PREFACE

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Guest Editor

Congenital adrenal hyperplasia (CAH) is among the most common and best characterized inborn errors of metabolism. This volume brings together an outstanding group of authors from the disciplines of psychology, clinical and molecular medicine, and surgery in an attempt to present a comprehensive and current summary of various forms of CAH.

In the first article, New presents data on prenatal treatment from her group's extensive experience. These data indicate no irreversible serious adverse effects in mothers, fetuses, or infants who have undergone such treatment. There are still long-term safety concerns surrounding prenatal administration of dexamethasone, but in approximately 12 years' follow-up the offspring of these treated pregnancies have been generally healthy. Half of the girls who were prenatally treated were born with normal genitalia, while most of the others show considerably less genital ambiguity compared with their elder non-prenatally-treated sisters. Given the powerful and far-reaching psychosexual impact of a child born with abnormal genitals, judicious use of prenatal dexamethasone to pregnancies at 25% risk for classic CAH is advised with careful monitoring.

Therrell discusses the now widespread practice of neonatal screening for CAH by hormonal assays. Pitfalls do exist in these diagnostic studies, but refined algorithms accounting for altered cut-off values of 17-hydroxyprogesterone in low birth weight and repeated sampling to detect delayed disease expression help clarify the picture for clinicians. Since about 95% of CAH alleles are readily identified in most populations, genotyping may ultimately improve the accuracy of screening. Screening undoubtedly rescues some infants who would have been unrecognized with the disease. Surprisingly, we do not know the exact rate of infant mortality for CAH.

CAH is most often caused by deficiency of steroid 21-hydroxylase; less common causes include deficiencies of 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, and 17α-hydroxylase/17,20-lyase. Genetic characterization of these disorders has enhanced our understanding of phenotypic differences among patients. These topics are discussed in individual chapters authored by myself, and White, Pang, and Auchus, highlighting areas of recent clinical and research interest.

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Gene therapy is still a distant gleam on the horizon. Replacing glucocorticoids and, as needed, mineralocorticoid, remains the cornerstone of therapy for CAH. Merke and Cutler present data supporting the use of new multidrug regimens including lower doses of the standard drugs, hydrocortisone and fludrocortisone, and drugs to block androgen action (flutamide) and reduce estrogen production (testolactone). Schnitzer and Donahoe detail the surgical approach to the child with genital ambiguity, with special attention to the CAH female. There is hope that newer surgical techniques may improve functional outcome; however, since these procedures have been employed only for the past decade, there are insufficient long-term data.

Meyer-Bahlburg points out that although much attention has been focused on psychosexual pathology, most CAH women function without problems. We also ought to recognize that the health professionals counseling parents of infants regarding "optimal gender policy" are not always experienced in dealing with problems of gender and sexuality prominent in adolescents and adults. The newly established North American Task Force on Intersexuality will begin to address the issue of long-term psychosexual outcome for individuals with genital anomalies, including CAH, in a cooperative multicenter study involving psychologists, endocrinologists, geneticists, and surgeons.

Berenbaum reviews the data on cognitive function, and concludes that the studies demonstrating IQ differences among CAH patients compared with siblings are methodologically weak. Perhaps the only measure credibly distinguishing brain function in CAH females is visual-spatial abilities, presumably enhanced in these individuals by prenatal exposure to androgens. The cohort of prenatally treated girls with CAH should provide an interesting control group for future psychological studies.

Migeon and Wisniewski argue for restraint in using suppressive doses of glucocorticoids, especially during the first year of life. The goal of medical therapy should be to promote a good linear growth outcome, while avoiding adiposity and virilization.

A final indicator of how far we have come in the management of CAH is the article from Lo and Grumbach, analyzing pregnancy outcome among affected women. These authors stress the need for titrating the dose of hydrocortisone or prednisone in order to maintain serum testosterone in the high normal range for pregnancy (i.e., approximately 200 ng/dl after the first trimester), and using stress doses during and immediately following labor and delivery. Most offspring have been healthy, and because of the protective effect of placental aromatase, virilization of female fetuses is very rare.

I wish to thank all the contributors to this volume for their efforts, and John Vassallo and the editorial staff at W.B. Saunders for initiating and facilitating this project. This valuable collection of articles should serve as a focal point for discussions of practice guidelines for CAH, and trigger interest in further investigations aimed at improving patient outcomes.

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PRENATAL TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

The United States Experience

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Congenital adrenal hyperplasia (CAH) refers to a family of inherited disorders in which defects occur in one of the enzymatic steps required to synthesize cortisol from cholesterol in the adrenal gland. Because of the impaired cortisol secretion, adrenocorticotropic hormone (ACTH) levels rise owing to impairment of a negative feedback system, which results in hyperplasia of the adrenal cortex. The enzyme defects are translated as autosomal recessive traits, with the enzyme 21-hydroxylase deficient in more than 90% of CAH cases.[23] Owing to the blocked enzymatic step, cortisol precursors accumulate in excess and are converted to potent androgens, which are secreted and cause in utero virilization of the genitalia in affected female fetuses in the classic form of 21-hydroxylase deficiency. These effects are also seen in 11β-hydroxylase deficiency, which is the second-most common cause of CAH.

STEROIDOGENESIS

Aldosterone, cortisol, and testosterone are derived from cholesterol, and many of the same enzymes are used for their synthesis in the

Significant sections of the work herein were supported by United States Public Health Service grant HD00072 and Children's Clinical Research Center grant 06020.

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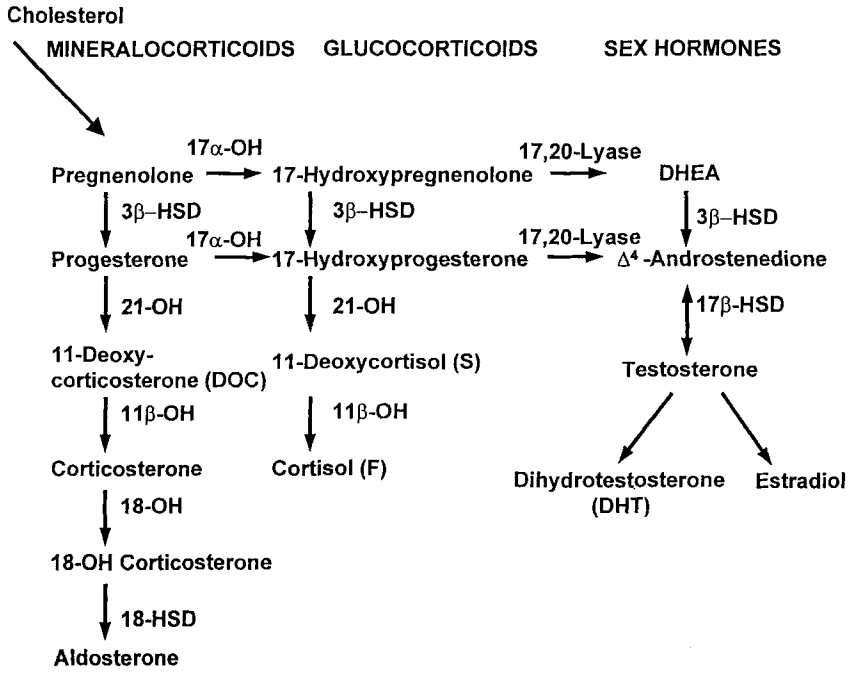

Figure 1. Adrenal steroidogenesis. OH = hydroxylase (for enzymes); HSD = hydroxysteroid dehydrogenase (for enzymes); DHEA = dehydroepiandrosterone.

adrenal cortex (Fig. 1). Deficiencies in any of the enzymatic steps that are common to the synthesis pathway of these hormones can result in the loss of a combination of some or all of their production, or unchecked negative feedback loops can lead to overproduction. In 21-hydroxylase deficiency (and 11β-hydroxylase deficiency), the enzyme deficiency creates the effect of a dam behind which steroid precursors accumulate, which then overflow into biosynthetic pathways unaffected by the block, resulting in the production of excess androgens.

Cortisol synthesis is regulated by a negative feedback loop in which high serum levels of cortisol inhibit the release of ACTH from the pituitary, whereas low serum levels of cortisol stimulate the release of ACTH. This loop defines the hypothalamic-pituitary-adrenal axis. The central nervous system determines the hypothalamic set point for the expected plasma cortisol level. Plasma cortisol levels lower than the hypothalamic-pituitary set point will increase the rate and intensity of ACTH secretory pulses (the net ACTH release has basal, diurnal, and stress-induced components). A deficiency of 21-hydroxylase, causing impaired synthesis and decreased secretion of cortisol, leads to chronic elevations of ACTH with overstimulation and consequent hyperplasia of the adrenal cortex. Because the pathways for testosterone, dehydroepiandrosterone, and Δ^4-androstenedione preceding the 21-hydroxylase step

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are unblocked, the precursors (17-hydroxyprogesterone, pregnenolone, 17-hydroxypregnenolone, and progesterone) are routed to these pathways, androgens are oversecreted in the adrenals in utero, and the genitals of the female fetus are masculinized.

21-HYDROXYLASE DEFICIENCY

Classic

In the classic form of CAH owing to 21-hydroxylase deficiency, androgen excess causes external genital ambiguity in newborn females (female pseudohermaphroditism), who may present with a urogenital sinus, scrotalization of the labia majora, labial fusion, or clitoromegaly. After birth, males and females exhibit progressive postnatal virilization, which can include central precocious puberty later in childhood, progressive penile or clitoral enlargement, precocious pubic hair, hirsutism, acne, advanced somatic and epiphyseal development, reduced fertility, menstrual abnormalities in women, and small testes in men. There are two forms of classic steroid 21-hydroxylase deficiency: simple virilizing and salt-wasting. Three fourths of classic cases are salt-wasting.[23] To some extent, the symptoms can be arrested or reversed by treatment with glucocorticoids, which suppresses ACTH stimulation of the adrenal cortex. Patients with aldosterone deficiency require treatment with salt-retaining steroids as well.

Nonclassic

Nonclassic 21-hydroxylase deficiency refers to the condition in which partial deficiencies of 21-hydroxylation produce late-onset, less extreme hyperandrogenemia and milder or no symptoms. Females do not demonstrate genital ambiguity at birth, although males and females may manifest signs of androgen excess at any phase of postnatal development. Short stature, premature development of pubic hair, insulin resistance, acne, reduced fertility, and, in women, polycystic ovaries, hirsutism, and male pattern baldness, may be seen in untreated patients. Some individuals are never affected with overt signs of androgen excess and require no treatment. Symptomatic nonclassic patients respond to treatment with a glucocorticoid to suppress ACTH, thereby suppressing the androgens.

Frequency

Analysis of CAH incidence data from almost 6.5 million newborns screened in the general population worldwide has demonstrated an overall incidence ranging from 1 in 13,000 to 1 in 15,000 live births for

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the classic form of CAH.[24, 25] Applying the Hardy-Weinberg equilibrium formula, the carrier rate for a classic CAH mutation is approximately 1 in 63.[24]

The overall frequency of nonclassic 21-hydroxylase deficiency is high. Speiser and co-workers[30] assessed the population genetics of the nonclassic disorder and found nonclassic 21-hydroxylase deficiency to be more common than the classic deficiency causing CAH. In fact, it was the most common human autosomal recessive disease trait. The disease frequency in the general heterogeneous population of New York City is 1 in 100, and 1 in 7 persons is a carrier. The highest ethnic frequency is found among Ashkenazi Jews, occurring in 1 in 27 persons. Other specific ethnic groups also exhibit high disease frequency: 1 in 40 Hispanics, 1 in 50 Slavs, and 1 in 300 Italians. These results have been confirmed by other studies.[29, 42]

Diagnosis at Birth

Patients who have CAH present with a unique hormonal profile owing to their enzymatic deficiency. The best diagnostic test for 21-hydroxylase deficiency is the ACTH stimulation test measuring the serum concentration of 17-hydroxyprogesterone. A logarithmic nomogram has been developed and provides hormonal standards for assignment of the type of 21-hydroxylase deficiency by relating baseline to ACTH-stimulated serum concentrations of 17-hydroxyprogesterone.[22] The nomogram can clearly distinguish patients with classic CAH from those with nonclassic CAH, as well as identify classic and nonclassic heterozygotes.

The ACTH test to measure 17-hydroxyprogesterone levels in suspected CAH cases should not be performed during the initial 24 hours of life because samples from this period are typically elevated in all infants and may yield false-positive results. Aldosterone, plasma renin, and serum sodium and potassium levels are measured to assess salt-preserving ability. Karyotyping or other genetic analysis will establish the genetic sex in cases of ambiguous genitalia. Imaging the internal anatomy by pelvic sonography will reveal a uterus, fallopian tubes, and ovaries in females affected with CAH. Such findings are often the first indication that the infant with genital ambiguity is a genetic female.

The diagnosis of 11β-hydroxylase deficiency is made on the basis of elevated serum deoxycorticosterone (DOC) levels or 11-deoxycortisol (compound S) levels, as well as marked urinary elevation of their tetrahydrometabolites. Further characterization by molecular genetic analysis can be performed. Diagnosis in a newborn is difficult because the characteristic hypertension does not generally appear during the newborn period. Distinction from 21-hydroxylase deficiency on the basis of steroid patterns is also problematic at this age because 17-hydroxyprogesterone levels will typically be elevated in cases of 11β-hydroxylase deficiency;

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however, in 21-hydroxylase deficiency, deoxycortisol and DOC are not elevated.

MOLECULAR GENETICS

21-Hydroxylase Deficiency

The gene encoding 21-hydroxylase (a microsomal cytochrome P450 called CYP21, previously known as P450c21) is located on the short arm of chromosome 6 in the human lymphocyte antigen (HLA) complex.[9] The gene for the 21-hydroxylase enzyme, *CYP21,* and its homologue, the pseudogene *CYP21P,*[21] alternate with two genes called *C4B* and *C4A*[6, 36] that encode the two isoforms of the fourth component (C4) of serum complement.[2] *CYP21* and *CYP21P,* which each contain 10 exons, share 98% sequence homology in exons and approximately 96% sequence homology in introns.[14, 37]

Approximately 50 mutations in the *CYP21* gene causing 21-hydroxylase deficiency have been identified thus far.[15] The author and her colleagues have demonstrated deletional mutations of the 21-hydroxylase deficiency genes and characterized specific point mutations in many patients.[1, 13, 31, 33, 34, 37, 38] The most common mutations are the result of two types of recombination events between *CYP21* and *CYP21P*: (1) misalignment and unequal crossing over, resulting in large-scale DNA deletions; or (2) apparent gene conversion events that result in the transfer to *CYP21* of smaller-scale deleterious mutations present in the *CYP21P* pseudogene.[32]

Correlation/Noncorrelation of Genotype to Phenotype

In general, there is a good correlation between the severity of the clinical disease and the discrete mutations observed. In 1995, the author and her colleagues compared the genotypes and phenotypes in approximately 200 patients and divided them into mutation-identical groups.[39] That study and another by Krone and co-workers[16] demonstrated that the 10 most common mutations observed in *CYP21* cause variable phenotypic effects and are not always concordant with genotype. Subsequently, the author has genotyped over 600 patients and identified 85 mutational groups, 23 of which had more than one phenotype. DNA sequencing analysis ruled out rare undetected mutations on the same allele.

The noncorrelation of genotype to phenotype may present a difficulty for the clinician in directing prenatal treatment.

