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21-HYDROXYLASE DEFICIENCY: CLASSICAL & NONCLASSICAL CONGENITAL ADRENAL HYPERPLASIA

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SEARCH STRATEGY AND SELECTION CRITERIA

Data for this review were identified by searches of MEDLINE, Knowledge Finder, and references from relevant articles;

Numerous articles were identified through searches of the extensive files of the authors. Search terms were "congenital adrenal hyperplasia", "21-hydroxylase deficiency", "CYP21″.

SUMMARY

Congenital adrenal hyperplasia (CAH) is a family of inherited disorders of adrenal steroidogenesis owing to a deficiency in the 21-hydroxylase enzyme. CAH is considered to exist in three forms – salt wasting, simple virilizing, and nonclassical. Nonclassical 21-hydroxylase deficiency is one of the most common autosomal recessive diseases in the world. This paper will first review adrenal steroidogenesis and the pathophysiology of 21-hydroxylase deficiency. The three forms of CAH will then be discussed in terms of their clinical presentation, diagnosis and treatment, as well as the genetic basis of these disorders. Prenatal diagnosis and treatment will also be reviewed.

INTRODUCTION

Congenital adrenal hyperplasia (cah) is a family of inborn errors of steroidogenesis, each disorder characterized by a specific enzyme deficiency that impairs cortisol production by the adrenal cortex. Numerous investigators have unraveled the mechanisms of adrenal steroid synthesis and the associated enzyme defects responsible for the clinical syndromes. The enzyme most often deficient is 21-hydroxylase, the focus of this seminar, which accounts for over 90% of cah cases, and is the most

common cause of genital ambiguity in the newborn. Less often, cah is caused by deficiencies of 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, aldosterone synthase, 17α-hydroxylase/17,20-lyase, and the steroidogenic acute regulatory protein (lipoid hyperplasia).

Correctly identifying the form of cah is achieved by the observation of clinical syndromes reflecting distinct hormonal patterns, and is measured quantitatively as abnormally low or high glucocorticoid, mineralocorticoid, and androgen levels. In the severe, or classical form of cah owing to 21-hydroxylase deficiency (21-ohd), adrenal androgen overproduction causes prenatal virilization in females and continued masculinization postnatally in both sexes. There are two types of classical cah, including simple virilizing and salt-wasting. The less severe, nonclassical form of 21-hydroxylase deficiency does not cause genital ambiguity in females and is characterized by signs of postnatal androgen excess. Approximately 60 mutations in the gene for 21-hydroxylase, cyp21, have been identified to cause classical and nonclassical cah. These identifications have important implications for early prenatal diagnosis and prenatal treatment. The current direction of cah research is focusing on improved therapeutic management of patients, including promising new treatment protocols, as well as exploring the possibility of gene therapy.

ADRENAL STEROIDOGENESIS AND REGULATION

The adrenal cortex produces three main types of hormones under the control of independent regulatory systems: glucocorticoids (cortisol), mineralocorticoids (aldosterone) and androgens (testosterone). The cortex is divided into three distinct zones — the outer zona glomerulosa, the middle zona fasciculata, and the inner zona reticularis. The synthesis of cortisol occurs in the zona fasciculata of the cortex, while the mineralocorticoid aldosterone is dependent upon enzymatic activity limited to the zona glomerulosa. Sex steroids are synthesized in the zona reticularis. Figure 1 is a schematic diagram of steroidogenesis in the adrenal cortex.

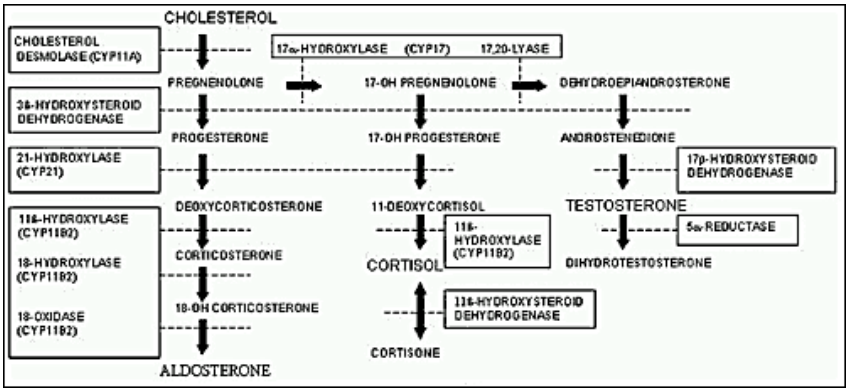

Figure 1. Schema of adrenal steroidogenesis. (1)

Cortisol is synthesized under the trophic control of adrenocorticotropic hormone (ACTH), forming a negative feedback loop in which high serum cortisol centrally inhibits and low serum cortisol stimulates release of ACTH, which defines the hypothalamic-pituitary-adrenal axis. Net ACTH release has basal, diurnal, and stress induced components. The pulsatile release of ACTH is in turn modulated by corticotropin releasing hormone (CRH), while the central nervous system determines the hypothalamic set point for the expected plasma cortisol level. A deficiency of 21-hydroxylase impairs production of cortisol resulting in increased pituitary secretion of ACTH. Chronic elevations of ACTH levels stimulate the accumulation of precursor steroids in the impeded pathways and cause excessive

steroid synthesis in other adrenal biosynthetic pathways unaffected by the enzyme deficiency.

