female patients 12 years of age and older, affected and unaffected with CAH, who were treated with dexamethasone partially or to term was conducted. To date, this is the largest study evaluating the long-term effects of dexamethasone. No adverse effects such as increased risk for cognitive defects, disorders of gender identity and behavior, sexual function in adulthood, hypertension, diabetes, and osteopenia were found (127).

Prenatal Diagnosis and Treatment of 11β-OHD CAH

Several approaches to prenatal identification by measuring steroid precursors in affected fetuses have been used (145-147). Advances in genotyping of the *CYP11B1* gene have made molecular genetic studies of fetal DNA extracted from maternal blood, the ideal method to diagnose 11β-OHD CAH in the fetus (148, 149). The established protocol of prenatal diagnosis and treatment in 21OHD CAH can be applied to 11β-OHD CAH. Reduced virilization of affected females in prenatal diagnosis and treatment in 11β-OHD CAH have been reported (128, 148).

TREATMENT

HORMONE REPLACEMENT

The goal of therapy in CAH is to both correct the deficiency in cortisol secretion and to suppress ACTH overproduction (104). Proper treatment with glucocorticoid reduces stimulation of the androgen pathway, thus preventing further virilization and allowing normal growth and development. The usual requirement of hydrocortisone (or its equivalent) for the treatment of classical 21OHD form of CAH is about 10-15 mg/m2/day divided into 2 or 3 doses per day. Conventionally, oral hydrocortisone tablets with the smallest available dose of 5 mg have been preferred. To administer small doses to infants and young children, 10 mg and 5 mg tablets have to be cut and crushed. Hydrocortisone granules and tablets at smaller doses are becoming more widely available allowing for ease of administration and avoidance of excess dosing (150).

Dosage requirements for patients with NC-21OHD CAH are typically less. Adults may be treated with the longer-acting dexamethasone or prednisone, alone or in combination with hydrocortisone. A small dose of dexamethasone at bedtime (0.25 to 0.5 mg) is usually adequate for androgen suppression in non-classical patients. Anti-androgen treatment may be useful as adjunctive therapy in adult women who continue to have hyperandrogenic signs despite good adrenal suppression. Females with concomitant PCOS may benefit from an oral contraceptive, though this treatment would not be appropriate for patients trying to get pregnant. Treatment of adult males with NC-21OHD may not be necessary, though our group has found that it may be helpful in preventing adrenal rest tumors and preserving fertility. Optimal corticosteroid therapy is determined by adequate suppression of adrenal hormones balanced against normal physiological parameters. The goal of corticosteroid therapy is to give the lowest dose required for optimal control. Adequate biochemical control is assessed by measuring serum levels 17-OHP and androstenedione; serum testosterone can be used in females and prepubertal males (but not in newborn males). It is recommended that hormone levels are measured at a consistent time in relation to medication dosing, usually 2 hours after the morning corticosteroid. Titration of the dose should be aimed at maintaining androgen levels at age and sex-appropriate levels and 17-OHP levels of <1000 ng/dL. Analysis of diurnal and 24-hour urine collection of 17-OHP metabolites can be utilized to further assess adrenal control. (151) Concurrently, over-treatment should be avoided because it can lead to Cushing syndrome. Depending on the degree of stress, stress

dose coverage may require doses of up to 50-100 mg/m2/day (1).

Patients with salt-wasting CAH have elevated plasma renin in response to the sodium-deficient state, and they require treatment with the salt-retaining 9α-fludrocortisone acetate. The average dose is 0.1 mg daily, ranging from 0.05 mg to 0.2 mg daily. Infants should also be started on salt supplementation, as sodium chloride, at 1-2 g daily, divided into several feedings. Although patients with the SV and NC form of CAH can make adequate aldosterone, the aldosterone to renin ratio (ARR) has been found to be lower than normal, though not to the degree seen in the salt-wasting form (152)(155). It has not been customary to supplement conventional glucocorticoid replacement therapy with the administration of salt-retaining steroids in the SV and NC forms of CAH, though there has been some suggestion that adding fludrocortisone to patients with elevated PRA may improve hormonal control of the disease (153). The requirement for fludrocortisone appears to diminish with age, and over-suppression of the PRA should be avoided, to prevent complications from hypertension and excessive mineralocorticoid activity. Measurements of plasma renin and aldosterone are used to monitor the efficacy of mineralocorticoid therapy in all patients with the salt wasting form of the disease (1). The use of systemic cortisol injection for impending adrenal crises is discussed below.