11β-Hydroxylase Deficiency

The disorder of 11β-hydroxylase deficiency, which has a frequency of approximately 1 in 100,000 live births,[41] is caused by an autosomal

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recessive defect of the enzyme protein-encoding gene *CYP11B1*. The *CYP11B1* gene comprises nine exons. Approximately 30 mutations in *CYP11B1* have been identified in cases of 11β-hydroxylase deficiency.[15] The gene is located on chromosome 8q22, about 40 kb from the highly homologous gene *CYP11B2*, which encodes aldosterone synthase. Mutations in the *CYP11B1* gene have been identified throughout the coding region, but there is clustering around exons 2, 6, 7, and 8, suggestive of mutational hot spots.[19] Although gene conversions do occur between *CYP11B1* and *CYP11B2*,[19, 20] most of the mutations found in *CYP11B1* are random point mutations.[35] These mutations have been identified from diverse ethnic backgrounds, with the highest incidence among a highly inbred group of Moroccan (Sephardic) Jews.[26, 27]

PRENATAL DIAGNOSIS AND TREATMENT

Breakthrough in Prenatal Diagnosis and Treatment in Congenital Adrenal Hyperplasia

When it was discovered that CAH-affected fetuses had elevated 17-hydroxyprogesterone and Δ^4-androstenedione in their amniotic fluid, measuring the levels of these substances by amniocentesis and hormonal assay became the first method of prenatal diagnosis for this disorder; however, amniocentesis is performed in the second trimester, and, to prevent prenatal virilization of an affected female, treatment must begin before 10 weeks' gestation. After beginning prenatal treatment, dexamethasone for the developing fetus would suppress 17-hydroxyprogesterone in amniotic fluid; hence, this hormonal test could not be relied on for diagnosis. When HLA was found to be linked to CAH, diagnoses were made using HLA genetic linkage marker analysis. This method resulted in many diagnostic errors owing to recombination or haplotype sharing. The method generally used now is direct DNA analysis of the 21-hydroxylase gene (*CYP21*) with molecular genetic techniques.

Establishment of Algorithm

Prenatal treatment of 21-hydroxylase deficiency with dexamethasone has been performed for approximately 15 years to suppress excess adrenal androgen secretion and prevent virilization should the fetus be an affected female. An algorithm has been developed for the prenatal diagnosis of 21-hydroxylase deficiency and CAH using direct molecular analysis of the 21-hydroxylase locus and dexamethasone treatment (Fig. 2). When properly administered, dexamethasone is effective in preventing ambiguous genitalia in the affected female and has been shown to be safe for the mother and fetus.[18] The largest human studies thus far have shown no congenital abnormalities, and the birth weight, birth length, and head circumference were not different in the offspring of

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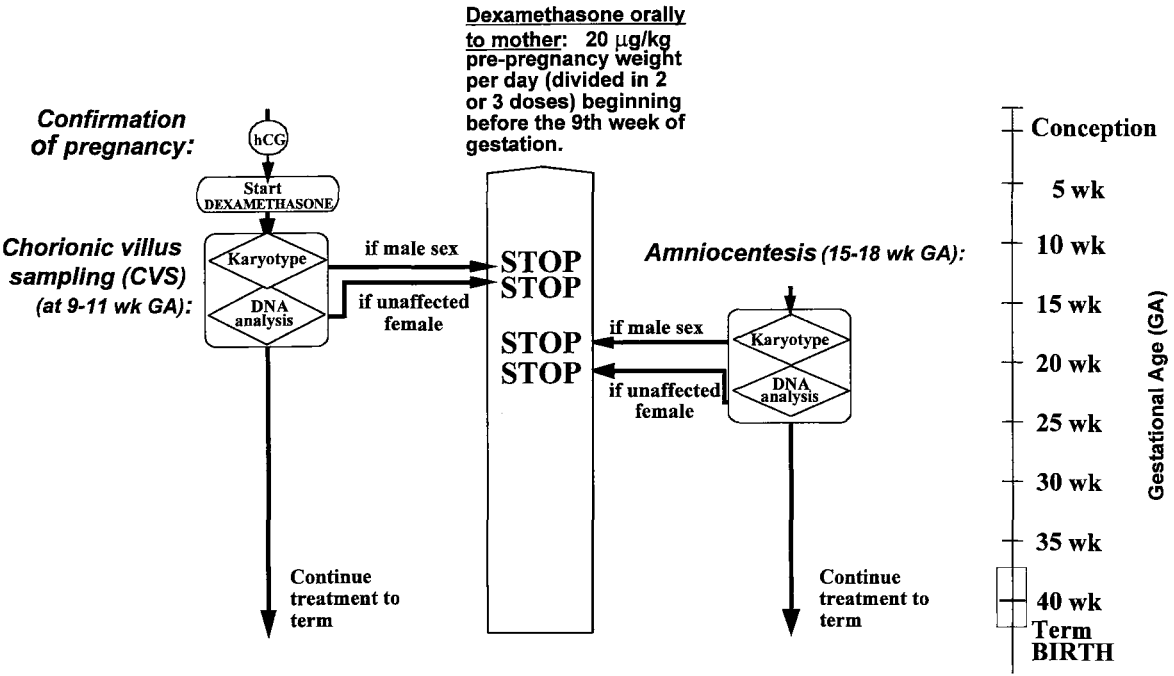

Figure 2. Prenatal management of pregnancy in families at risk for a fetus affected with 21-OHD. hCG = human chorionic gonadotropin; GA = gestation. Neonatal confirmation/postnatal follow-up for all treated and untreated cases:

1. Clinical, Recording of birth weight, birth length, head circumference, developmental assessment, and other pertinent physical findings;

2. Hormonal, Measurement of 17-hydroxyprogesterone levels (cord blood), post-72 hour blood sample, either dried blood spot on filter paper or whole blood (serum), electrolytes and plasma renin activity (PRA) for affected infants;

3. Molecular, Blood sample (peripheral blood) for DNA analysis.

(*From* Mercado AB, Wilson RC, Cheng KC, et al: J Clin Endocrinol Metab 80:2014–2020, 1995; with permission.)

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dexamethasone-treated pregnancies when compared with a nontreated group,[11, 17, 18] provided patients and physicians adhered to the recommended therapeutic protocol.

Dexamethasone (20 μg/kg/d in three divided doses) is administered to the pregnant woman not later than the ninth week of gestation, blind to the sex or the affected status of the fetus (Fig. 2). Diagnosis by DNA analysis requires chorionic villus sampling at approximately the 10th week of gestation, or sampling of amniotic fluid cells obtained by amniocentesis in the second trimester. The fetal DNA is used for specific amplification of the *CYP21* gene using polymerase chain reaction (PCR).[40] If the fetus is determined to be an unaffected female on DNA analysis or a male on karyotype analysis, treatment is discontinued; otherwise, treatment is continued to term.

Monitoring of the pregnant woman during prenatal treatment reduces the risk of complications to her and her fetus. Blood pressure, insulin resistance, and weight gain should be evaluated periodically throughout pregnancy. During the second half of pregnancy, maternal serum or urinary cortisol and estriol levels can be measured to ensure suppression of the maternal and fetal pituitary-adrenal axis.[11]

The New York Hospital–Cornell Medical Center Experience

From 1986 to 1998, prenatal examination for CAH owing to 21-hydroxylase deficiency was carried out in 403 pregnancies at The New York Hospital–Cornell Medical Center. In 280 pregnancies, diagnoses were made by amniocentesis; in 123, chorionic villus sampling was performed. The rapid allele-specific PCR was used for DNA analysis in some cases.[40] The Intron2 mutation was the most common in the New York City population, followed by a 30-kb deletion encompassing most of *CYP21*. Figure 3 describes other genotype combinations. Because of genetic variability, there is no shared mutation that distinctly identifies a CAH diagnosis.

Of the 403 pregnancies evaluated, 84 fetuses were found to be affected with classic 21-hydroxylase deficiency. Fifty-two of these fetuses were female, 36 of whom were treated prenatally with dexamethasone. Dexamethasone administered before 10 weeks' gestation (23 affected female fetuses) was effective in reducing virilization. Thirteen cases had affected female sibs (Prader stages 1–4). Six these 13 fetuses were born with entirely normal female genitalia, whereas 6 others were significantly less virilized (Prader stages 1–2) than their sibs. One fetus was Prader stage 3 (Fig. 4). Among the rest, for whom the index cases were either cousins or male sibs, four were born with normal genitalia, three were Prader stages 1 to 2, and three were Prader stages 3 to 4. The newborns who were Prader stages 3 and 4 were born to women who were either extremely obese (and, because of a limit on the amount of dexamethasone given prenatally, undertreated) or were noncompliant

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Mutation Group	n	Mutation Group	n
Int2/Int2	10	Ex1 Int2 Ex3/ND*	1
Del/Ex1 Int2 Ex3	7	Del/Ex7v Ex7T Ex8318 Ex8356	1
Del/Int2	6	Ex1 Int2 Ex3/Ex7v	1
Ex1 Int2 Ex3/Int2	5	Ex1 Int2 Ex3/Ex8318	1
Ex4/Int2	4	Del/Del	1
Del/Ex7v	4	Ex3/Ex3	1
Del/Ex4	4	Ex3/Ex6	1
Del/Ex8318	3	Ex3/Int2	1
Ex6/Ex6	2	Ex4/ND*	1
Ex8318/Int2	2	Ex4/Ex8318	1
Ex1 Int2 Ex3/ Ex1 Int2 Ex3	2	Ex4/Ex8356	1
Ex8356/Int2 Ex7v	2	Ex6/Int2	1
Int2/ND*	2	Ex7v/Ex8356	1
Del/Int2 Ex3	2	Ex8318/ND*	1
Del/Ex3	1	Ex1 Int2 Ex3/Ex8356	1
Del/Ex8356	1		

Figure 3. Mutation groups identified in the CYP21 locus in the prenatal 21-hydroxylase deficiency congenital adrenal hyperplasia referrals (n = 72). Del = gene deletion; Ex1 = Exon 1 (P30L); Ex3 = Exon 3 (8 base pair deletion); Ex4 = Exon 4 (172N), Ex6 = Exon 6 (cluster: I236N, V237Q, M239K); Ex7v = Exon 7 (V281L); Ex7T = Exon 7 (T306 insertion); Ex8318 = Exon 8 (Q318X); Ex8356 = Exon 8 (R356W); Int2 = Intron 2 (A or C to G).* ND = no mutation detected.

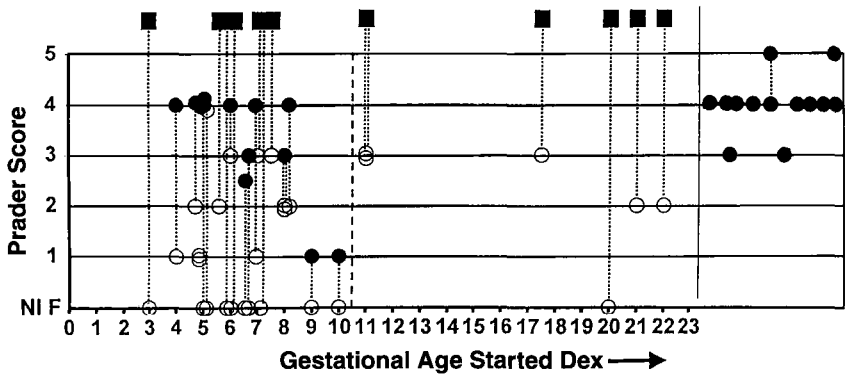

Figure 4. Prader stages of affected female infants in monitored, dexamethasone (Dex) prenatally treated pregnancies, in relation to gestational age when dexamethasone was started. Affected untreated siblings are shown attached by a dotted line. Affected female siblings are represented by solid circles, affected male siblings are represented by solid squares, and dexamethasone-treated pregnancies are represented by open circles. The solid circles in the right-hand column indicate affected untreated female referrals.

and stopped treatment. Overall, the average Prader score for affected females treated prenatally was 1.7 (including the partially treated). In contrast, the average score for untreated affected females was 3.9. Insufficient data exist to correlate the degree of genital ambiguity with mutation group in prenatally treated patients.

No significant or enduring side effects were noted in the fetuses who were treated prenatally. Fetal demise was approximately the same for the dexamethasone-treated and untreated groups (1 female at 35 weeks and 1 male at 19 weeks prenatally treated, and 2 females [13 and 36 weeks] and 1 male at 24 weeks untreated). In addition, as reported in previous studies, prenatally treated newborns did not differ significantly in birth weight from untreated newborns. The mean birth weight for dexamethasone prenatally treated fetuses was 3.4 kg in comparison with 3.5 kg for untreated fetuses ($P = 0.26$) (mean for treated affected fetuses, 3.3 kg, and for unaffected fetuses, 3.4 kg; mean for untreated affected fetuses, 3.6 kg, and for unaffected, 3.5). Quantitative follow-up studies are currently in progress regarding cognition, gender, temperament, and handedness (an indicator of prenatal androgen effect) in children and adults prenatally treated with dexamethasone.

There were no significant differences in side effects in the women treated with dexamethasone when compared with those in the women not treated, which had been a concern of some investigators, except in weight gain. By self-report, women who were not treated with dexamethasone gained an average of 28.6 pounds, whereas treated mothers gained an average of 36.8 pounds, which was statistically significant ($P < 0.005$). No statistically significant differences were found for the presence of striae ($P = 0.14$), edema ($P = 0.56$), hypertension ($P = 0.60$), or gestational diabetes ($P = 0.42$) by the self-reports. In a random survey of the mothers, 14 who had CAH-affected girls and who were prenatally treated with dexamethasone were satisfied with the treatment outcome. Of the 35 mothers asked whether they would take dexamethasone again if they got pregnant, 33 said yes, 2 said no, and 1 said she would abort.

11β-Hydroxylase Deficiency

The experience in prenatal treatment for 11β-hydroxylase deficiency is limited when compared with the experience with 21-hydroxylase deficiency. In 1989 Bouchard and co-workers[3] reported the first attempt at prenatal treatment with dexamethasone in an affected female with 11β-hydroxylase deficiency, which failed to prevent ambiguous genitalia in the newborn. Subsequently, prenatal diagnosis for 11β-hydroxylase deficiency was performed in four additional families. In three of the cases, the fetus was either a heterozygote or unaffected. Two families were reported on by Curnow and co-workers,[8] one by Geley and co-workers,[12] and another by Cerame and co-workers.[7] In 1999 Cerame and colleagues reported the first prenatal diagnosis and treatment of an affected female with 11β-hydroxylase deficiency and CAH. The treatment was successful because the newborn had normal female external genitalia.

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Controversy of Prenatal Treatment

A recent report has questioned the safety of long-term prenatal glucocorticoid treatment of fetuses potentially affected with CAH.[28] The report claims that prenatal treatment with dexamethasone contributes to low birth weight, fetal demise, serious maternal complications, and cognitive and developmental deficiencies. The cited references are predominantly based on animal studies in which excess glucocorticoid dosages were used. The author finds these claims to be unfounded based on her experience with the largest number of treated human pregnancies in the world, in addition to the results of other large studies.[10, 11]

The risk-to-benefit ratio in view of no enduring side effects in the mother or child favors prenatal treatment. Additionally, males and unaffected females treated with short-term dexamethasone show no side effects.[18] Treatment of affected females alleviates potential sex misassignment, repeated genital surgeries that cannot easily recreate natural genital structures, and psychologic effects. Long-term studies, currently in progress, are needed to determine the outcome of treatment conclusively.