Steroids in 21-hydroxylase deficiency

Deficiency of 21-hydroxylase occurs in three forms: 1) simple virilizing, 2) salt-wasting, and 3) nonclassical. The simple virilizing and salt-wasting forms of 21-hydroxylase deficiency are characterized by excess adrenal Deficiency of 21-hydroxylase occurs in three forms: 1) simple virilizing, 2) salt-wasting, and 3) nonclassical. The simple virilizing and salt-wasting forms of 21-hydroxylase deficiency are characterized by excess adrenal androgen biosynthesis in utero, which causes prenatal virilization of the genetic female and postnatal virilization of both boys and girls. Biochemically, the conversion of 17ü -hydroxyprogesterone (17-OHP), the main substrate of the 21-hydroxylase enzyme, to 11-deoxycortisol in the pathway of cortisol synthesis is impaired (Figure 1). Baseline and ACTH stimulated serum levels of cortisol precursors may be extremely elevated in untreated patients, principally, 17-OHP. The 21-hydroxylase enzyme defect can also impair the conversion of progesterone to 11-deoxycorticosterone (DOC) in the pathway of aldosterone synthesis, as occurs in the salt-wasting form. Aldosterone production is not sufficient for sodium reabsorption by the distal renal tubules causing low serum sodium, high serum potassium, in addition to increased levels of adrenal androgen and cortisol precursors seen in simple virilizers. In salt-wasting CAH, both newborn boys and girls are subject to early, life-threatening, salt-wasting crises within the first few weeks of life.

Hormonal diagnosis of 21-hydroxylase deficiency is established by comparison of baseline and ACTH stimulated serum levels of 17-OHP. (2) A nomogram provides hormonal standards for assignment of the 21-hydroxylase phenotype (Figure 2). Because of the diurnal variation in l7-OHP, an early morning serum concentration of l7-OHP may be useful as a screening test for genotyping 21-OHD. ACTH stimulation, however, remains the most definitive hormonal diagnostic test. An ACTH 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.

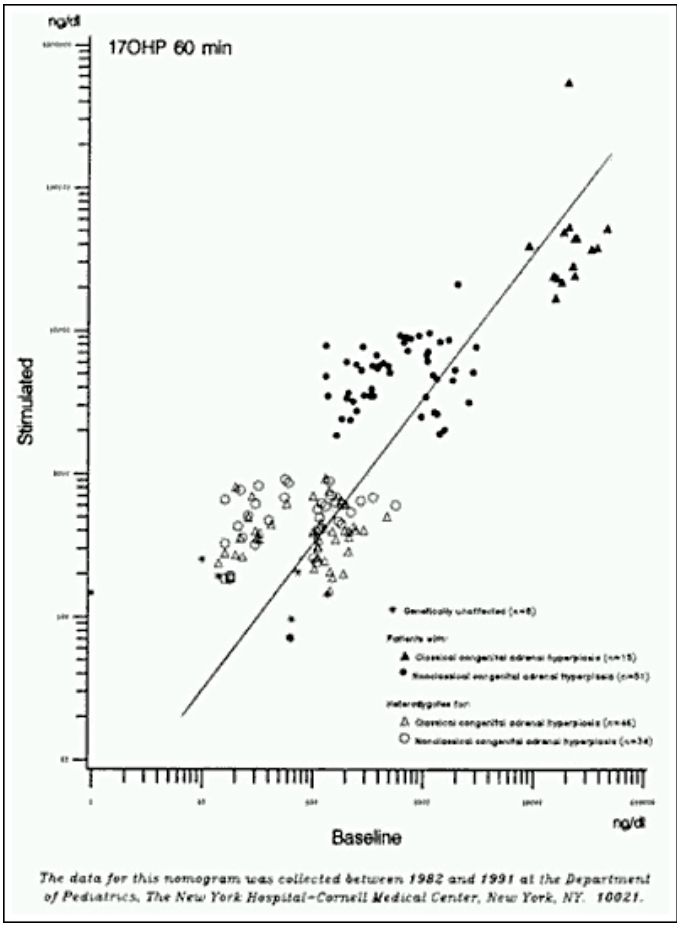

Figure 2. Nomogram for hormonal diagnosis of 21-hydroxylase deficiency relating baseline to ACTH-stimulated serum concentrations of 17-OHP. Scales are logarithmic. A regression line for all data points is shown.

Nonclassical CAH, sometimes called late-onset, occurs when there is only a mild deficiency of enzyme 21-hydroxylase. These patients do not waste salt, and females are not virilized at birth. Nonclassical 21-hydroxylase deficiency may present at any age postnatally, with patients exhibiting moderately high androgens and stimulated levels of 17-OHP. (3)

The various clinical and biochemical features associated with the different forms of 21-hydroxylase deficiency are presented in Table 1.

Prenatal Virilization

In utero virilization occurs in genetic females (46, XX) affected with simple virilizing or salt-wasting 21-hydroxylase deficiency. Excessive adrenal androgens masculinize the external genitalia (female pseudohermaphroditism), so that an affected newborn female may present with varying degrees of virilization including a urogenital sinus, scrotalization of the labia majora, labial fusion, or clitoromegaly. In rare cases, the masculinization is so profound that the urethra is penile. (4) A five stage classification by Prader (5) is used to represent different degrees of virilization (Figure 3), where on a scale of 1 to 5 (I-V) the genitalia can be scored from slightly virilized (e.g., mildly enlarged clitoris) to that resembling a male with a penile urethra. Most females with 21-hydroxylase deficiency are born with Prader IV genitalia. As females with CAH have a 46, XX karyotype and do not have testes, anti-müllerian hormone (AMH) is not secreted, and müllerian ducts develop normally into a uterus and fallopian tubes. Thus, females with 21-hydroxylase deficiency have the potential for fertility.