In managing 11β-OHD, glucocorticoid administration provides cortisol replacement and decreases ACTH, as it does in 21OHD. This in turn removes the drive for over secretion of deoxycorticosterone (DOC) and 11-deoxycortisol and, in most cases, normalizes blood pressure. A thorough examination undertaken by endocrine challenge and suppression studies to evaluate zonal differences has shown that in 11β-OHD CAH, the zona fasciculata exhibits reduced 11β-hydroxylation and 18-hydroxylation, while both functions appear to be spared in the zona glomerulosa

(154). This demonstrates that the zona glomerulosa and the zona fasciculata function as two physiologically, and likely genetically, separate glands. Glucocorticoid treatment produces natriuresis and diuresis, normalizes plasma volume and thus increases the plasma renin to levels that stimulate aldosterone production in the zona glomerulosa. In addition to normalizing blood pressure, the goal of treatment is to replace deficient steroids and in turn minimize adrenal sex hormone excess, prevent virilization, optimize growth, and protect potential fertility. Serum DOC and 11-deoxycortiol are thus the principal steroid index of the 11β-OHD. Plasma renin activity is useful as a therapeutic index as well. In poorly controlled 11β-OHD patients, DOC is elevated, whereas plasma renin is suppressed; both are normal in well-controlled patients. As in patients with 21OHD, oral hydrocortisone at a dose of 10-15 mg/m2 divided into two to three daily doses is the preferred treatment. Long-acting glucocorticoids may be used at or near the completion of linear growth. In patients who have had ongoing hypertension for some time before diagnosis is made, adding spironolactone, calcium channel blockers or amiloride may be necessary (60).

In patients with 3β-HSD deficiency, glucocorticoid administration also reduces the excess production of androgens. In addition, these patients have mineralocorticoid deficiency and require treatment with the salt-retaining 9α-fludrocortisone acetate. Patients with the StAR protein deficiency or SCC deficiency (lipoid form of CAH) classically have severe adrenal insufficiency with mineralocorticoid deficiency and salt wasting; they require both glucocorticoid and mineralocorticoid replacement. Patients with 17 α -hydroxylase/17,20 lyase deficiency typically have excess DOC and low-renin hypertension, and treatment with a glucocorticoid should normalize serum DOC level and lead to normalization of blood pressure. In several conditions, such as StAR protein deficiency, 3β-HSD, 17 α -hydroxylase/17,20 lyase deficiency and cytochrome P450 oxidoreductase

deficiency, patients require sex steroid replacement. Sex steroids should be added at a developmentally appropriate time to allow patients to resemble their peers.

Because patients with CAH are at risk for short stature as adults, other adjunct therapies are being utilized. Two studies have demonstrated significant improvement in growth velocity, final adult height prediction (17) and final adult height (15) with the use of growth hormone in conjunction with a GnRH analogue.

In non-life-threatening periods of illness or physiologic stress, the corticosteroid dose should be increased to 2 or 3 times the maintenance dose for the duration of that period. Each family should be given injection kits of hydrocortisone for emergency use (25 mg for infants, 50 mg for children, and 100 mg for adults). In the event of a surgical procedure, a total of 5 to 10 times the daily maintenance dose may be required during the first 24-48 hours, which can then be tapered over the following days to the normal preoperative schedule. Stress doses of dexamethasone should not be given because of the delayed onset of action. It is not necessary for increased mineralocorticoid doses during these periods of stress (1, 104).

It is imperative for all patients who are receiving corticosteroid replacement therapy, such as patients with CAH, to wear a Medic-Alert bracelet, medallion, or equivalent identification that will enable correct and appropriate therapy in case of emergencies. Additionally, all responsible family members should be trained in the intramuscular administration of hydrocortisone.

Bone Mineral Density

In order to adequately suppress androgen production in patients with CAH, the usual requirement of hydrocortisone is generally higher than the endogenous secretory rate of cortisol. Chronic therapy with glucocorticoids at supraphysiologic levels can result in diminished bone accrual and lead to osteopenia and osteoporosis. Glucocorticoid induced bone loss is a well-known phenomenon and is the most prevalent form of secondary osteoporosis (155, 156).

Unlike other diseases treated with chronic glucocorticoid therapy, however, the effect of glucocorticoid replacement in CAH on BMD is unclear. Previous studies of patients with 21OHD have reported increased, normal, or decreased BMD (157-160). It has been postulated that the elevated androgens typically found in patients may have a protective effect on bone integrity, but the precise mechanism is unknown. The increased adrenal androgens, which are converted to estrogens, may counteract the detrimental effects of glucocorticoids on bone mass. This may explain why older CAH women, particularly those who are post-menopausal, are at higher risk for osteoporosis than younger CAH patients. It has been proposed that the inhibitory effect of corticosteroid therapy on bone formation is counteracted by estrogen's effect on bone resorption through the RANK-L/osteoprotegerin (OPG) system (161).