SUMMARY

Based on the author's experience, prenatal diagnosis and treatment of 21-hydroxylase deficiency is safe and effective in significantly reducing or eliminating virilization in the affected female, and the same outcome seems to be true in the treatment of 11β-hydroxylase deficiency. Prenatal treatment spares the newborn female the consequences of genital ambiguity, genital surgery, sex misassignment, and gender confusion. Of the monogenic disorders, steroid 21- and 11β-hydroxylase deficiency are two of the few in which prenatal treatment is effective and influences postnatal life.

ACKNOWLEDGMENT

The author wishes to express her appreciation to Laurie Vandermolen and Andrea Putnam for their extensive editorial assistance.

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COGNITIVE FUNCTION IN CONGENITAL ADRENAL HYPERPLASIA

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Cognition may be affected in various ways by congenital adrenal hyperplasia (CAH). Patients who have CAH have been reported to have higher than average intelligence, lower than average intelligence, higher than average rates of learning disabilities, and specific patterns of cognitive enhancements and impairments. Cognitive changes might be expected to result from several factors that are atypical in CAH.[84] The disease produces changes in hormones that might affect brain development and behavior, such as excess exposure to androgens and adrenocorticotropic hormone (ACTH) and reduced cortisol. Because children are not always diagnosed early or given appropriate treatment, they may be at risk for salt-wasting crises or hypoglycemia, which can adversely affect the brain. Even when children receive optimal treatment, they may be exposed to excess glucocorticoids because of difficulties involved in mimicking the rhythmicity of cortisol production.

This article reviews the evidence for cognitive changes in patients with CAH owing to 21-hydroxylase deficiency and considers the mechanisms that might produce these changes. The intention is not to provide the details of all studies but to summarize the state of knowledge and gaps and to highlight methodologic issues that affect the interpretation

Work for this article and the author's research here were supported by National Institutes of Health grant HD 19644.

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of results (for earlier reviews, the reader is referred to references 7, 13, 21, and 64).

THE NATURE OF COGNITIVE CHANGES IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

Enhanced Intelligence

Intelligence in patients with CAH was first discussed by Money and Lewis[62] who noted that their patients had intelligence quotient (IQ) scores that were shifted toward the high end of the distribution. Sixty percent of patients with CAH had an IQ above 110 compared with 25% of the general population. IQ was not related to sex, disease symptoms, the age at treatment, or residential proximity to the hospital.

Subsequent studies that included siblings provided some explanation for the apparent IQ advantage in patients with CAH. Several investigators confirming findings of an elevated IQ in patients with CAH also found an elevated IQ in the siblings of the patients, with no differences in IQ between patients and unaffected siblings.[3, 49, 57, 74, 89] These studies illustrate the importance of including sibling controls.

Various explanations have been proposed for the IQ advantage in patients and unaffected siblings. Some invoke an advantage for heterozygotes owing to the gene that causes CAH, including direct genetic effects and effects of slightly increased androgens, but these explanations do not easily fit the data. The most likely reasons for the apparent IQ advantage in patients with CAH and their unaffected siblings are methodologic, that is, outdated test norms or selective sampling. Children from families with a low IQ may not receive good medical care and may die before diagnosis.[74] Families with a higher than average IQ may be treated at university-affiliated facilities where most studies are conducted. Many, although not all, participating families have had an above average socioeconomic status. In support of the possibility of selective sampling in clinic-based studies, two recent studies of representative samples did not find IQ elevations in either patients with CAH or their siblings.[38, 45] The best test of the sampling explanation will come from studies of children with CAH detected through newborn screening, who represent the population, rather than a sample, of patients with CAH.[85]

Impaired Intelligence in Patients with the Salt-Wasting Form

Rather than having an IQ advantage, patients with CAH have been suggested to have some cognitive compromise caused by complications from the disease. Nass and Baker[64] hypothesized that cognitive impairments could occur in patients with salt-wasting CAH as a result of

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hypotension, dehydration, and hyponatremia. A few studies have found that patients with salt-wasting CAH have lower IQs than patients with simple-virilizing CAH.[38, 64] There are similar trends in other studies,[57, 62, 89] which were not significant, perhaps owing to small effects, large variability among patients, and low statistical power with small samples. Nevertheless, it seems unlikely that salt-wasting status alone is associated with a substantially lowered IQ, given other studies showing an above average IQ in groups of patients with CAH, the majority of whom had salt-wasting CAH.

It is possible that the IQ will be impaired in children with uncontrolled CAH, especially with frequent salt-wasting or hypoglycemic episodes. It is also likely that any intellectual impairment associated with salt-wasting symptoms will be greater in males than in females with salt-wasting CAH, because boys are generally identified through salt-wasting crises, whereas girls are likely to be detected by their ambiguous genitalia.[84] Previous studies have been unable to assess these possibilities because of small sample size and limited medical information. The issue of cognitive compromise in children with salt-wasting CAH is worthy of study.

Elevated Rates of Learning Disabilities or Differential Cognitive Deficits

There has been recent interest in the possibility that children who have CAH are at increased risk for learning disabilities when compared with children who do not have CAH. The basis for this suggestion comes from two different perspectives. The first perspective represents a variant of the hypothesis that patients with CAH are more likely to have lower IQs than unaffected relatives as a result of brain insult from disease complications. Some of these illness factors, such as hypotension and electrolyte imbalance, are limited to patients with salt-wasting CAH, whereas others, such as hypoglycemia, may occur in patients with the salt-wasting or simple-virilizing form. Males and females sustain these complications; therefore, increased learning disabilities would be expected in both sexes, although males may be particularly predisposed because they may be more ill than females early in life.

The second perspective on learning disabilities in patients with CAH derives from the hypothesis that the male predominance among the population of children with learning disabilities results from exposure to high levels of androgens during prenatal development. Although cognitive effects of androgens have been considered for many years, the topic was popularized by Geschwind and Galaburda[32] and specifically applied to learning disabilities. They hypothesized that prenatal testosterone has differential effects on the two cerebral hemispheres (retarding development of the left and enhancing development of the right), lead-

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ing to learning disabilities and left-handedness. Extensive research in non-CAH samples has tested these hypotheses regarding associations among left-handedness, learning disabilities, and immune dysfunction, most of which have not been confirmed.[18, 56] According to theories of androgen effects on behavior (proposed by Geschwind and Galaburda or others), only females with CAH would be at increased risk for learning disability as a result of their exposure to above average levels of androgens during prenatal development. Males with CAH should have a rate of learning disabilities similar to that of males without CAH because they are already exposed to high levels of androgens (not different from typical males).[70] Males with and without CAH and females with CAH should all be more likely to have a learning disability than females without CAH.

The results of three studies have been interpreted to indicate that patients with CAH are at increased risk for learning disabilities,[26, 65, 72] whereas two studies suggest that they are not.[71, 83] The methodology in all of these studies (positive and negative) limits the interpretation of the results. The primary concern is that because most of the studies failed to include a standard psychoeducational assessment of learning disabilities, it is unclear whether the observed differences reflect some other behavioral problem (such as a low IQ or altered profile of cognitive abilities) or rater bias. The one study with targeted assessment[71] is dated and used measures that are not as diagnostic as contemporary tests. Other concerns in some of the studies include inappropriate or absent comparison groups, the failure to separate males and females, and the lack of formal statistical analyses.

Four studies were designed to test androgen effects on learning disabilities. Three studies appropriately focused on the question in females or separated the sexes for analysis,[65, 71, 83] whereas the other did not separate the sexes.[72] In one early study, there was no evidence of learning disabilities in girls with CAH,[71] but the sample was small and the measures less diagnostic than tests currently available. In a more recent study, females with CAH were found to have a discrepancy in verbal and performance IQ considered indicative of a learning disability,[65] but there is reason to question the diagnostic validity of a discrepancy score,[61] especially because the high prenatal androgen levels characteristic of females with CAH would be expected to produce enhanced spatial ability, which is a component of performance IQ. The replicability of this finding can be assessed in studies examining IQ but not designed to look at learning disabilities per se. The finding of a relatively higher performance than verbal IQ has been replicated by some researchers[36, 45, 74] but not others.[3, 49, 57, 83] It is unclear what factors account for the discrepant findings, but they may include the overall IQ of the study participants (a higher IQ is generally associated with a higher verbal than performance IQ), the proportion who have salt-wasting CAH, and the proportion who have serious complications from the disease.

Another study on learning disabilities focused on illness characteristics (not androgen) in relation to the assumed high incidence of learning

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difficulties,[26] but there was no comparison group to establish that the incidence was, in fact, significantly higher than what would be expected. The investigators made good use of the variation within their sample of learning difficulties and disease complications to examine possible sources of the learning problems. When compared with children without problems, children with physician-reported learning difficulties had lower blood glucose at presentation, a greater incidence of convulsions owing to documented hypoglycemia, and worse growth in the first year of life; they were also more ill at presentation, but the differences in the incidence of presenting features were not significant. These results suggest that learning difficulties might occur in children with severe and poorly treated CAH but not in most patients.

Overall, problems inherent in the studies on learning disabilities make it difficult to determine whether children with CAH are truly at increased risk for a learning disability when compared with their unaffected relatives. Even if the differences observed in all studies are "real," it is unclear what they mean. It is not known whether the differences reported actually reflect lower intellectual abilities in unselected samples of patients with CAH when compared with unaffected siblings secondary to disease complications, or a true learning disability (which occurs in the presence of normal intelligence). It is unlikely that the differences reflect androgen effects, given the fact that apparent sex differences in at least some learning disabilities reflect referral bias rather than true sex differences in prevalence.[82] Further study is necessary to document the nature of the differences, and several factors need to be considered. Because the population base rate of diagnosable learning disabilities is relatively low, large samples are needed to see differences between patients and controls. It may be more reasonable to look at variations in school achievement, rather than the categorical diagnosis of learning disability. An assessment of learning disabilities requires specific and detailed evaluation, which is costly and time consuming. To understand the causes of any increased learning disability, there needs to be good documentation of medical history, especially with respect to disease control, including the symptoms of salt loss and hypoglycemia. Such record keeping is not always easy to accomplish, especially in the United States where patients often change clinics frequently during childhood. If at least some of the learning disabilities result from late diagnosis and poor treatment, problems are less likely to exist now than in the past, especially with the advent of newborn screening for CAH.[85]

Even if children with CAH are not at increased risk for learning disabilities, they may have specific cognitive or learning deficits that reflect consequences of the disease or its treatment. These deficits would occur in both sexes but might be more common in males than in females. Their relation to disease would depend on their cause (e.g., from salt-wasting versus hypoglycemic events). Deficits would be expected to relate to the differential vulnerability of different brain systems at different points in development.

One specific deficit in number or computation ability has been

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reported[3] but generally not confirmed.[38, 71, 75] It seems unlikely that nonreplications reflect methodologic differences (e.g., test used, subject age) because there is no theoretic reason to expect a selective deficit in number ability and no post hoc explanation of it.

Studies addressing the question of learning disabilities or differential cognitive impairments in patients with CAH might use as a model the studies conducted by Rovet and colleagues[78, 79] in children with congenital hypothyroidism. Children with congenital hypothyroidism who are detected and treated early in life have IQs that are within the normal range but 5 to 10 points lower than the IQs of controls, with the degree of deficit depending on disease and treatment factors reflecting the degree of thyroid deficiency (e.g., disease severity, etiology, starting dose of thyroxine). Even treated children with congenital hypothyroidism who have normal IQs have delays or deficits in specific abilities, and these deficits are differentially associated with the timing of thyroid deficiency, reflecting the timetable of brain development. Children who have CAH are unlikely to have deficits as severe as those seen in children with congenital hypothyroidism, but they could be evaluated with some of the same tests to determine whether they are at risk for specific learning or cognitive disabilities that might be related to disease complications.

Enhanced Spatial Abilities in Female Patients Owing to Excess Androgens

There is good reason to expect that female patients with CAH will show alterations in the pattern of cognitive abilities. These hypothesized variations reflect the fact that there are population sex differences in the pattern of cognitive abilities and the likelihood that, in humans as in other species, behavioral and neural sex differences are influenced by androgens acting on the developing brain during prenatal life and perhaps continuing to act on the brain later in life. Sex differences in cognitive abilities are well documented.[35, 46] On average, men and boys are better than women and girls in mathematical reasoning and spatial ability, especially three-dimensional rotation, whereas women and girls are better than men and boys in aspects of verbal ability (especially fluency), memory, perceptual speed, and decoding of emotions.

Many studies in a variety of nonhuman mammalian species, including rodents and primates, have shown that sex hormones have a major effect on the development of sex differences in the brain and in behaviors as diverse as sexual behavior, rough play, aggression, maternal behavior, and spatial learning.[1, 5, 6, 33] Regions of the rat brain thought to subserve aspects of spatial learning, including the hippocampus, have been shown to be affected by sex hormones.[43, 77, 90] In rhesus monkeys, androgens present in the prenatal or postnatal periods affect learning abilities that

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show sex differences and the maturation of the cortical regions that subserve these abilities.[2, 19]

Females who have CAH provide a unique opportunity to study whether early androgens have similar effects on human brain and behavior. Females with CAH are masculinized and defeminized in a variety of ways.[9, 60] When compared with their sisters or other same-sex controls, females who have CAH engage in more male-typical childhood play[12, 15, 24, 28]; have more male-typical interests in adolescence[8]; are more likely to report the use of physical aggression in conflict situations[14]; are less interested in infants,[48] marriage, motherhood, and feminine appearance[24, 28]; score lower on measures related to empathy, intimacy, the need for social relations, maternal/nurturant behavior, and succorance[39, 74]; and are less likely to engage in heterosexual activity and more likely to fantasize about, and be aroused by, other women.[95] Females with CAH are not sex atypical in all ways. For example, most have female-typical gender identity.[28, 95] The reader is referred to the article by Meyer-Bahlburg elsewhere in this issue for a detailed discussion of these issues.

Given the pattern of sex differences in cognition, females who have CAH should have differential cognitive enhancements and reductions if these abilities are influenced by the amount of androgen present during prenatal or neonatal development. When compared with their unaffected sisters, females with CAH should have relatively high scores on spatial, mathematical, and mechanical abilities and relatively low scores on verbal fluency, memory, perceptual speed, and emotion decoding.

Several studies have addressed the effects of androgen on cognition in females with CAH.[3, 36, 38, 53, 58, 71, 75, 83] Two studies were specifically designed to test the hypothesis that spatial abilities would be higher (and other female-superior abilities lower) in females with CAH when compared with control females.[36, 75] Several features of these studies maximized their ability to test the hypothesis. The cognitive tests were chosen on the basis of known population sex differences in the age range of the study participants; the studies assessed sex differences in control males and females; the control subjects were siblings; patients and siblings were compared separately by sex; and there were control tasks of general intelligence (on which differences were not expected).

Females with CAH were found to have better spatial abilities than unaffected female relatives in childhood[36] and in adolescence and adulthood.[75] The differences were almost as large as the sex differences. The cognitive advantage was unique to spatial ability; female patients and controls had similar overall intelligence and similar (or lower) scores on other cognitive abilities.