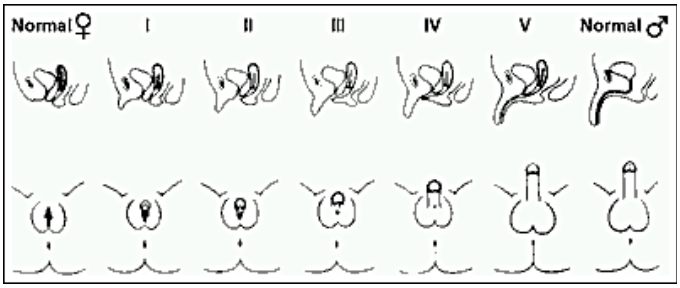

Figure 3. Diagrams representing five degrees of virilization affecting the urogenital sinus and external genitalia in females. (5)

It is not possible to distinguish between simple virilizing classical 21-OHD and salt-wasting classical 21-OHD based solely on the degree of virilization of an affected female at birth. In pregnancies at risk of a female child affected with congenital adrenal hyperplasia, successful suppression of fetal adrenal androgen production has resulted by giving the mother dexamethasone, a glucocorticoid able to cross the placental barrier. (6)

CLASSICAL CONGENITAL ADRENAL HYPERPLASIA

Simple virilizing congenital adrenal hyperplasia

The salient features of classical simple virilizing 21-OHD are prenatal virilization and progressive postnatal masculinization with accelerated growth and advanced bone ages but no evidence of mineralocorticoid deficiency.

Diagnosis at birth of a female with simple virilizing CAH usually is made immediately because of the apparent genital ambiguity. As differentiation of the external genitalia is not affected in newborn males, only hyperpigmentation may suggest increased ACTH secretion and cortisol deficiency. Diagnosis at birth in males usually depends on prenatal or newborn screening. If glucocorticoid replacement therapy does not begin postnatally, female genitalia may continue to virilize because of excess adrenal androgens, and pseudoprecocious puberty can occur. Signs of hyperandrogenism in children affected with CAH include early onset of facial, axillary, and pubic hair, adult body odor, and rapid somatic growth. This early growth spurt is accompanied by premature epiphyseal maturation and closure, resulting in a final height that is below that expected from parental heights. (7-11) CAH patients are tall children but short adults. In adolescents and adults, signs of hyperandrogenism may include temporal balding, severe acne, irregular menses, hirsutism, and infertility. Poor control of the disease in males with both simple virilizing and salt-wasting CAH has been associated with reduced sperm counts. (10, 12-14) Gonadal dysfunction usually occurs because the excess adrenal androgens are aromatized peripherally to estrogens, which suppress pituitary gonadotropins and impair the growth and function of the testes.

When classical CAH is diagnosed and adequately treated in infancy, the onset of puberty in both girls and boys usually occurs at the expected chronological age. (15, 16) However, physiologic secretion of gonadotropins may be abnormal. Studies suggest that excess adrenal androgens (aromatized to estrogens) inhibit the pubertal pattern of gonadotropin secretion by the hypothalamic-pituitary axis. (12, 17)

Although the expected age of menarche may be delayed in females with classical CAH, (18) many who are adequately treated have regular menses after menarche. (8, 16) Menstrual irregularity and secondary amenorrhea with or without hirsutism occur in a subset of postmenarchal females, especially those in poor hormonal control. (8, 19-21) Primary amenorrhea or delayed menarche can occur if a woman with classical CAH is untreated, inadequately treated, or over treated with glucocorticoids. Short stature in patients who are over treated result from glucocorticoid induced growth suppression. (15)

Fertility problems in females with classical CAH arise for various reasons including anovulation, secondary polycystic ovarian syndrome, irregular menses, non- suppressible serum progesterone levels, and inadequate introitus. (22, 23) However, there have been several reports of treated women who have had successful pregnancies with the delivery of healthy, full-term infants. (16, 24, 25) Adequacy of glucocorticoid therapy is probably an important variable with respect to fertility outcome. As inadequate vaginal introitus may affect a third of classical CAH adult females, (22) it has been the practice to delay vaginoplasty until sexual intercourse is regular or when the patient can be expected to assume responsibility for vaginal dilatation.

Successfully treated male patients with CAH may have normal pubertal development, normal testicular function, and normal spermatogenesis and fertility. (10, 19, 26, 27) However, in some adequately treated patients as well as those in poor hormonal control, the presence of gonadal abnormalities in adult males with 21-hydroxylase deficiency has been reported in the form of testicular nodules, testicular atrophy, azoospermia, and the suppression of gonadotropin secretion. (10, 12-14, 28) Testicular nodules caused by expanding adrenal rests are another frequently reported complication in postpubertal boys with classical CAH and inadequate hormonal control. (29-32) These nodules develop during periods of sustained elevations of ACTH and decrease in size during administration of glucocorticoids. (30, 31, 33) Regular testicular examination and periodic testicular ultrasonography are recommended for early detection of testicular lesions.