Surgery

The decision for genital surgery in females with virilizing forms of CAH and males with forms of CAH associated with under virilization should be made by parents or patients themselves with the guidance of a multidisciplinary team involving pediatric endocrinology, urology, genetics, and psychology. As part of a case-by-case approach, attention should be given to gender identity, quality of life and potential for fertility. The aim of surgical repair in females with atypical genitalia caused by CAH, if the decision is made, is generally to preserve the sexually sensitive glans clitoris, decrease frequency of urinary tract

infections and provide a vaginal orifice that functions adequately for menstruation, intromission, and delivery. A medical indication for early surgery other than for sex assignment is recurrent urinary tract infections as a result of pooling of urine in the vagina or urogenital sinus.

In the past, it was routine to recommend early corrective surgery for neonates born with atypical genitalia. However, in recent years, the implementation of early corrective surgery has become increasingly controversial due to lack of data on long-term functional outcome. Data show that genital sensitivity is impaired in areas where feminizing genital surgery had been done, leading to difficulties in sexual function (162). Another study showed that patients with more severe mutations in the *CYP21A2* gene, i.e., those with the null genotype and thus those more severely virilized, had more surgical complications that those less severely virilized and were less satisfied with their sexual life (163). Because of the scarcity of these data, the role of the parents in sex assignment becomes crucial in all aspects of the decision-making process, and should include full discussion of the controversy and all possible therapeutic options for the intersex child, particularly early versus delayed surgery. A large repository of data concerning long-term outcomes in patients affected by CAH, including psychosexual well-being, has been enhanced by the establishment of disease registries (164).

In a study of intersex individuals ≥ 16 years old, 66% of individuals with CAH thought that the appropriate age of genital surgery was infancy or childhood (165). In a study of caregivers of female infants with CAH who underwent genitoplasty, 2/3 of caregivers reported no regret over their decision-making. However, 1/3 reported some level of regret in the process (165).

Other Treatment Strategies and Novel Therapies

Glucocorticoid replacement has been an effective treatment for CAH for over 50 years and remains its primary therapy; however, the management of these patients presents a challenge because both inadequate treatment, as well as over suppression, can cause complications. In forms of CAH with decreased cortisol production, increased ACTH production stimulates excessive synthesis of adrenal products in those pathways unimpaired by the enzyme deficiency such as androgens. The goal of therapy in CAH is to both correct the deficiency in cortisol secretion and to suppress ACTH overproduction which drives androgen production (104).

Often, doses of hydrocortisone higher than physiologic replacement or dosing in reverse diurnal pattern are required to suppress androgen production driven by ACTH. Alternatives to oral glucocorticoid administration 2-3 times a day include continuous subcutaneous pump and newer modified release formulations. Physiologic cortisol secretion patterns can be mimicked with varied infusion rates of hydrocortisone via continuous subcutaneous pump administration. This more intense method of administration should be considered in those with rapid cortisol metabolism or poor gastrointestinal absorption (166). Modified release and delayed release formulations of hydrocortisone were developed recently (167). When taken at bedtime, these formulations approximate the diurnal pattern of physiological cortisol production with an early morning peak and aim to prevent the ACTH surge at dawn that drives androgen production (168).

Adjunct treatments targeting hypothalamic and pituitary signaling to the adrenal glands are in development. To suppress ACTH production, corticotrophin-releasing factor (CRF) antagonists have been developed. CRF produced in the hypothalamus stimulates the release of ACTH from

the pituitary gland. CRF antagonism can reduce ACTH production and reduce ACTH driven androgen production in patients with CAH. With the adjunct use of CRF antagonists, lower doses of hydrocortisone can be used for physiologic replacement only. Two oral agents are currently undergoing clinical trials in patients with classical CAH (169, 170).

To reduce androgen production, the use of abiraterone acetate indicated for prostate cancer was proposed for patients with CAH. (171) Abiraterone acetate is a potent inhibitor of steroid enzyme 17-hydroxylase/17,20-lyase (CYP17A1) needed for androgen production and is being studied in clinical trials as adjunct therapy in adult and soon pediatric subjects. (164, 172)

Bilateral adrenalectomy is a radical measure that can be effective in some cases. A few patients who were extremely difficult to control with medical therapy alone showed improvement in their symptoms after bilateral adrenalectomy (164, 165). Because this approach renders the patient completely adrenal insufficient, however, it should be reserved for extreme cases and is not a good treatment option for patients who have a history of poor compliance with medication.

CONCLUSION

The pathophysiology of the various types of CAH (the most common being 21OHD) can be traced to specific, inherited defects in the genes encoding enzymes for adrenal steroidogenesis. Clinical presentation of each form is distinctive and depends largely on the underlying enzyme defect, its precursor retention and deficient end products. Treatment of CAH is targeted to replace the insufficient adrenal hormones, notably cortisol and salt retaining hormones, and to suppress the excess precursors. With proper hormone replacement therapy, normal and healthy development may be expected. Glucocorticoid and, if necessary, mineralocorticoid replacement, has been the mainstay of treatment for CAH, but new treatment strategies continue to be developed and studied to improve care. Molecular genetic techniques used postnatally and prenatally along with well described genotype phenotype correlations can help guide clinical management.

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