The results of other studies examining specific cognitive abilities in patients with CAH are difficult to interpret. In most of these studies, the groups were not found to be significantly different. The primary problems in these studies (and the reasons why they did not find differences) concern the use of cognitive tests that do not show sex differences and small samples, producing low statistical power. Sex differences in cognitive abilities are moderate to large in size (0.3 to 1 standard devia-

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tions) when compared with the very large sex differences in activities and interests (2 to 3 standard deviations) and sexual orientation (5 to 6 standard deviations), and the differences between patients with CAH and controls are likely to be smaller than the sex differences.[9] It is easier to find differences between females with CAH and female controls in interests and sexual behavior[9, 60] than in cognitive abilities. Studies assessing cognitive abilities generally do not have sufficient statistical power to detect differences between patients and controls.

Other methodologic limitations in studies of specific cognitive abilities in patients with CAH make conclusions difficult. In one study,[38] patients had lower overall intelligence than controls, making it difficult to detect enhancement of specific abilities. In other studies,[53, 72] groups were different on measures that do not show sex differences (the differences probably reflect type I error or effects owing to other aspects of CAH or its treatment), or males and female patients were not considered separately (so the differences between female patients and controls would be confounded with similarities between male patients and controls).

It is difficult to interpret the studies with small samples that did not find differences, and it is reasonable to give more weight to the studies that did find differences. The latter studies used measures that showed big sex differences. The differences that were found between females with CAH and control females were specific and were hypothesized. These hypotheses were based on similar evidence from other species and are consistent with other findings of behavioral masculinization in females with CAH.[9, 60] Findings of enhanced spatial ability in females with CAH are consistent with evidence from two other sources regarding the effects of androgens on spatial ability. First, males with low early androgen levels (owing to idiopathic hypogonadotropic hypogonadism) have lower spatial ability than do controls,[42] which seems to reflect reduced androgen levels early in development. Spatial ability correlates with testicular volume, does not improve with androgen replacement in males with idiopathic hypogonadotropic hypogonadism, and is not reduced in males with acquired (late-onset) hypogonadism.[42] Second, spatial ability in a sample of 7-year-old girls was related to their testosterone levels in utero (assessed in amniotic fluid at 14–16 weeks' gestation). Girls with high levels of prenatal testosterone had faster mental rotation (an aspect of spatial ability) than girls who had low levels.[34] These confirming studies support the notion that enhanced spatial ability in females with CAH results directly from effects of androgens on the developing brain and not from social responses to the girls' virilized genitalia or other abnormalities of the disease. For a detailed discussion of these alternative explanations, the reader is referred elsewhere.[9, 36, 73]

Additional study might elucidate the nature of the spatial advantage in females with CAH. It would be valuable to understand the basic cognitive processes involved in superior performance on standard spatial tasks and to determine the relative influence of prenatal versus

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postnatal androgen excess. Currently, it is unknown whether spatial ability is linearly related to the degree of prenatal androgen exposure, as has been found for childhood toy play.[11, 69] It is also unclear whether spatial ability is increased by later androgen exposure (e.g., with undertreatment), as might be expected from data in typical women showing positive correlations between testosterone and spatial ability.[47]

There is little evidence to indicate whether females with CAH perform more poorly than their unaffected female relatives on aspects of cognition that typically show female superiority and that are hypothesized to be inhibited by prenatal androgens. The single finding of reduced perceptual speed in girls with CAH[36] requires replication, especially because the reduction was also observed in boys with CAH, and because the expected sex difference (female superiority) in control children was not seen. Sex differences in memory and emotion have only recently been empirically documented, and there have been no studies of those abilities in females with CAH. Such studies will be challenging to perform because sex differences in these abilities are smaller than those in spatial ability, and large samples (more than 50 per group) will be necessary to ensure power to detect any effects of early androgens.

Some data suggest that males with CAH have lower spatial abilities than their unaffected male relatives (significantly so in one study,[36] but not in the other[75]). There are at least two viable explanations for this result. Studies in male rodents show that high levels of early androgens may sometimes produce demasculinization, although they often have no effect.[4, 23] Males with CAH may be sex typical in many ways[8, 9, 14, 15] but sex atypical in some aspects of cognition. Alternatively, their reduced spatial ability may reflect consequences of the disease (which are likely to be more severe in males than in females).

Cognitive Effects Owing to Other Hormonal Abnormalities

Several other hormones are abnormal in CAH and might be expected to affect psychologic functioning, including cognition. ACTH is increased in untreated CAH, in the salt-wasting and simple-virilizing forms, in males and females. ACTH might have permanent effects on behavior through prenatal effects on the developing brain or acute effects on behavior in later life. With respect to the cognitive consequences of prenatal ACTH increases, prenatal stress is associated with elevations in ACTH, and it is reasonable that high levels of ACTH during early development might produce some of the same adverse effects as prenatal stress. Changes could occur to the hippocampus, with sequelae for learning and memory. There have been no studies in patients with CAH to test this possibility.

With respect to the possible cognitive consequences of acute elevations of ACTH, some evidence suggests that ACTH may facilitate some

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aspects of attention and memory.[16] In the only relevant study in patients with CAH, elevated ACTH was associated with a faster reaction time, considered to reflect enhanced stimulus encoding or overt responding.[87] This finding was confirmed in two sets of within-subject analyses. Correlations were seen between reaction time and endogenous ACTH level, and reaction time differed during two treatment phases: on cortisol replacement (low ACTH) and off medication (high ACTH). ACTH levels were not associated with other measures of visual and verbal attention or memory. This study is intriguing, but given the small sample size (six patients), it needs to be replicated. It also should be extended to delineate the selective nature of ACTH effects and some proposed moderators of the effects (including sex and disease characteristics).

Cognitive Impairments Secondary to Glucocorticoid Excess

Recent studies on the adverse effects of glucocorticoids raise the possibility that the treatment of CAH might produce cognitive impairments. It is likely that the nature and degree of effects depend on the timing and extent of exposure (i.e., neonatal versus later postnatal, ongoing versus transient).

Studies in nonhuman species indicate that excess glucocorticoids during early development or later in life can have adverse behavioral effects, including impaired learning and memory and heightened responsivity to stress, and that these behavioral effects are mediated by glucocorticoid effects on the hippocampus.[51, 54] Accumulating evidence suggests that glucocorticoids have similar adverse effects in humans.[17, 51, 52, 54, 80] Although memory and attention seem to be most affected by glucocorticoids, other cognitive functions may be impaired. These effects occur with corticosteroids from exogenous[44, 63, 93, 94] and endogenous sources (Cushing's syndrome[31]).[51, 52]

It is possible that patients with CAH might have subtle cognitive or mood dysfunctions secondary to their treatment. It is unlikely that the impairments would be severe; otherwise, they probably would have been noticed. Although treatment is intended to normalize cortisol levels, it is difficult to achieve true physiologic replacement,[59] and the focus on reducing levels of 17-hydroxyprogesterone may result in excess glucocorticoid exposure and adrenal oversuppression.[40, 59] No studies have been designed to assess cognitive or affective changes in patients with CAH in relation to glucocorticoid excess, and it is difficult to obtain this information from published studies.

The accumulating evidence about the adverse effects of glucocorticoids makes it important to study specific behavioral changes in patients with CAH that result not just from the disease (prenatal androgens or postnatal salt-wasting or hypoglycemic episodes) but from the consequences of standard treatment of the disease. Such studies should include children with severe forms of CAH, some of whom may be treated

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with excessive doses of glucocorticoid.[40, 59] They should also include children with mild forms of CAH who are detected earlier and treated more aggressively and at earlier ages than in the past, particularly when newborn screening for CAH is available.[85] Given the possibility that memory could be impaired by other aspects of the disease (e.g., increased ACTH, prenatal androgens in females with CAH), it may be difficult to determine when deficits directly reflect glucocorticoid effects; therefore, it is important to detail associations between treatment and cognition. It will be interesting to see whether these deficits are not found when other treatment regimens are used.[59] Given the behavioral effects of prenatal glucocorticoid excess, it is important to study cognition in children at risk for CAH who are treated with dexamethasone in utero[30, 68] and in the offspring of women with CAH in relation to the treatment received during pregnancy.[50]

NEURAL SUBSTRATES OF COGNITIVE CHANGES IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

The neural substrates of the cognitive changes in patients with CAH are currently unknown, but studies in other species and from other areas of research in humans suggest some possibilities that could be tested empirically. Active investigation in this area should produce answers to some of these questions in the near future.

Neural Mechanisms for Androgen Effects

There is considerable interest in documenting sex differences in the human brain and in determining how these brain differences underlie behavioral sex differences and are affected by sex steroid hormones. This work follows logically from studies in other species, in which a substantial number of brain sex differences have been described and related to behavior and to exposure to sex steroid hormones. For example, sex differences in spatial learning in rats may be mediated by early androgen effects on the developing hippocampus.[43, 77] In other species, gonadal hormones have been shown to affect neuronal size, survival, and outgrowth; synapse number and organization; dendritic branching patterns; gross nuclear volume; cortical thickness; and neurotransmitter systems.

A variety of sex differences have been reported in human brain structure and function and have been suggested to underlie sex differences in cognition.[27, 29, 76] In terms of structure, for example, the splenium of the corpus callosum has been reported to be wider and more bulbous in females than in males,[22] and the relative size of the language areas larger.[37] Females have been reported to have greater neuronal density than males in regions of the temporal lobe known to subserve language

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functions.[92] Functional sex differences have been observed with brain imaging techniques during the performance of simple cognitive tasks. For example, during the processing of rhymes, females have been reported to activate the left and right inferior frontal gyrus regions, whereas males activate only the left.[81] Sex differences in cerebral hemispheric specialization (lateralization) have been observed with noninvasive measures in typical samples of normal individuals.[29, 76, 91]

The results of studies of structural and functional sex differences are exciting and have encouraged investigators to examine these differences in patients, especially females, with CAH (D. Merke, MD, personal communication, May 2000).[72] For several reasons, these studies have not yet been informative. It has proven difficult to replicate results regarding sex differences, which may reflect statistical issues associated with the methods and samples typically used in imaging studies. Many regions of the brain are examined in imaging studies, and it is likely that some of these areas will show sex differences in a given sample simply as a result of sampling fluctuation and multiple comparisons. Nonreplications may result from low statistical power; even moderate-to-large differences require larger samples than is usually feasible in imaging studies.

Brain sex differences have not been specifically found to relate to behavioral sex differences. For example, sex differences in cortical activation while subjects solve a rhyming task do not translate into sex differences in performance. This observation may result, in part, from the fact that the tasks used in imaging studies are simple, producing little variation across participants. Nevertheless, other factors are involved because this problem is not unique in human studies. Studies in rodents show that there may be no behavioral effect of destroying regions that show sex differences and are sensitive to early androgens.

The relations between variations in brain structure/function and variations in cognition are complex. There is fairly good evidence documenting the brain regions that subserve some of the cognitive abilities that show sex differences and that are likely to be affected by early hormones. For example, spatial abilities involve frontal and parietal structures; verbal memory involves the left temporal lobe, particularly the hippocampus; verbal fluency involves left frontal regions; and decoding of emotion depends on the right hemisphere. It has been difficult to show that individual differences in these regions are associated with individual differences in behavior. Consider two examples. In imaging studies, increased activation of a particular area has been associated with abilities subserved by that area in positive and negative ways. Enhanced performance with increased activation is interpreted to reflect better brain functioning associated with better performance, whereas reduced performance with increased activation is interpreted to reflect the need for greater effort in persons with low ability. Despite compelling evidence that the hippocampus is crucially involved in aspects of verbal memory that show sex differences, removing the hippocampus does not eliminate the sex difference.[10]

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One sex-differentiated aspect of brain function has been studied in patients with CAH—lateralization as assessed by handedness and hemispheric specialization. Left-handedness is generally associated with reduced lateralization for language and is slightly more common in males than in females. Consistent with an androgen effect on handedness, females with CAH have been reported to be more likely to be left-handed than their unaffected female relatives, although there is large variability among patients.[66] The sex difference in handedness is small, as is the difference between female patients with CAH and controls; therefore, the difference is difficult to detect in the small-to-moderate samples typically studied. In fact, in the initial report, increased left-handedness in females with CAH was only found when sister pairs were analyzed,[66] and the power increased. It has been difficult to replicate the finding of increased left-handedness in females with CAH.[41, 74] Even apparent replications are difficult to interpret[45, 86] because males and females are not considered separately. Increased left-handedness in males with CAH would not likely result from androgen exposure but might reflect brain damage associated with the disease.

Lateralization in patients with CAH has also been studied with measures of perceptual asymmetries (generally, visual field asymmetries and ear asymmetries under conditions of dichotic listening), which show small-to-moderate sex differences. The results have been inconsistent, probably because the studies do not have sufficient statistical power to detect what are likely to be small effects.[41, 45, 91] Given the fact that lateralization differences (sex differences and CAH–control female differences) are small, these differences cannot account for differences in patterns of cognitive abilities. The two different aspects of lateralization studied—handedness and cerebral hemispheric specialization—show opposite patterns of sex differences. Males are less lateralized in their handedness but more lateralized in their hemispheric specialization. Any relations among androgens, handedness, and brain asymmetry are complex.

Neural Effects Owing to the Disease or Its Treatment

Increasing attention is focused on brain changes in patients with CAH that might reflect complications of the disease or its treatment. In contrast to the effects associated with androgens, these changes would be equally common in males and females with CAH, or perhaps more common in males with CAH whose diagnosis may be delayed and who may present with more severe disease. Brain damage caused by hypotension and hyponatremia would clearly be expected to be higher in patients with the salt-wasting versus simple virilizing form of disease. Damage caused by hypoglycemia would be expected in patients with salt-wasting or simple virilizing CAH. Damage resulting directly from steroid treatment (hypothesized to include myelin or temporal lobe changes[67]) would also be expected in patients with the salt-wasting or

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simple virilizing form, although damage might be greater in patients with salt-wasting CAH because they are likely to be treated with high doses of glucocorticoid.

In two studies, patients with CAH had an increased frequency of white-matter abnormalities[67, 83] (although the latter study did not conduct formal statistical analyses) and temporal lobe atrophy.[67] In neither study could the abnormalities be related to clinical characteristics. This failure to see associations may reflect the relatively small samples (39 and 19 patients) and the difficulty in obtaining reliable information about past events.

Additional research should indicate whether patients with CAH have damage in brain regions known to be affected by glucocorticoids[51, 52, 54, 80] and whether brain changes are related to indicators of treatment and to performance on cognitive abilities known to be subserved by those regions. For example, it might be hypothesized that patients who have been consistently overtreated would show changes in the hippocampus that would correlate with impairments in verbal memory.