Table 1. Clinical and Biochemical Features of Various Disorders of Adrenal Steroidogenesis

Deficiency (Adrenal Disorder)	Genital Ambiguity	Postnatal Virilization	Diagnostic Hormones	Treatment
I. 21-Hydroxylase				
A. Classic				
1. Salt wasting (SW) [b]	F	Yes	17OHP D [4] -A Aldosterone	HC, 10-15 mg/m [2] /day orally (PO), and 9 a FF, 0.05 – 0.2 mg/day PO
2. Simple virilizing (SV)	F	Yes	17-OHP, D [4] -A	HC (same); addition of 9aFF (same) if renin
B. Nonclassic (symptomatic and asymptomatic)	No	Yes	17-OHP, D [4] -A	HC, 10-15 mg/m [2] / day or dex, 0.25-0.5 mg/day h.s., or prednisone 5-10 mg/day
II. 3 b -Hydroxysteroid dehydrogenase				
A. Classic	M (±F)	Yes	17-OHP D [5] 17-OHP DHEA D [4] -A	HC and 9 a FF as for SW 21-OHD
B. Nonclassic	No	Yes	17-OHP DHEA	HC as for nonclassic 21-OHD

III. 11ß-Hydroxylase				
A. Classic (hypertensive CAH)	F	Yes	DOC S D ^{4} -A PRA	HC, 10-15 mg/m ^{2} /day
B. Nonclassic	No	Yes	S DOC	HC, dex, or prednisone as for nonclassical 21-OHD
III. 17 a -Hydroxylase/ 17,20-lyase	M	No	DOC B	HC, 10-15 mg/m ^{2} /day ^{a}
IV. Steroidogenic acute regulatory protein (StAR; congenital lipoid hyperplasia) ^{b}	M	No	None	HC, 10-15 mg/m ^{2} / day 9 a FF, 0.05-0.2 mg/day ^{a}

17-OHP: 17-Hydroxyprogesterone; D 4-A: D 4-Androstenedione; B: Corticosterone; S: 11-Deoxycortisol; DOC: Deoxycorticosterone; DHEA: Dehydroepiandrosterone; HC: Hydrocortisone; D 5 17-OHP: 17-hydroxypregnenolone; 9 a FF: fludrocortisone acetate; dex: dexamethasone; PRA: Plasma renin activity ^{a} With addition of sex steroidreplacement at puberty; ^{b} Salt wasting is a risk

Salt-wasting congenital adrenal hyperplasia

Inadequate secretion of the mineralocorticoid aldosterone causes salt wasting in approximately three-quarters of all classical CAH patients. Aldosterone secretion is insufficient for the reabsorption of sodium by the distal renal tubule resulting in salt wasting, characterized by hyponatremia, hyperkalemia, inappropriate natriuresis, and low serum and urinary aldosterone with concomitantly high plasma renin activity (PRA). In addition, hormonal precursors of 21-hydroxylase may act as mineralocorticoid antagonists and provoke salt wasting. (34, 35) Untreated infants with renal salt-wasting have poor feeding, weight loss, and dehydration which can progress to azotemia, vascular collapse, shock and death. Adrenal crises occur in the newborn period as early as one to seven weeks of life.

It is important to recognize that the extent of virilization in an affected female may be the same in simple virilizing and salt-wasting CAH. Thus, even a mildly virilized newborn with 21-hydroxylase deficiency should be observed carefully for signs of a potentially life-threatening crisis within the first few weeks of life.

NONCLASSICAL CONGENITAL ADRENAL HYPERPLASIA

Nonclassical 21 hydroxylase deficiency may present at any age with a variety of hyperandrogenic symptoms. This form of CAH results from a mild deficiency of the 21-hydroxylase enzyme and is diagnosed by serum elevations of 17 OHP that plot on the nomogram between range for unaffected individuals and levels observed for classical CAH patients (Figure 2).

Similar to classical CAH, nonclassical 21 hydroxylase deficiency 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 nonclassical CAH. (36, 37)

Women may present with symptoms of androgen excess, including hirsutism, temporal baldness, and infertility. Menarche in females may be normal or delayed, and secondary amenorrhea is a frequent

occurrence. Polycystic ovarian syndrome may be seen in these patients. It is likely that excessive levels of adrenal sex steroids disrupt gonadotropin release and with direct effects on the ovary, ultimately lead to the formation of ovarian cysts, which may continue autonomously to produce androgens.

In males, early beard growth, acne, and growth spurt may prompt the diagnosis of nonclassical CAH. A reliable indication of an adrenal, as opposed to testicular, source of androgens is the proportionate small size of the testes compared to the phallus that results from suppression of the hypothalamic pituitary gonadal axis. Symptoms of nonclassical CAH in adult males may be limited to short stature or oligozoospermia and diminished fertility.

EPIDEMIOLOGY

There is a worldwide incidence of approximately 1:15,000 live births for classical CAH owing to 21-hydroxylase deficiency. (38) The prevalence in specific populations based on newborn screening results are 1:10,000 – 1: 23,000 in the United States and Europe (38-41), 1:18,000 in Japan (42), and 1:23,000 in New Zealand. (43)

Nonclassical 21 hydroxylase deficiency has a much higher frequency than the classical forms of CAH. (44, 45) Nonclassical 21 hydroxylase deficiency has an ethnic specific frequency of 1:27 for Ashkenazi Jews, 1:53 for Hispanics, 1:63 for Yugoslavs, 1:333 for Italians, and 1 in 100 in a diverse Caucasian population. The ethnic specific frequency of nonclassical CAH indicates that it may be the most frequent monogenic disease in humans (Figure 4) and may be the commonest cause of reduced fertility.