SUMMARY

Cognition in patients with CAH has not been as well studied as other aspects of psychologic function.[9, 60] Nevertheless, it is possible to make some conclusions and to offer a number of hypotheses for further study (Table 1). First, patients with CAH do not seem to have an overall intellectual advantage as a direct consequence of the disease. The high IQs reported in some groups of patients with CAH are also reported in their siblings and probably reflect sampling bias. Second, it is possible that, on average, patients with salt-wasting CAH have lower overall ability than patients with the simple-virilizing form, but both groups are well within the normal range, and there is considerable variability among both groups. Third, the evidence to date does not confirm that patients with CAH are more likely to have diagnosable learning disabilities when compared with their unaffected relatives, but this issue has not been well studied with the appropriate psychoeducational assessments. It is unlikely that patients with CAH are at substantially increased risk for frank learning disabilities, but they may be likely to have problems in specific areas. Fourth, females with CAH seem to have enhanced spatial ability as a result of exposure to high levels of androgens early in development. The neural substrate of this advantage is unknown but a subject of active research. It is unclear whether when compared with their unaffected siblings, females with CAH are better in other abilities that are typically performed best by males or worse in abilities typically performed best by females. Fifth, it is likely that patients with CAH have other cognitive changes as a consequence of disease characteristics (besides androgens) and of the treatment of the disease. Some evidence suggests that patients with CAH are more likely to have white-matter brain changes produced by the disease and its

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Table 1. SUMMARY OF CONFIRMED AND HYPOTHESIZED COGNITIVE CHANGES IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA AND THEIR CAUSES

Cognitive Change	Subgroups	Confirmed?	Hypothesized Cause
High intelligence		High also in sibs	Sample bias
Slightly reduced intelligence	SW-CAH	Likely in some patients	Disease complications
Learning disabilities	SW > SV	No	
Specific disabilities		Possible	Disease complications
Increased spatial ability	Females only	Yes	Prenatal androgens
Reduced verbal fluency, memory, emotion decoding	Females only	Hypothesized	Prenatal androgens
Impaired memory		Hypothesized	Excess glucocorticoid (hippocampus)
Attentional changes		Hypothesized, confirmed in one study	Effects of ACTH

SW-CAH = Salt-wasting congenital adrenal hyperplasia; SV = simple virilizing.

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treatment. This has not been well studied but should be because of the potential clinical implications. It is reasonable to hypothesize that there will be cognitive changes that reflect effects of undertreatment (e.g., ACTH effects on attention) and other changes that reflect effects of overtreatment (e.g., glucocorticoid effects on memory). Some of these effects may be transient, reflecting acute brain changes, whereas others may become chronic as a result of permanent brain changes with repeated exposure.

There is need for continuing study of cognition in patients with CAH. Such studies will provide basic information about hormonal effects on cognition and the neural mechanisms that mediate those effects. They will also provide important clinical information to guide psychologic and medical treatment of patients.

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CONGENITAL ADRENAL HYPERPLASIA OWING TO 21-HYDROXYLASE DEFICIENCY

Growth, Development, and Therapeutic Considerations

Claude J. Migeon, MD, and Amy B. Wisniewski, PhD

Congenital adrenal hyperplasia (CAH) owing to 21-hydroxylase deficiency is a disorder that results in decreased biosynthesis of cortisol and, in some cases, aldosterone. CAH also results in increased secretion of the anabolic steroid androstenedione and the mildly salt-wasting steroids progesterone and 17-hydroxyprogesterone.[7]

The goal of treatment is to replace the hormones a patient is missing and to suppress the hormones produced in excess. Both the lack of therapy and overtreatment can result in major problems with growth.

This article describes the specific steroids relevant to untreated patients with CAH that are important to growth and optimal hormone treatment. Long-range follow-up of growth in adults is reported.

This work was supported by a grant from the Genentech Foundation for Growth and Development (98-33C to CJM), National Institutes of Health (NIH) National Research Service Award F32HD08544 (to ABW), and by NIH, National Center for Research Resources, General Clinical Research Center Grant RR-00052.

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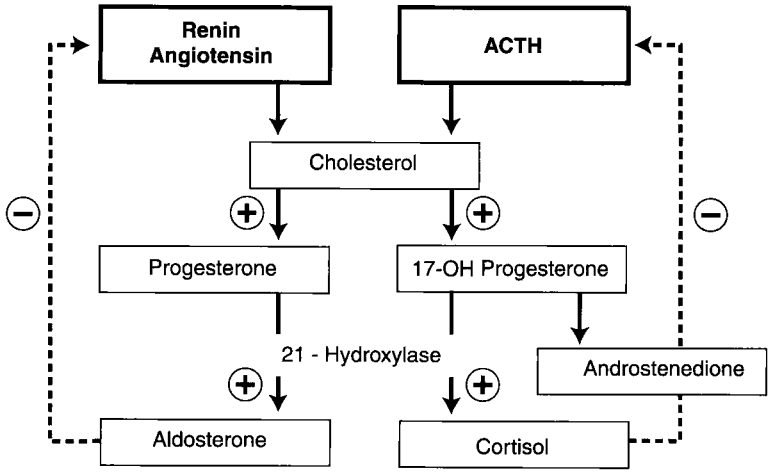

Figure 1. Cortisol and aldosterone production. ACTH = adrenocorticotropic hormone; OH = hydroxy.

BASAL STEROID IMBALANCES ASSOCIATED WITH CONGENITAL ADRENAL HYPERPLASIA

Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency is the most common form, representing more than 90% of cases. It is the only form discussed in this article. Decreased or absent cortisol secretion results in decreased negative feedback of the hypothalamic-pituitary axis, which, in turn, leads to increased secretion of corticotropin-releasing hormone (CRH) and corticotropin (ACTH). Increased ACTH is responsible for hyperplasia of the adrenal cortex and increased production of cortisol precursors, particularly 17-hydroxyprogesterone, progesterone, and androstenedione (Fig. 1).

The elevated secretion of 17-hydroxyprogesterone and progesterone produces a salt-losing tendency.[15] If the 21-hydroxylase deficiency is partial, the adrenal cortex can increase aldosterone secretion to compensate for the salt loss (simple virilizing form of CAH). In contrast, if the 21-hydroxylase deficiency is complete, no aldosterone is secreted, and a salt-losing crisis develops (salt-losing form of CAH).

In untreated CAH, the deficiency in cortisol and aldosterone produces abnormalities of gluconeogenesis and electrolytes, respectively, as seen in Addison's disease. The overall effect is negative as far as growth is concerned. At the same time, increased secretion of androstenedione, 10% of which is metabolized to testosterone, is expected to result in marked anabolism.

MODALITIES OF GLUCOCORTICOID REPLACEMENT

The goal of therapy is to suppress the hyperplastic adrenal glands of patients by suppressing the secretions of CRH and ACTH. This

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suppression is accomplished by administering an appropriate dose of cortisol that will ideally equal the amount secreted over a normal 24-hour period by the patient. Investigators have attempted to determine the daily cortisol secretion rate of normal individuals and have used these values to determine optimal replacement rates for patients with CAH.

Multiple methods have been used to determine normal rates of cortisol secretion. The authors have used a technique called urinary production rate by isotope dilution.[16, 17] Studies of normal subjects of various ages and sizes indicated that secretion rates were similar across subjects when corrected for variation in body surface area. The mean cortisol secretion rate plus or minus one standard deviation (SD) was determined to be 12 ± 2 mg/m²/24 h with this technique. Of note is the large standard deviation observed; the third to 97th percentile values ranged from 8 to 16 mg/m²/24 h. An additional problem was associated with the circadian variation of the clearance rate of cortisol.[22] This variation contributed an overestimation of 1.4 to 3.8 mg/m²/24 h of the original value. A more accurate secretion rate was approximately 10 mg/m²/24 h.

Zumoff and co-workers[45] compared various methods of determining rates of cortisol secretion. Specifically, urinary production rates were compared with plasma production rates. The plasma methods were found to yield 10% to 25% lower estimates than the urinary methods.

More recently, cortisol production has been investigated using stable isotope dilution/mass spectrometry.[8] With that method, values for 12 normal subjects were 5.7 ± 1.5 mg/m²/24 h. Kerrigan and co-workers[18] reported that deconvolution analysis resulted in cortisol secretion values similar to stable isotope dilution/mass spectrometry. A value of 5.7 ± 1.5 mg/m²/24 h was reported for 18 normal males at Tanner stages I to IV. With this type of technique, a slight underestimation (10% to 20%) can occur when plasma endogenous cortisol is so low that it is not detected by laboratory methods. A summary of the literature findings indicates that the mean cortisol secretion rate ranges between 5.7 and 10 mg/m²/24 h.

In addition to the large range of values reported for the normal cortisol secretion rate, one must consider the effect of the route of cortisol administration on the dose needed for therapeutic effects. A perfect administration would result in mimicking the diurnal rhythm of secretion along with its episodic variations and would probably require continuous intravenous administration. Clearly, this type of administration is not feasible, and one must consider alternate routes. Intramuscular preparations are no longer available, leaving only oral preparations of cortisol, cortisone, prednisone, or prednisolone. These oral preparations cannot be expected to reproduce physiologic conditions of secretion. Although physiologic levels of blood cortisol may not be necessary to suppress ACTH secretion, it is certain that oral replacement administered every 8 or 12 hours will not reproduce normal blood levels.

It is also necessary to consider the ability of gastric acidity to destroy cortisol partially. Experience with addisonian subjects suggests that

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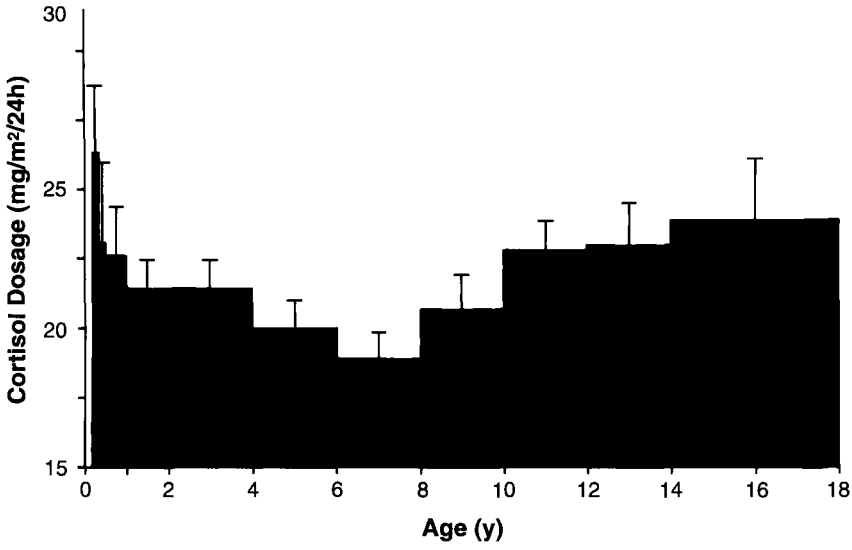

Figure 2. Variation (mean ± SEM) in the cortisol dosage with age in patients with congenital adrenal hyperplasia. (*From* Sandrini R, Jospe N, Migeon CJ: Temporal and individual variations in the dose of glucocorticoid used for the treatment of salt-losing congenital virilizing adrenal hyperplasia due to 21-hydroxylase deficiency. Acta Paediatr (Suppl) 388; 56–60, 1993; with permission.)

about one-half of cortisol administered orally actually reaches the blood; therefore, oral glucocorticoid replacement must exceed the calculated normal daily production rate.

Cortisol blood levels double or triple at times of stress; therefore, it is recommended that basal cortisol doses be increased in infections with fever. Such infections can occur frequently during the first 2 to 3 years of life and probably result in higher replacement doses administered at that period of development (Fig. 2).

The previous discussion of the difficulties intrinsic to determining the appropriate dose of oral glucocorticoid replacement illustrates the types of problems that may occur in the treatment of patients with CAH. For practical purposes, a dose is elected as a starting level, and adjustments are made by monitoring adrenal suppression and somatic growth.

MODALITIES OF MINERALOCORTICOID REPLACEMENT

Aldosterone secretion at various ages is rather constant throughout life, independent of body size.[29] In fact, aldosterone secretion is somewhat greater during the first year of life than at any other time. The

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proportionally high levels in infancy have been explained by the fact that the newborn kidney is partially resistant to mineralocorticoids. Additionally, infant diets contain little sodium, usually 9 mEq/d.

Only one preparation for salt retention is available, 9∝-fluorocortisol acetate (Florinef acetate), prescribed at a dose of 0.05 to 0.15 mg orally usually once daily. In contrast to the variation of cortisol dose with body size, a continuous dose of Florinef is usually given throughout life in salt-losing patients.

MONITORING TREATMENT

Because of the difficulties in determining the theoretically optimal dose of cortisol, physicians must determine the appropriate replacement dose by monitoring growth rate and adrenal suppression. Measuring appropriate growth presents a problem because infants usually do not reach their growth-rate centile until 9 to 18 months of age. After that time, a normal child is expected to continue growth at the same percentile of the normal height curve. A reasonable approach to monitoring treatment is to maintain the child at the length percentile calculated from the midparental height. At the same time, the pediatrician should attempt to maintain the bone age between ± 1 SD for age.

Simultaneously, blood steroids should be checked every 3 to 4 months. Before puberty, plasma androstenedione varies by age and pubertal status but generally is maintained at 20 to 50 ng/dL, whereas 17-hydroxyprogesterone is maintained at 500 to 1000 ng/dL. Experience has shown that full suppression of these steroids can only be obtained with a cortisol dose that results in growth retardation. The mineralocorticoid dose is checked by measuring serum electrolytes and blood pressure. Unfortunately, blood pressure is difficult to check in a crying child.

FINAL ADULT HEIGHT

Most patients who have CAH, regardless of the age at diagnosis or the quality of endocrine treatment, achieve a final adult height that is shorter than predicted from mean parental height. This shorter height has been observed in all medical centers that have observed patients, as reviewed by Blizzard[2] and reported by others.[1, 3, 6, 19, 33, 39] In the authors' clinic, the final adult height for men with CAH was significantly less than the height of the population of American men in general and that of the midparental height (Fig. 3).[41]

Similar observations of less-than-predicted final adult height were true for women with 21-hydroxylase deficiency observed at the authors' center (Fig. 4). Women with salt-losing CAH were slightly, yet significantly, taller than women with the simple virilizing form.[20, 31] Presumably, this difference occurred because female infants who were salt losers exhibited greater masculinization of their external genitalia at birth and

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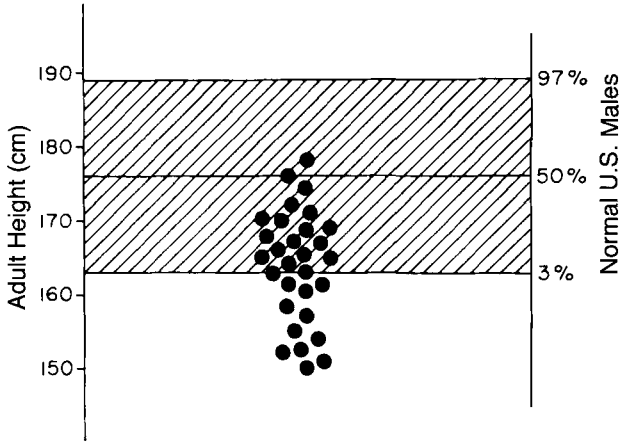

Figure 3. Adult height in men with congenital virilizing adrenal hyperplasia (CVAH). The hatched area represents the mean ± 2 SD for adult height of unaffected American men. (*From* Urban MD, Lee PA, Migeon CJ: Adult height and fertility in men with congenital virilizing adrenal hyperplasia. N Engl J Med 299:1392–1396, 1978; with permission.)