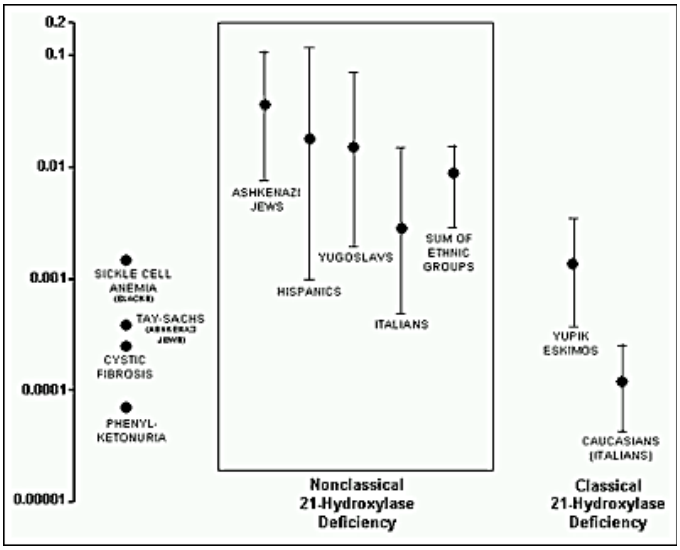

Figure 4. Frequency of nonclassical and classical 21-hydroxylase deficiency in comparison with other autosomal recessive disease incidences. Speiser #2056]

GENETICS

The gene encoding the enzyme 21-hydroxylase, CYP21, is a microsomal cytochrome P450 located on the short arm of chromosome 6 (46) in the human lymphocyte antigen (HLA) complex. (47.) CYP21 and its homologue, the pseudogene CYP21P, alternate with two genes called C4B and C4A (48, 49) that encode the two isoforms of the fourth component (C4) of serum complement. (50) CYP21 and CYP21P, which each contain 10 exons, share 98% sequence homology in exons and approximately 96% sequence homology in introns. (51, 52)

Approximately 60 mutations in the CYP21 gene causing 21-OHD have been identified thus far. (53) 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 CYP21 of smaller-scale deleterious mutations present in the CYP21P pseudogene.

Both classical and nonclassical 21-hydroxylase deficiency are inherited in a recessive manner as allelic variants. Classical 21-hydroxylase deficiency results from the presence of two severely affected alleles and nonclassical 21-hydroxylase deficiency results from the presence of either two mild 21-hydroxylase deficiency alleles or one severe and one mild allele. A list of common CYP21 mutations causing the different forms of CAH is listed in Table 2. It is important to note, however, that the 10 most common mutations observed in CYP21 cause variable phenotype effects and are not always concordant with genotype. (54, 55)

In the three major publications on the issue of nonconcordance, (54, 56-58) it is clear that although there is nonconcordance of phenotype to genotype, it is rare (~10%). It is true that this rare nonconcordance of genotype to phenotype complicates prenatal diagnosis and parents must be alerted to this possibility. In our extensive experience with prenatal diagnosis and treatment, we have not encountered the problem of genotype not predicting phenotype to date. (54) We are, however, prepared to deal with it as caveats are added to the consent forms and letters. Oftentimes, the indication for prenatal diagnosis is an affected sibling. Thus, we have an opportunity to examine genotype-phenotype correlations in living family members.

In recent years, 21-hydroxylase deficiency has been added to the newborn screening panels in 31 states. It is an ideal disorder for newborn screening as the salt-wasting form has a high mortality and morbidity in the neonatal period and can be treated easily with replacement medication. Detection is usually accomplished by measurement of 17-OHP levels on day 2 or 3 of life.

TREATMENT

There is no doubt that controversy exists regarding surgery before the 1st year of life. The Intersex Society of North America (ISNA) initially proposed that even sex assignment should not be done in the newborn period. They have now modified their position stating that sex assignment should be done in the newborn period although surgery should be postponed. However, this does not apply to CAH, a condition in which 46, XX females are usually ambiguous but do not have complete virilization of the genitalia. The ambiguity of the genitalia often requires surgical correction because urine collects in the vagina or in the urogenital sinus and can cause repeated infections. As fertility is not impaired if control of adrenal androgens is maintained after genitoplasty, the 46, XX female is capable of having a child as she has all the organs necessary for becoming a mother (i.e. uterus, fallopian tubes, ovary and vagina). It is one of the few intersex conditions in which fertility is possible. We are in the midst of an evaluation of 200 patients born with genital ambiguity and who have reached adult life. Preliminary analysis suggests that assignment to the female sex is accepted by these women who maintain a female gender. These data were presented at the conference entitled, “Hormonal and Genetic Basis of Sexual Differentiation Disorders,” held in Tempe, AZ in May 2002.(59)

Lifelong glucocorticoid replacement therapy is the mainstay of treatment for classical and symptomatic nonclassical CAH patients. Glucocorticoids not only replace cortisol but also reduce the overstimulation of the adrenal cortex by reducing the release of ACTH, thereby suppressing the overproduction of adrenal androgens. Hydrocortisone is usually chosen for infants and children, as it is shorter acting than prednisone or dexamethasone, thus less likely to comprise growth. Excessive

glucocorticoid administration should be avoided as it can cause cushingoid facies, growth retardation, and inhibition of epiphyseal maturation.

Oral hydrocortisone administration of 10-20 mg/m2 is conventionally given in daily divided doses, as it is believed divided doses better suppress the production of adrenal androgens. The dosage is increased 2 to 3 times that of the normal daily dosage during times of non life threatening illness or stress. Up to 5 to 10 times the daily dosage may be required during surgical procedures. Patients with salt wasting CAH may also require mineralocorticoid replacement. A cortisol analogue, fluorohydrocortisone (Florinef), is used for its potent mineralocorticoid activity. Patients with non-classical 21-OH deficiency are only treated with glucocorticoids if they manifest symptoms of androgen excess. In cases of excess ovarian androgen production, the use of progestational and estrogenic agents may be necessary for suppression of gonadotropin release. In the author's clinic, serum concentrations of Δ5-17-hydroxypregnenolone, 17-OHP, Δ4-androstenedione, dehydroepiandrosterone (DHEA), testosterone, estradiol, deoxycorticosterone (DOC), corticosterone, cortisol, and aldosterone in all CAH patients, as well as levels of PRA in salt-wasting patients, are monitored every three months.

Successful treatment of CAH children hinges on the delicate balance of suppressing adrenal androgen secretion with glucocorticoid administration while maintaining normal growth. However, CAH causes short stature in adults even when good adrenal hormonal control is maintained throughout childhood and puberty. The adult height of CAH patients may be as low as 2 standard deviations (SD) below the mean. (7, 8, 60) Elevated adrenal androgens causing advanced epiphyseal maturation and premature epiphyseal fusion, as well as over treatment with glucocorticoids, are likely causes of short adult stature. One recent study found that growth hormone therapy alone or in combination with gonadotropin-releasing hormone analog (GnRHa) was effective in improving growth rate, height deficit, and height in children with CAH. (61) More recently, Lin-Su et al (2005) demonstrated that the combination of growth hormone and LHRH analogue improved final adult height by 8 cm when compared to CAH subjects treated only with glucocorticoid and mineralocorticoid therapy.(62)

Another new approach for management of CAH patients is the use of an antiandrogen (flutamide) and an aromatase inhibitor (testolactone) in combination with a reduced hydrocortisone dose. (63-65) This treatment regimen was successful in normalizing linear growth and bone maturation in children over two years of treatment. However, the use of flutamide is prohibited from use in any young patient outside of the NIH because of severe liver toxicity. Second, the use of antiandrogens in women of reproductive age carries the risk of causing incomplete virilization in affected males. Antiandrogens are not employed as treatment in many centers.

Bilateral adrenalectomy has been reported to improve symptoms in a few patients who were extremely difficult to control with medical therapy alone.(66-68) An adult female with CAH who had long-term amenorrhea resumed regular menses following bilateral adrenalectomy.(69) Because this approach induces complete adrenal insufficiency, 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.

Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency may prove to be an excellent candidate for gene therapy. The disorder is monogenic and 21-hydroxylase is only expressed in the adrenal glands. Indeed, a mouse model for steroid 21-hydroxylase deficiency is currently being used to develop gene therapy for congenital adrenal hyperplasia. (70, 71) An adeno-associated vector demonstrated a longer period of expression and much lower immune response than previously used adenovirus vector. (72) Gene therapy would eliminate the difficulties of adequate adrenal androgen suppression without hypercortisolism.

PRENATAL DIAGNOSIS AND TREATMENT

Prenatal diagnosis and treatment of 21-hydroxylase deficiency has been utilized for over 15 years and is appropriate in families where a previous family member has been affected (Figure 5).(73) Dexamethasone (20 µg/kg/day in 3 divided doses) is administered to the pregnant mother before the 9th week of gestation, or ideally before the 7th week, blind to the sex or affected status of the fetus. This suppresses excess adrenal androgen secretion and prevents virilization should the fetus be an affected female. As the urogenital sinus formation begins at approximately the ninth week of gestation, (74) prenatal treatment must begin as soon as the pregnancy is confirmed in order to prevent virilization of external genitalia in the affected female. Dexamethasone is used because it crosses the placenta, crossing from the maternal to the fetal circulation.