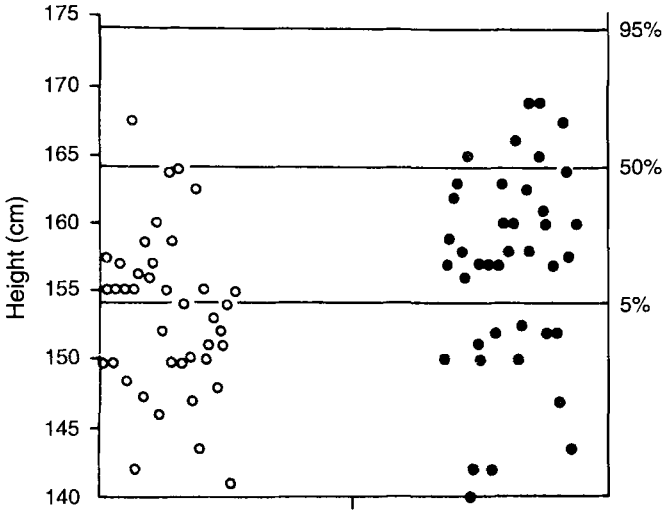

Figure 4. Adult height of 80 women with congenital adrenal hyperplasia. Patients with the simple virilizing form are represented by open circles and those with the salt-losing form, by solid circles. The fifth, fiftieth, and ninety-fifth percentiles for unaffected American women are shown. Average height for patients with salt loss was significantly greater than height for those with simple virilization. (*From* Mulaikal RM, Migeon CJ, Rock JA: Fertility rates in female patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N Engl J Med 316:178–182, 1987; with permission.)

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were diagnosed and treated sooner than infants with the simple virilizing form.

It has been suggested that a high cortisol dose was responsible for the short stature observed in these patients. Indeed, Rappaport and co-workers[33] showed that patients treated with doses of 27 to 55 mg/m^2/24 h did not grow as well as patients receiving 15 to 36 mg/m^2/24 h. Using even smaller doses, Girgis and Winter[9] reported that 15 patients with 21-hydroxylase deficiency who were compliant with treatment had below normal height (Z-score, -0.96) and a delayed mean bone age (Z-score, -1.63). Eleven patients with fair-to-poor compliance had a mean height that did not significantly differ from that of patients who were well treated. These poorly controlled subjects did have slightly advanced bone ages.

In a randomized crossover investigation, the influence on growth of 15 or 25 mg/m^2 of daily oral hydrocortisone supplemented with 0.1 mg/d of flurocortisone, each for a 6-month period, was studied. This study included 26 children with CAH aged 3.5 to 15 years.[37] The doses represented accepted lower and upper limits of oral hydrocortisone treatment, respectively. Greater growth velocity was observed when the children received the 15 mg/m^2 dose of daily oral hydrocortisone, but their 17-hydroxyprogesterone blood concentrations were markedly elevated. The problem with such a study is whether increased anabolic steroids will advance bone age and result in early closure of bone epiphyses.

To alleviate this drawback, a second randomized crossover study involved administration for 6 months of an antiandrogen (flutamide) and an inhibitor of the conversion of androgen to estrogen (testolactone), with either a reduced dose of cortisol (8 mg/m^2/d) or a dose of 12.9 ± 2.1 mg/m^2/24 h.[24] These children were 1 year 10 months to 12 years 2 months old.

Most of the patients studied by Laue and co-workers[25] were salt losers and received larger doses of 9α-fluorocortisol (193 ± 82 μg/d). The growth and bone maturation rates were 8.5 ± 2.3 cm/yr and 1.4 ± 0.9 bone-age year/chronological-age year, respectively, with cortisol treatment alone in contrast to 6.1 ± 1.3 cm/yr with the lower dose of cortisol along with flutamide and testolactone. Treatment with the lower doses of cortisol supplemented with flutamide and testolactone seemed to decrease the rate of growth and bone age maturation. It remains to be seen whether catch-up growth will occur later in this group to improve final adult height.

A Finnish study[14] is of interest because it illustrates that 92 patients with CAH had a mean body length that was greater than average ($+0.8$ SD) at birth but less than average (-1.0 SD) at 1 year of age. The mean adult height for this same group of subjects was less than average for women (-1.0 SD) and men (-0.8 SD). Loss of statural growth occurred for this group in the first 12 months of life. As noted previously (see Fig. 2), during this period, the largest cortisol doses are administered

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owing to frequent neonatal infections and subsequent doubling or tripling of the replacement basal dose.

EFFECTS OF PRENATAL THERAPY ON GROWTH

In a group of children with CAH treated prenatally with dexamethasone, birth weight, birth length, and head circumference did not differ from findings in untreated matched controls.[23] Postnatal height and weight (at 3 months, 1 year, and 4 years of age) and head circumference (at 3 months and 1 year of age) fell within the normal range. Additionally, unaffected children treated prenatally with dexamethasone showed normal growth parameters. These findings suggest that prenatal therapy does not affect somatic growth.

WEIGHT

Changes in height and body composition were evaluated retrospectively in prepubertal boys and girls with CAH between 1 and 10 years of age.[5] Measures of body composition included body mass index (BMI) and *adiposity rebound* (nadir of BMI). The median peak BMI value occurred at 6.8 months of age for subjects with CAH compared with 8 months for unaffected children. Adiposity rebound occurred at 1.74 years for subjects with CAH compared with 5.5 years for controls. This finding is relevant because individuals with early adiposity rebound are at increased risk for obesity.[35] In a separate investigation, males with CAH (aged 8–32 years) had greater fat to lean mass ratios as determined by dual energy x-ray absorptiometry (DXA) when compared with control males.[4]

Weight during childhood was negatively correlated with adult height in 21-hydroxylase–deficient patients.[14, 44] In a clinical trial of young children with CAH, weight velocity decreased when patients received a low dose of hydrocortisone along with flutamide and testolactone compared with conventional treatment.[25]

Twenty-two women with CAH (aged 17–34 years) were compared with unaffected, age-matched women for BMI.[11] The women with CAH had a significantly greater BMI than controls. Concentrations of sex hormone–binding globulins (SHBG) were decreased in obese individuals[42]; however, SHBG concentrations were normal for the participants with CAH despite their increased BMI.

Related to the topic of weight in individuals with CAH is the topic of weight gain in pregnant women treated with dexamethasone to prevent virilization of potentially affected fetuses. Women who receive such treatment gain weight more rapidly during their first trimester of pregnancy when compared with untreated women; however, the total weight gain over the entire pregnancy is not greater in dexamethasone-treated women.[23]

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PENILE LENGTH

Paradoxically, premature exposure to elevated androgen levels leads to micropenis in adult rats.[12, 28] The mechanism underlying short penile length in this animal model is thought to be a decrease of penile androgen receptors. Boys with the simple virilizing form of CAH are often diagnosed after 2 to 3 years of age and are exposed to elevated androgen levels before the start of treatment. This condition may be extended throughout development if the treatment is less than optimal, and it is reasonable to consider adult penile length in this subgroup of patients as it relates to excess androgen exposure.

Two studies have evaluated stretched penile length in men with CAH owing to 21-hydroxylase deficiency. In the first study, 2 of 12 men (17%) had an adult, stretched penile length less than 2.5 SD below the mean.[26] Final adult height for these two men was below the third centile. The remaining 10 men (83%) had adult, stretched penile lengths in the normal range. In a second study, mean stretched penile length was documented in nine men[40] and did not differ from the general population. Correlations between penile length and overall somatic growth with serum androgen levels were not performed in either study. In both studies, final adult height for participants was less than predicted.

BONE MINERAL DENSITY

Bone mineral density (BMD) is increased by excess androgen exposure and decreased by excess glucocorticoid exposure. Whole-body and regional BMD as determined by DXA were investigated in a group of male and female patients with CAH (aged 8–32 years).[4] All of the patients were considered to have received optimal treatment as reflected by 17-hydroxyprogesterone concentrations. Whole-body BMD measures did not differ between patients with CAH and controls; however, men with CAH had lower spinal BMD scores when compared with unaffected men.

A regional BMD study in a larger group of prepubertal adolescent and young adults with the salt-wasting form of CAH revealed no differences in lumbar spine (L2-L4) BMD scores as evaluated by DXA when compared with those of controls.[10] This lack of a group difference in lumbar BMD score was true even for patients who had reached a final adult height that was lower than that for the general population. Similarly, BMD of the total body, lumbar spine (L2-L4), arms, and legs in a group of young adults with 21-hydroxylase deficiency did not differ from that in controls.[30]

Lumbar spine (L2-L4) BMD was assessed in patients (aged 4–22 years) with 21-hydroxylase deficiency in relation to their level of metabolic control with oral cortisol (good, fair, or poor).[9] BMD scores did not differ according to the level of metabolic control, nor were they related to 17-hydroxyprogesterone levels, height, or growth velocity.

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Although multiple studies clearly show that BMD is not adversely affected by cortisol replacement in children and young adults with CAH, concern does exist for older patients. Older adults (aged 16–52 years) with CAH had significantly lower L2-L4 and femoral neck BMD scores when compared with controls.[13] Specifically, all of the subjects with glucocorticoid doses greater than 18 mg/m^2/24 h (hydrocortisone equivalents) had significantly decreased femoral neck BMD scores and patients with doses greater than 20 mg/m^2/24 h had decreased L2-L4 BMD scores. Most of the subjects receiving the highest glucocorticoid dose also received dexamethasone.

Despite the need for life-long hydrocortisone replacement, young patients with 21-hydroxylase deficiency were not at increased risk for low BMD; however, older adults receiving high doses of dexamethasone therapy were reported to have decreased BMD. One should closely monitor bone density in this subgroup of patients.

POSSIBLE MECHANISMS RESPONSIBLE FOR SHORT STATURE IN CONGENITAL ADRENAL HYPERPLASIA

Adrenal Androgens

The main androgen hypersecreted in patients with CAH is androstenedione, an androgen that does not bind to the androgen receptor; therefore, it would not be expected to exert androgenic effects on its own. Nevertheless, 5% to 15% of androstenedione can be metabolized to testosterone and then dihydrotestosterone; hence, untreated subjects grow to be shorter than their expected genetic potential. Lack of androstenedione suppression results in early accelerated growth leading to short adult height.

Case reports of estrogen resistance and aromatase activity defects indicate that androgens alone are not sufficient for epiphyseal fusion.[38] Additionally, the excessive growth rate in boys with familial testotoxicosis, a condition in which androgen concentrations are elevated throughout development, is suppressed by treatment with testolactone (a blocker of androgen-to-estrogen conversion) despite continuation of elevated testosterone concentrations.[24] Estrogens arising from excess adrenal androgen production throughout development may act as a factor leading to premature epiphyseal fusion in boys and girls with CAH.

The lack of effect of age at the initiation of endocrine treatment on the final adult height in men[41] or of the level of metabolic control with oral cortisol on current growth velocity[9] makes it difficult to suggest a major role for adrenal androgens in decreasing the height of patients with CAH. More likely, overtreatment with glucocorticoids during neonatal development compromises growth later in life. This theory is supported by the observation that height at 2 years of age predicts adult height,[9] and by the data of Jääskeläinen and Voutilainen,[14] who reported a significant decrease in height centile during the first year of life.

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Glucocorticoid Therapy

Growth hormone production can be reduced as a result of exposure to excessive amounts of glucocorticoids,[43] and glucocorticoid-induced growth suppression has been reported to be reversible in response to growth hormone treatment.[21] The explanation that the shorter-than-expected adult height of patients with 21-hydroxylase deficiency is caused by altered growth hormone production consequent to hydrocortisone therapy is unsatisfactory because patients with CAH exhibit normal growth hormone responses to arginine and insulin stimulation.[33] This observation is true even for 21-hydroxylase–deficient patients receiving high doses of hydrocortisone. It is unknown whether certain aspects of growth hormone action other than production, such as receptor regulation or signal transduction, are altered in patients with CAH during long-term therapy with hydrocortisone.[34]

In addition to influencing growth hormone production, glucocorticoids can adversely affect growth by suppressing osteoblast function.[27] It is difficult to conclude that this effect occurs in patients with CAH because they show little evidence of decreased BMD scores. Perhaps the anabolic action of the adrenal androgens protects these patients from this side effect.

CAN ADULT HEIGHT BE IMPROVED IN CONGENITAL ADRENAL HYPERPLASIA?

Patients with CAH do not reach their optimal height potential. Absent or inappropriate treatment results in rapid growth but short adult height. Excessive therapy results in impaired development. Various suggestions have been offered for improving the final height of patients with CAH.

Administering an Appropriate Dose of Cortisol

It is surprising how difficult it is to maintain replacement cortisol therapy at a dose that promotes normal growth. Problems arise in determining normal hormone secretion and in attempting to administer replacement in a physiologic fashion. A third consideration is patient compliance for individuals faced with a lifetime of therapy.

Use of Antiandrogens or Androgen-to-Estrogen Conversion Blockers

The use of antiandrogens or androgen-to-estrogen conversion blockers constitutes a theoretically sound approach; however, the use of additional therapeutic agents is not conducive to better compliance. The side effects of prolonged administration of these substances is not known, and occasional hepatic toxicity has been reported. No data are

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available on the adult height of patients who received these experimental therapies.

Adrenalectomy

Because 21-hydroxylase deficiency results in insufficient secretion of cortisol and aldosterone and oversecretion of androgens and salt-wasting hormones, it has been proposed that adrenalectomy would eliminate the hormones produced in excess, making therapy easier for affected individuals. Such an approach does not resolve the difficulty of administering an appropriate dose of replacement cortisol.

Growth Hormone Therapy

In view of the fact that growth hormone secretion has been reported to be normal in patients who have CAH, it is unclear how growth hormone therapy could improve height. Perhaps the limited success obtained with Turner syndrome patients justifies such an experimental protocol. In a review of growth hormone application to novel conditions, Rosenfeld and Buckway[36] report that growth velocity increased in children with CAH treated with growth hormone for 1 to 4 years. Seven subjects received treatment for 4 years, resulting in a 1.1 standard deviation gain in height. Although promising, these data do not reveal if true improvements can be expected for final adult height.

SUMMARY

In the absence of long-term results of experimental therapies, a common sense approach toward dealing with the growth of patients who have CAH is desirable. First, an effort can be made to decrease the replacement cortisol dose during the first year of life. Doubling, rather than tripling, the basal dose at times of stress could be helpful. The use of adjunctive therapy for infections could result in fewer fevers.

After 1 year of age, mean parental height could be used to establish at which centile the child should theoretically grow. The dose of cortisol could be adjusted to maintain the bone age between ± 1 SD. Plasma androstenedione levels should not rise above 50 ng/dL, and 17-hydroxyprogesterone should not be totally suppressed but be maintained between 500 and 1000 ng/dL. Compliance with therapy should be encouraged, particularly for adolescent patients. In the final analysis, a realistic expectation for patients would be a height between the 50th and third percentile of the normal growth curve and, in some cases, slightly below the third percentile when the genetic potential is slight.

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PREGNANCY OUTCOMES IN WOMEN WITH CONGENITAL VIRILIZING ADRENAL HYPERPLASIA

Joan C. Lo, MD, and Melvin M. Grumbach, MD

Since the landmark introduction of glucocorticoid therapy for the treatment of congenital adrenal hyperplasia (CAH) 5 decades ago[11, 125, 126] and subsequent advances in the early diagnosis of this disorder, the improvement in morbidity and mortality of affected infants and children has been dramatic.[44] Despite these striking advances, several long-term clinical outcomes remain to be addressed, particularly as these patients enter adulthood. In adult female patients with virilizing CAH, low fertility rates have traditionally been reported and are attributable to a variety of factors. Data on gestational management and pregnancy outcomes remain limited. Nevertheless, successful pregnancies have been reported in women with classic 21-hydroxylase deficiency, and issues concerning reproduction and pregnancy are increasingly becoming important in the medical care of these patients as adults. This article discusses the gestational management and reported pregnancy outcomes in women with virilizing CAH, focusing primarily on women with 21-hydroxylase deficiency, the steroidogenic defect that accounts for over 90% of cases.[123]

This work was supported in part by grant R01-HD-02335 and M01-RR-01271 from the National Institutes of Health, Bethesda, Maryland.