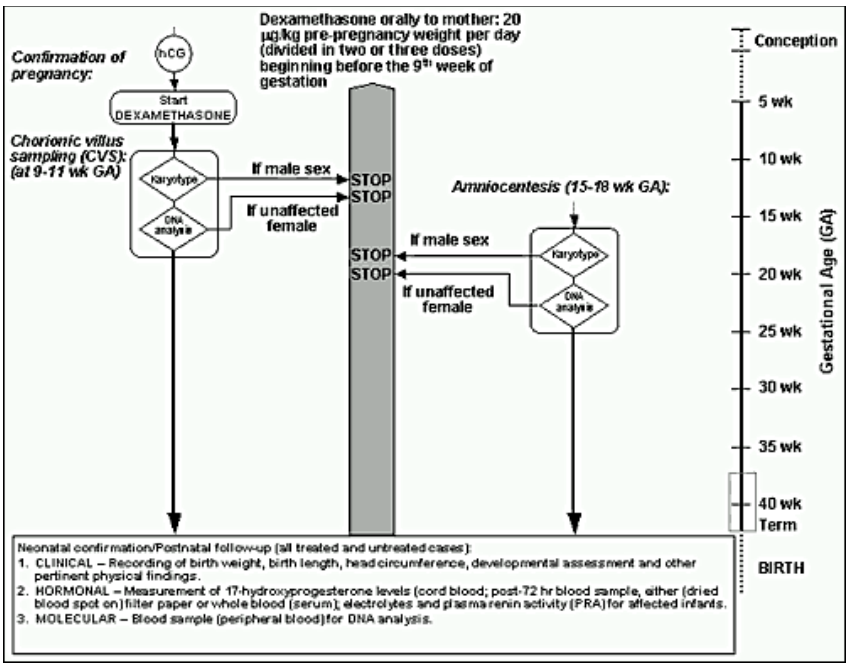

Figure 5. Algorithm depicting prenatal management of pregnancy in families at risk for a fetus with 21-OHD. (6)

Prenatal diagnosis by DNA analysis (75, 76) requires chorionic villus sampling in approximately the 9th week of gestation. Amniotic fluid cells obtained by amniocentesis in the 16th – 20th week of gestation may also be used for DNA analysis. If the fetus is determined to be a male upon karyotype or an unaffected female upon DNA analysis, treatment is discontinued. Otherwise, treatment is continued to term. This is the first instance of an inborn metabolic disorder to be successfully treated prenatally. The largest human studies have found that dexamethasone administered in proper doses at or before the 9th week of gestation is effective in reducing virilization in the genetic female so that genitoplasty was not needed in the majority of cases (Figure 6). (6, 77, 78)

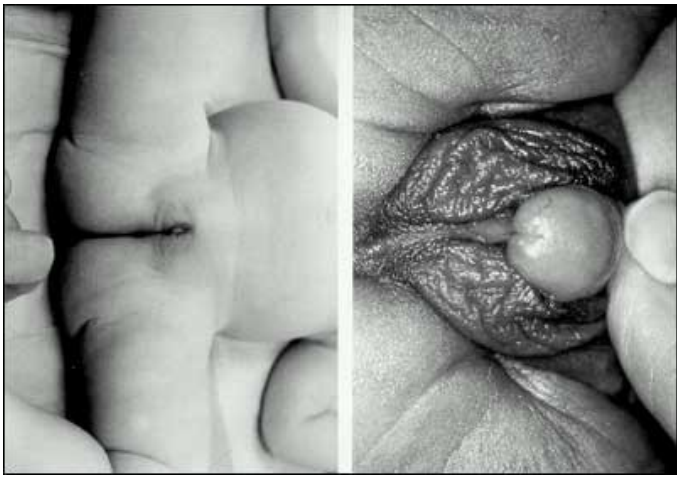

Figure 6. Genitalia of prenatally treated (bottom) and untreated (top) siblings with Classical CAH owing to 21-hydroxylase deficiency. The prenatally untreated girl (top) has virilized genitalia with an enlarged clitoris and scrotalization of the labia majora (Prader IV). The prenatally treated sib was born with normal female genitalia.

Since prenatal treatment became available, the majority of studies have proven it to be effective and safe for both mother and child, provided patients and physicians adhere to the recommended therapeutic protocol. (6) Some results from animal studies, in which excess glucocorticoid dosages were used, suggest there are maternal complications of glucocorticoid treatment.(79) However, results from one of the largest studies in humans, indicate there are no enduring maternal complications (i.e. edema, striae, excessive weight gain, spontaneous abortion).(6) In fact, all maternal complications disappeared after delivery and all mothers contacted (save three, who did not desire another child) stated that they would repeat their care with dexamethasone if they were pregnant again.

To date, the majority of studies show no major effect of glucocorticoid therapy on fetal birth weight, growth or development; treated newborns have been shown to reach developmental milestones at the appropriate ages. (6, 77, 78) In a study by Lajic et al., (78) in which only 44 pregnancies were studied as compared to 595 reported by New et al.,(80) although weight gain by the mother increased in the first trimester, overall weight gain was not different from untreated. There was no increase in spontaneous abortions. In monkeys, degeneration of the hippocampus was observed with administration of dexamethasone to the mother. However, the dose given was 200 times that used in human prenatal treatment. Cognition and postnatal growth reported by Lajic et al. were normal in humans. A long term follow-up study of 44 children treated prenatally in Scandinavia demonstrated normal pre- and post natal growth compared to matched controls.(78) In our study, prenatally treated newborns also did not differ in weight, length, or head circumference from untreated, unaffected newborns.(80, 81)

In one study by Forest et al., only 2 of 37 dexamethasone-treated newborns were abnormally small, and in both cases, it was undetermined if it was treatment-related or a result of underlying factors (i.e. the parents being undersize).(77) Studies undertaken with monkeys only demonstrated fetal growth retardation in the range of 40-160 ü g/kg/d, a dose 2-8 times that of the highest human dose. (77) However, the largest human studies have shown that birth weight, birth length and head circumference were not significantly different in the offspring of dexamethasone treated pregnancies and those not treated; the average difference in birthweight was only 300 grams, a clinically insignificant value. (6, 77, 82, 83)