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PREGNANCY IN WOMEN WITH CLASSIC 21-HYDROXYLASE DEFICIENCY

Factors Contributing to Lower Fertility Rates

Women with CAH owing to 21-hydroxylase deficiency have decreased fertility rates, particularly women with the salt-wasting variant of this syndrome. In 1987 Mulaikal and co-workers[91] published fertility data obtained from 80 adult women with CAH owing to 21-hydroxylase deficiency who had been previously evaluated at Johns Hopkins Hospital in a follow-up to an earlier study of this same population.[66] Of 80 affected women, 40 manifested the salt-wasting variant of this syndrome. Overall, 40% of the women had irregular menstrual cycles or amenorrhea, and a large proportion of women in the salt-losing group reported an inadequate introitus. Half of the women reported an adequate vaginal introitus and heterosexual activity, and, among this group, 15 of 25 women (60%) with the simple virilizing form became pregnant, whereas only 1 of 15 women (7%) with the salt-losing form became pregnant.[91] Shortly thereafter, two additional salt-losing patients reported pregnancy, bringing the overall fertility rate to 20% among salt losers.[84] These historical data, however, may not reflect true pregnancy rates among women who desire conception and have had an adequate trial (e.g., 6–12 months) of unprotected intercourse, particularly in light of current data[58a, 74] and increasing evidence that the cause of low child-bearing rates in women with CAH is complex and multifactorial.[83]

Several contributing factors have been suggested to explain the low fertility rates observed among women with virilizing CAH. These factors include masculinization of the external genitalia, an inadequate introitus, and other structural factors related to genital reconstructive surgery, which may reduce heterosexual activity (e.g., poor surgical repair, vaginal stenosis, and clitoral dysfunction),[2, 7, 8, 26, 67, 91, 110] in addition to a number of hormonal factors. Women with classic 21-hydroxylase deficiency are frequently anovulatory owing to increased levels of adrenal androgens or progestational steroids and, in some cases, secondary ovarian hyperandrogenism.[10, 51, 55, 66, 91, 102, 106] Alternatively, the increased basal level of progestational steroids in these patients may have an independent contraceptive effect with prevention of conception even in the presence of ovulation.[51] Luteal phase deficiency in the setting of inadequate glucocorticoid treatment has been reported as a cause of infertility.[122] Because fewer marriages, lower rates of heterosexual activity, sexual behavioral differences, and, in some studies, somewhat increased rates of homosexuality have been observed in female patients with CAH, particularly those with evidence of salt-wasting, increasing attention has been directed toward the potential psychosexual effects of prenatal androgen excess on the fetal brain.[13, 14, 25, 30, 34, 68, 69, 83, 87]

Despite these factors, more recent fertility prognoses appear improved,[58a, 100] even among patients with the salt-wasting form, in part owing to earlier detection and treatment of 21-hydroxylase deficiency,

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surgical advances in genital reconstruction, and higher patient compliance rates.[100] Other studies similarly support an increased number of pregnancies among patients with salt-wasting 21-hydroxylase deficiency.[43, 74, 132] It has been suggested that women with CAH who are able to conceive at least one child seem to have normal fertility with respect to subsequent pregnancies.[68] These reports of successful pregnancy outcomes in women with classic 21-hydroxylase deficiency underscore the importance of careful attention and endocrine management during gestation, particularly in cases in which the fetus is female.[74]

Maternal Hyperandrogenism and the Female Fetus

The differentiation of male external genitalia is induced by testosterone/dihydrotestosterone during the first 12 weeks of fetal development.[46] Exposure of the female fetus to maternal androgen excess during the critical period of 7 to 12 weeks of fetal life can lead to variable masculinization of the genitalia.[47] The degree to which this occurs is determined by the stage of differentiation at the time of exposure, although, in severe cases, complete labioscrotal fusion and penile urethra formation have been observed.[47] The vagina separates from the urogenital sinus at about the 12th fetal week, and excess androgen exposure subsequent to this period can only result in labial hypertrophy and clitoromegaly in the female fetus (but not urogenital sinus formation or labial fusion). These latter effects are comparable with the changes seen with postnatal virilization.[46, 47] In addition, prenatal exposure to high androgen levels can putatively lead to masculinization of the female fetal brain.[14, 25, 53, 83]

Several mechanisms may protect the female fetus from the masculinizing effects of elevated endogenous androgens in the maternal circulation. The principal mechanism is placental aromatization of testosterone and androstenedione to estradiol and estrone, respectively, in which case the human placenta serves as a metabolic barrier to reduce fetal exposure to circulating maternal androgens and androgen precursors. The aromatase (CYP19, $P450_{arom}$) concentration and the total aromatase activity in the human placenta increase substantially during pregnancy (up to 16.5-fold by term pregnancy), conferring even greater fetal protection during late gestation.[65] This increase may explain the lack of infant virilization in reported cases of maternal hyperandrogenism owing to pregnancy luteoma, hyperreactio luteinalis, and polycystic ovary syndrome.[15, 52, 58, 80] On the other hand, there have been reports of masculinization of the external genitalia of female infants born to mothers with virilizing adrenocortical or ovarian tumors, including luteoma of pregnancy.[23, 50, 59, 64, 75, 93, 117, 120] In at least two reports, the female child had a developed penis.[93, 117] Presumably in these cases, placental CYP19 activity was insufficient for the excess androgen load.

Human placental aromatase activity also protects the pregnant woman and fetus from the virilizing effects of fetal androgen production

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by converting fetal androgen precursors to estrogens. As the fetal adrenal gland develops, production of dehydroepiandrosterone sulfate (DHEAS) increases. Fetal DHEAS is 16α-hydroxylated in the fetal adrenal and liver, and these androgen precursors are subsequently converted to estrogens (primarily estriol) by the human placenta.[20, 24] In pregnancies affected with placental aromatase deficiency owing to CYP19 gene mutation in the fetus, the inability to convert androgen precursors to estrogens leads to accumulation of placental testosterone and androstenedione (Fig. 1), and transfer of these androgens to the fetal and maternal circulation results in masculinization of the external genitalia of the female fetus and virilization of the mother.[24, 45, 90, 92, 109] A striking illustration is the occurrence of female pseudohermaphrodism in female infants with severe P450 aromatase deficiency, which emphasizes the importance of the fetoplacental unit in androgen metabolism as early as the first trimester.[24, 109] In affected pregnancies, maternal plasma and urinary estriol levels are typically low[92, 109] in comparison with the levels in normal gestation, in which estriol levels increase dramatically owing to fetal adrenal growth and increased placental aromatase activity (Fig.

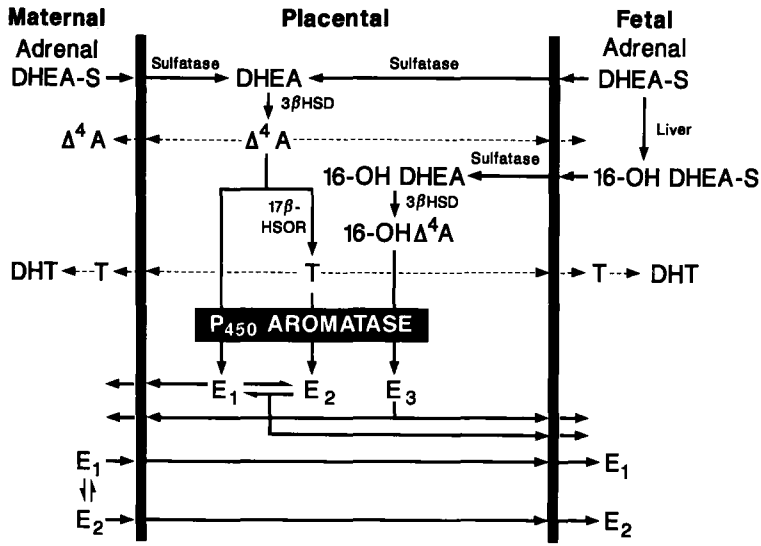

Figure 1. The placental synthesis of estrogens from C_{19}-androgen precursors by the fetoplacental unit. In P_{450} aromatase deficiency, there is little conversion of androgen precursors to estrogens, leading to accumulation of testosterone and androstenedione and transfer of these androgens to the fetal and maternal circulation. 3β-HSD = 3β-hydroxysteroid dehydrogenase II; 17β-HSOR = 17β-hydroxysteroid dehydrogenase III; DHT = dihydrotestosterone; Δ^4 A = androstenedione; E_1 = estrone; E_2 = estradiol; E_3 = estriol; DHEA-S = dehydroepiandrosterone sulfate; OH = hydroxy. (*Modified from* Conte FA, Grumbach MM, Ito Y, et al: A syndrome of female pseudohermaphrodism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutation in the gene encoding aromatase [CYP 19, P450arom]. J Clin Endocrinol Metab 78:1287–1292, 1994, The Endocrine Society; with permission.)

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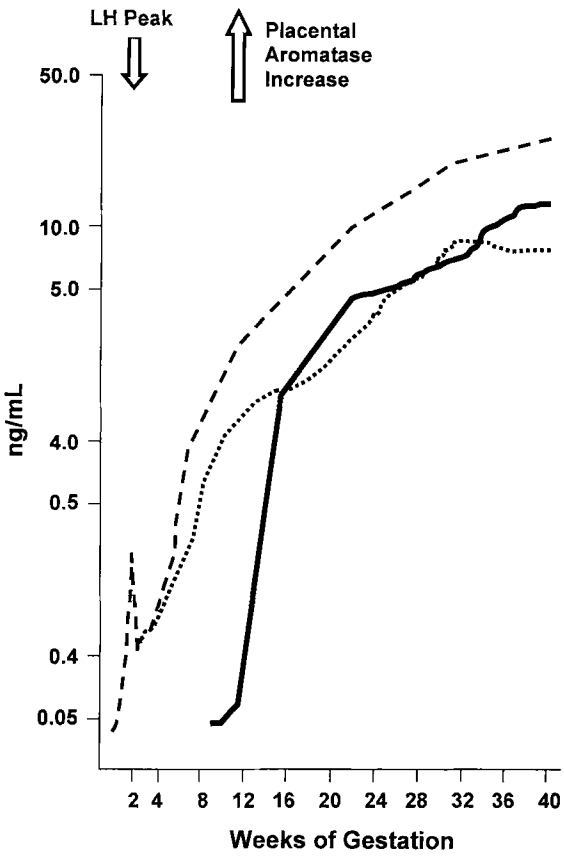

Figure 2. The pattern of plasma unconjugated estrogens throughout normal gestation. Note the log scale for plasma concentration; gestational age has been calculated from the last menstrual period. Estriol is an indicator of the synthesis of dehydroepiandrosterone (DHEA) and 16-hydroxy (OH) DHEA and their sulfates by the fetal adrenal cortex and liver and of the capacity for aromatization of 19-carbon steroids by the placenta. Plasma estriol was first detected at 0.05 ng/mL at 9 weeks' gestation and increased dramatically over the next 7 weeks (*solid line*). This is a time in gestation when placental aromatase activity increases. LH = luteinizing hormone. Dashed line = estradiol, dotted line = estrone. (*From* Grumbach MM and Auchus RJ,[45] *Modified from* Buster JE, Carson SA: Endocrinology and diagnosis of pregnancy. *In* Gabbe SG, Niebyl JR, Simpson JL [eds]. Obstetrics: Normal and Problem Pregnancies, ed 3. New York, Churchill Livingstone, 1996, pp 31–64; with permission.)

2).[19, 20, 98, 116] A pregnant woman who manifests progressive virilization beginning as early as the second trimester in the setting of high circulating androgens and low plasma and urinary estriol levels should be suspected of carrying a male or female fetus with a mutation in the CYP19 gene.[45]

The female spotted hyena provides an interesting comparative model of hyperandrogenism and deficient placental aromatase activity.[45]

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The female spotted hyena is a female pseudohermaphrodite; it urinates, copulates, and gives birth through a large penile clitoris and urogenital canal.[40] High levels of androstenedione are secreted from its androgenized ovary, resulting in greater circulating androstenedione concentrations in the female compared with the male.[40, 129] The female spotted hyena is dominant, more aggressive, and heavier than the male. During pregnancy, masculinization of the female genitalia of the fetus results from high in utero exposure to testosterone derived from placental metabolism of maternal androstenedione, in the setting of relatively low placental aromatase activity.[32, 72, 73, 129] The aromatase activity of the spotted hyena placenta is approximately 5% that of human placenta.[129] In contrast to human aromatase deficiency, the maternal ovary in the spotted hyena, not the fetal adrenal, serves as the source of androgens that cannot be adequately aromatized to estrogens.

In clinical states in which the capacity of the placental aromatase system to convert C19 steroids to estrogens is exceeded by androgens or androgen precursors from either the fetus or the pregnant woman, the female fetus is at risk for androgen-induced female pseudohermaphroditism. Synthetic nonaromatizable androgens and progestins escape this protective mechanism and can lead to masculinization of external genitalia of the female fetus at relatively low circulating levels in the pregnant woman.[47, 48, 124] Other factors that may contribute to protection of the female fetus during gestational hyperandrogenism, although to a much lesser degree, include the estrogen-induced increase in sex hormone–binding globulin (SHBG) levels, which reduces the fraction of bioavailable testosterone,[63, 96] and the potential antiandrogenic effects of progesterone.[31, 54, 79]

Pregnancy Outcomes

Table 1 summarizes the reported pregnancy outcomes among women with simple virilizing or salt-losing CAH. In 73 women (20 of whom had the salt-wasting form), there were 105 pregnancies resulting in 74 children. Approximately 10% of these pregnancies spontaneously aborted, and 10% were therapeutically terminated. The remaining pregnancies were carried to term (or near term) and delivered primarily by cesarean section. Only 19 vaginal births were reported (the method of delivery was not indicated in 15 births). In the majority of pregnancies, the woman maintained the same glucocorticoid dosage before gestation; however, some women, most of whom were salt losers, required higher steroid doses owing to symptoms of adrenal insufficiency[74, 131] or increased adrenal androgen levels.[74, 113, 132] In a few cases, the increased glucocorticoid dose was associated with weight gain, hypertension, glucose intolerance, or cushingoid features. Preeclampsia developed in three patients, but the relationship of preeclampsia to the underlying adrenal disorder or steroid therapy in these pregnancies was unclear.[74, 99, 131] In two infants, transient adrenal suppression was suspected,[70, 132] but the data were equivocal and complicated by other factors; furthermore, the

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infants (whose mothers had been treated with cortisone or hydrocortisone) recovered without sequelae, and it is unlikely they had clinical adrenal insufficiency. The placenta has a high concentration of 11β-hydroxysteroid dehydrogenase type II, an enzyme efficient in converting cortisol to the inactive cortisone, thus protecting the fetus from high plasma concentrations of maternal cortisol.[108] None of the 74 infants had evidence of classic CAH. Paternal carrier status was evaluated by either cosyntropin stimulation testing or CYP21 genotype studies in at least three cases at the time of pregnancy diagnosis.[74, 132] Thus far, only one female infant (born to a mother with simple virilizing CAH who had ceased glucocorticoid therapy before conception) has had ambiguous genitalia owing to maternal androgen excess.[62] Another female infant born to a woman with salt-losing CAH had rugose labia majora but no clitoromegaly.[132]