Miller and Seckl raise the specter of prenatal dexamethasone therapy leading to hypertension in adulthood. (79) It is not clear, however, that hypertension is the result of low birth weight or large

placental size, as previous studies arrive at contradictory conclusions. One study with 449 subjects concluded that there was no correlation with adult blood pressure and birth weight alone, but that low birth weight and large placental weight together were predictors of adult high blood pressure.(84) The highest adult blood pressures were found in those who were small babies born with large placentas. (84) In another study of 1511 subjects, in which they adjusted for height and body mass index, Whincup et al. showed that birth weight had a smooth graded inverse relation with blood pressure in 9-11 year olds. (85) In Whincup's report, subjects with low birth weight and high placental weight did not have particularly high mean blood pressures, contradicting the former Barker report.(84) Thus, it is difficult to correlate low birth weight with hypertension, as these studies examine low birth weight due to malnutrition and placental data is often not included. (84, 86-89)

Miller and Seckl also state the possibility that dexamethasone prenatal treatment contributes to fetal death, but this claim is unsupported by other authors.(90) In the 1993 report by Forest et al., there is strong evidence to the contrary.(83) In this study, there was 9% incidence of spontaneous abortion of 64 treated pregnancies, while 14% incidence in untreated pregnancies, suggesting that fetal death and spontaneous abortion cannot be attributed to dexamethasone treatment in every case.

Despite the claim that surgical reparation is necessary in CAH patients, (90) in the large experience of our CAH patients who were prenatally treated with dexamethasone, none to date have required reparative surgery of the genitalia as long as compliance has been assured. Cases of unsuccessful treatment are almost always correlated with a late treatment start date, too small a dosage of dexamethasone, or interrupted treatment. Therefore, the solution for effective outcome is to monitor it meticulously. When treatment is properly administered, genitoplasty has not been necessary. It should be clear that there are no long-term studies to date of surgical correction of ambiguous genitalia.

It has also been reported previously that children treated prenatally with dexamethasone are adversely affected cognitively and developmentally. (91) However, the discussion in the paper in which Trautman et al. state that the differences they found in treated versus untreated were not necessarily negative changes, but differences. Trautman et al. also state that these results should be considered preliminary until they can be replicated by a larger sample. A recent survey of 174 prenatally dexamethasone-exposed children, ages 1 month to 12 years, (including 48 with CAH) compared to 313 unexposed children (including 195 with CAH) found no differences in cognitive or motor development between the two groups.(92) Therefore, we believe that proper prenatal treatment of fetuses at risk for CAH can be considered effective and safe. Thus, long-term follow-up studies are necessary to evaluate cognition, gender, temperament, and handedness (an indicator of prenatal androgen effect) in children and adults prenatally treated in order to evaluate the long-term consequences of prenatal dexamethasone treatment. These studies are being prepared for publication. The oldest known patient is now 20 years of age, and she suffers no cognitive or developmental defects (New, pers. com.).

PSYCHOLOGICAL ASPECTS OF CAH

Several studies have shown that compared to their unaffected sisters, girls with CAH tend to prefer more male-typical toy choices, such as construction and transportation toys, and more male-typical play behavior, such as rough outdoor play.(93, 94) Meyer-Bahlburg et al (2004) demonstrated that CAH girls age 5-12 years have masculinization of gender-related behavior, but no gender identity issues.(95) Adult women with CAH have also been demonstrated to have masculinized behavior, being most pronounced in SW-CAH, slight but demonstrable in SV-CAH, and questionable in NC-CAH. For the majority of the adult women with CAH, gender identity was clearly female; however, gender dysphoria was identified in 3 out of 42 women with SW-CAH.(96) Long et al (2004) also demonstrated that CAH girls preferred male-typical toys and male playmates, but found that masculinity decreased

across developmental stages, such that by adulthood, there was no significant difference in masculinity between controls and women with CAH.(97)

Male predominance of left-handedness has been attributed to early androgen exposure. In support of this theory, girls with CAH have been found to be more left-handed biased than their unaffected sisters.(98) There is also some evidence that CAH women have a post-pubertal spatial advantage.(99)

In both children and adults, psychological adjustment does not appear to be compromised in females with virilized genitalia who are treated early in life and reared as females.(100) However, there are reports of less satisfaction with sexual function in women with classical CAH, particularly in those with the salt-wasting form.(94, 101-103)

CONCLUSION

Ambiguous genitalia in the newborn, often in combination with severe salt wasting, is the clinical hallmark of 21-hydroxylase deficiency. The pathophysiology of CAH can be traced to an inherited defect in the gene encoding the enzyme 21-hydroxylase. Treatment of patients with CAH is targeted at replacing cortisol, which is produced in insufficient quantity. With proper hormone replacement therapy, normal and healthy development may often be expected. Radioimmunoassay of serum and urinary steroid levels permit reliable diagnosis of the various forms of CAH. Prenatal treatment of affected females prevents potential sex misassignment, and repeated genital surgeries that cannot easily recreate natural genital structures. Based on our experience, proper prenatal diagnosis and treatment of CAH owing to 21-hydroxylase deficiency is safe and effective in significantly reducing or eliminating virilization in the affected female, thus making CAH one of the few monogenic disorders in which postnatal life is influenced by effective prenatal treatment. However, follow-up studies are still necessary to evaluate potential long-term consequences of prenatal treatment with dexamethasone.

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