The gestational changes in circulating androgen levels have not been well studied in women with virilizing CAH. The available data suggest that androgen levels increase, as would be expected in normal pregnancy, although these gestational increases may be substantial in some patients with the salt-wasting form of CAH despite compliance with glucocorticoid treatment.[74, 132] Although higher steroid doses may be necessary in some pregnancies owing to changes in glucocorticoid metabolism, in cases in which maternal androgen levels continue to rise dramatically despite increased steroid administration, the possibility of other contributing factors should be considered (e.g., nonadrenal or ovarian androgen production). It is uncertain whether corticotropin-releasing hormone (CRH) of placental origin has clinically significant effects on maternal pituitary-adrenal function during pregnancy.[39, 41] The exponential rise in third-trimester maternal CRH concentrations during late gestation is principally caused by increased CRH production by the placenta[41, 42] and has been implicated in the onset of labor.[81] Hypothetically, placental CRH may have contributed to the exceedingly high androgen levels reported during late gestation in a patient with salt-losing 21-hydroxylase deficiency,[74] either through an indirect mechanism (via adrenocorticotropin) or as a direct adrenal androgen secretagogue.[57] Unlike in the hypothalamus, placental CRH is stimulated by glucocorticoids.[61, 104]

Recommendations for Pregnancy Management

Preconception Issues

The need for glucocorticoid treatment compliance and careful endocrine monitoring should be discussed with all women with classic 21-hydroxylase deficiency who desire pregnancy. Normal ovulatory cycles and fertility may not be achievable in all patients despite effective glucocorticoid therapy, particularly in women with persistent adrenal hyperandrogenism or secondary ovarian pathology. In these patients, ovulation induction with clomiphene citrate or gonadotropin therapy

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Table 1. PREGNANCY OUTCOMES IN WOMEN WITH SIMPLE VIRILIZING OR SALT-LOSING CONGENITAL ADRENAL HYPERPLASIA*

Source	Number of Women and Classification	Total Number of Pregnancies	Birth Outcomes (Delivery Method)	Reference Number
Yamashita and Kozakae (1956)	1, Simple virilizing	1	1 Normal male (C)	130
de Alvarez and Smith (1957)	1, Simple virilizing	1	1 SAB	28
Wilson and Keating (1958)	2, Simple virilizing	2	2 Normal females (V, C)	128
Gans and Ser (1959)	1, Simple virilizing	1	1 Normal female (C)	38
Mason (1961)	1, Simple virilizing	1	1 Normal female (C)	76
Swyer and Bonham (1961)	1, Simple virilizing	1	1 Normal male (C)	114
Southren et al (1961)	1, Simple virilizing	2	2 SAB	111
Avin (1962)	1, Simple virilizing	2	1 Ruptured EP, 1 normal female (C)	4
Vant and Horner (1963)	1, Simple virilizing	2	1 SAB, 1 normal female (C)	119
Dewhurst and Gordon (1963)	1, Virilizing‡	1	1 Child†	29
Speroff (1965)	1, Simple virilizing	1	1 Normal female (V)	113
Harrison and Lister (1966)	1, Simple virilizing	1	1 Normal male (V)	49
Eyton-Jones (1968)	1, Simple virilizing	2	1 SAB, 1 normal male (V)	33
Mori and Miyakawa (1970)	1, Simple virilizing	1	1 Normal female (C)	89
Lev-Ran (1975)	1, Simple virilizing	1	1 Normal child†	71
Sotiropoulos et al (1976)	1, Virilizing‡	3	3 Children (3C)†	110
Kai et al (1979)	1, Simple virilizing	3	1 SAB, 1 normal male (V), 1 pseudohermaphrodite female (V)	62
Porter and de Swiet (1983)	1, Simple virilizing	1	1 Normal female (C)	99
Grant et al (1983)	1, Simple virilizing	1	1 Normal child (C)†	43
	3, Salt wasting	5	1 TAB, 4 normal children (4V)†	
Andersen et al (1983)	1, Salt wasting	1	1 Normal female (C)	3
Mulaikal et al (1987)	15, Simple virilizing	25	1 SAB, 1 TAB, 20 normal children (4V, >9C)†§	91
	1, Salt wasting	1	1 TAB	

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Blumberg et al (1988)	1, Salt-wasting	2	1 SAB, 1 normal female (C)	17
Helleday et al (1993)	1, Simple virilizing	1	Outcome not reported	51
Yarnell et al (1994)	1, Salt-wasting	1	1 Normal male (C)	131
Kuhnle et al (1995)	4, Simple virilizing	4	4 Children†	68
	2, Salt-wasting	2	2 Children†	
Migeon (1996)	2, Salt-wasting	2	Outcomes not reported	84
Ritzén and Wedell/Conway (1996)	1, Salt-wasting	1	Outcome not reported	103
Costa et al (1997)	2, Virilizing‡	2	2 Normal children (2C)†	26
Premawardhana et al (1997)	2, Simple virilizing 3, Salt-washing	8	5 TAB, 2 normal children (V, C)†	100
Zacharin (1999)	1, Salt-wasting	2	1 Normal male (C) 1 Female with rugose labia majora (C)	132
Lo et al (1999)	1, Simple virilizing	2	1 SAB, 1 normal female (V)	74
	3, Salt-wasting	5	2 TAB, 3 normal females (3C)	
Jääskeläinen et al (2000)	8, Simple virilizing	12	2 SAB, 6 normal females, 4 normal males (3V, 7C)	58a
	1, Salt-wasting	1	1 TAB	
Overall summary	73 Women with virilizing CAH 49, Simple virilizing 20, Salt-wasting 4, Salt-losing status not specified		105 Total pregnancies 11 SAB (10%) 11 TAB, 1 EP 74 Children (1 pseudohermaphrodite female) 8 Pregnancy outcomes not reported	

V = vaginal delivery; C = cesarean delivery; EP = ectopic pregnancy; SAB = spontaneous abortion; TAB = therapeutic abortion.

*The majority of patients were female pseudohermaphrodites diagnosed in infancy or early childhood.

†Gender unspecified.

‡Salt-losing status not specified.

§One infant was born premature. These pregnancy outcomes were reviewed in part by Klingensmith and co-workers[66] who reported 15 pregnancies in 10 women with virilizing CAH, resulting in 3 SAB, 2 TAB, 9 living children, and 1 infant with multiple birth defects who died shortly after birth. This earlier report may have included a woman with 11β-hydroxylase deficiency.

Modified from Lo JC, Schwitzgebel VM, Tyrrell JB, et al: Normal female infants born of mothers with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 84:930–936, 1999; © The Endocrine Society, with permission.

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may be helpful.[58a] For some patients, other assisted reproductive techniques, including in vitro fertilization, may be necessary for successful conception.[74] Bilateral adrenalectomy remains a therapeutic controversy,[118] but in one patient with salt-wasting 21-hydroxylase deficiency who had persistent elevations in serum progesterone levels despite adequate adrenal androgen suppression, removal of both adrenal glands ultimately led to spontaneous ovulation and subsequent fertility.[55, 103] Once pregnancy is achieved, it has been speculated that there may be a slightly higher rate of spontaneous miscarriage, perhaps related in part to inadequate corpus luteum activity and associated endometrial changes.[39] Nevertheless, the available data do not support an increased spontaneous abortion rate among glucocorticoid-treated women with classic CAH. In general, most pregnancies occurring among women with CAH have been carried successfully to term with a healthy outcome, although there has been at least one report of masculinization of the external genitalia in a female infant secondary to maternal hyperandrogenism (Table 1). Patients should be aware of the importance of careful hormonal monitoring throughout gestation and the need for close coordination of care between the obstetrician and endocrinologist.

Preconception counseling should include a discussion of the risk of having a child affected with 21-hydroxylase deficiency. For a woman with classic 21-hydroxylase deficiency, the risk of having an infant with the same disorder is approximately 1 in 120 births if the paternal status is unknown, based on an estimated 1 in 60 incidence of heterozygous individuals with a CYP21 mutation, derived from newborn screening data.[97] Accordingly, the risk of having an affected female child is 1 in 240 births. Genotype studies or a cosyntropin stimulation test to evaluate paternal carrier status may be informative in assessing the risk of having an affected fetus; however, the performance of genotype studies is not likely to be cost effective for random screening of individuals or populations. Although there is 20% to 30% overlap between serum 17-hydroxyprogesterone levels among normal individuals and heterozygote carriers of 21-hydroxylase deficiency following cosyntropin stimulation, a low hormone level in the partner is reassuring.[94] Serum 21-deoxycortisol (for which an assay is not currently available in commercial laboratories) provides a better discrimination of carrier status. It is unclear whether routine evaluation of paternal carrier status is warranted, although consideration is prudent in cases in which a history of consanguinity exists, or if the father is from an ethnicity/race background with a higher carrier frequency for classic 21-hydroxylase deficiency.

Gestational Management

In pregnant women with classic 21-hydroxylase deficiency, the authors recommend using a glucocorticoid that is metabolized by placental 11β-hydroxysteroid dehydrogenase type II for maternal adrenal steroid replacement. Hydrocortisone, cortisone acetate, prednisone, prednisolone, and methylprednisolone are metabolized by the placenta, minimizing placental transfer and fetal adrenal suppression. In contrast, dexa-

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methasone, which provides longer and more effective suppression of adrenal androgen production in women with 21-hydroxylase deficiency,[56, 102] is transferred across the placenta without oxidation of the 11-hydroxyl group and can suppress the fetal adrenal gland.[27, 108] Betamethasone is similarly transferred across the placenta without inactivation, and these glucocorticoids are generally not recommended for maternal glucocorticoid therapy during gestation. Obviously, the treatment considerations differ in pregnancies in which the fetus is at risk for CAH, in which case suppression of the abnormal fetal adrenal gland is the goal of prenatal therapy.[37, 82, 123] In such cases, when the father is a known heterozygote carrier and the mother homozygous for CYP21 deficiency, the risk of having an affected female fetus is 1 in 4 and early-onset prenatal dexamethasone therapy under an institutional review board–approved research protocol should be considered.[85]

Maternal clinical status, serum electrolytes, and circulating adrenal androgen levels should be assessed regularly during gestation to determine the need for increased glucocorticoid or mineralocorticoid therapy (Table 2). Excessive nausea, salt craving, and poor weight gain suggest adrenal steroid insufficiency. Although most patients with virilizing CAH do not require adjustment to their pre-pregnancy dose of glucocorticoid,[43, 58a, 60, 66] some women with severe or salt-wasting CAH may need increased doses to provide adequate steroid coverage during gestation, analogous to the management of an addisonian patient during pregnancy.[1, 74, 131] For the rare patient manifesting deficient hepatic 11β-hydroxysteroid dehydrogenase type I reductase activity, treatment with a glucocorticoid that does not require 11β-oxoreduction for activation (e.g., hydrocortisone or prednisolone) is necessary.[95] In selected patients, measurement of plasma renin activity may be helpful in optimizing adrenal steroid replacement; however, the clinician should remember that modest increases in plasma renin activity may occur during normal pregnancy.[127] One should monitor for hypertension and fluid retention in patients receiving mineralocorticoid therapy, particularly in the third trimester.[39] The authors recommend measurement of maternal serum total and free testosterone levels every 6 weeks during the first trimester, and every 6 to 8 weeks thereafter until delivery.[74] Sequential measurements should be performed in the same laboratory, at the same time of day (preferably in the morning), and at a consistent time after the last glucocorticoid dose.

The gestational changes in C19 and C21 steroid levels complicate the assesment of adrenal androgen excess in pregnant women with 21-hydroxylase deficiency.[74] In addition, there are potential confounding factors in measuring steroid hormone levels during pregnancy owing to interfering substances (e.g., SHBG and other steroid hormones) that may affect assay specificity, depending on the methodology.[19, 77] These concerns are particularly relevant in pregnant women with CAH in whom adrenal androgen precursor levels are elevated. It is advisable to use laboratories with expertise in steroid immunoassay and to compare these results with laboratory-specific reference values for pregnancy. Cross-sectional data from previous studies using specific radioimmuno-

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Table 2. SUGGESTED GUIDELINES FOR THE MANAGEMENT OF WOMEN WITH CLASSIC CONGENITAL ADRENAL HYPERPLASIA OWING TO 21-HYDROXYLASE DEFICIENCY DURING PREGNANCY AND DELIVERY

Gestational Management

Adrenal steroid replacement and adrenal androgen suppression

- Use a glucocorticoid that is metabolized by placental 11β-hydroxysteroid dehydrogenase II (e.g., hydrocortisone, cortisone acetate, prednisone, prednisolone, methylprednisolone).
- Assess clinical status, serum electrolytes, and serum androgen levels regularly to determine the need for increased glucocorticoid and/or mineralocorticoid therapy. Excessive nausea, salt craving, and poor weight gain suggest adrenal steroid insufficiency. In select cases, measurement of plasma renin activity may be helpful.
- Serum testosterone and free testosterone levels should be measured every 6 weeks in the first trimester and every 6 to 8 weeks thereafter. Target free testosterone levels to the high normal range for pregnancy; however, management must be individualized for each patient. Avoid inducing cushingoid effects from too high a dose of glucocorticoids.
- Fetal gender determination by ultrasonography may be helpful in guiding treatment goals; maternal androgen excess will have minimal effects on the male fetus.

Labor and delivery

Stress dose glucocorticoid therapy

- A soluble hydrocortisone ester (up to 50–100 mg IV every 8 hours) should be given at the initiation of active labor and continued until after delivery, followed by a rapid taper to previous maintenance doses.

Cesarean versus vaginal delivery

- Android pelvic characteristics may increase the risk for cephalopelvic disproportion.
- Elective cesarean section should be considered, especially for those patients who have had genital reconstructive surgery.

Evaluation of the infant

- Assess the infant for clinical signs of adrenal insufficiency (e.g., hypotension, hypoglycemia).
- Examine the infant for ambiguous genitalia. Female pseudohermaphroditism may either be a consequence of maternal hyperandrogenism or, if the father is a carrier, of fetal 21-hydroxylase deficiency.* Affected male infants may have enlarged external genitalia.
- If the external genitalia are ambiguous, appropriate laboratory studies in the infant should be carried out to exclude 21-hydroxylase deficiency.

*In an affected mother with 21-hydroxylase deficiency, the risk of having an affected female infant with fetal congenital adrenal hyperplasia is estimated to be approximately 1 in 240 based on an estimated 1 in 60 incidence for heterozygous individuals (see text for further details).

Adapted from Lo JC, Schwitzgebel VM, Tyrrell JB, et al: Normal female infants born of mothers with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 84:930–936, 1999; © The Endocrine Society, with permission.

assay (RIA) techniques with solvent extraction and chromatographic purification, including data from selected specialty reference laboratories, suggest that circulating total testosterone levels are increased substantially during normal pregnancy (up to threefold) when compared with nonpregnant values, in large part owing to the estrogen-induced increase in SHBG levels.[22,74] Androstenedione levels may also be substantially elevated,[74] although third-trimester androstenedione levels within the normal range have been reported.[22] More recent longitudinal studies

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demonstrate that circulating testosterone levels rise during normal pregnancy with a mean increase of 1.7-fold from 6 to 36 weeks' gestation. Androstenedione levels also increase, but to a lesser extent[63, 96]; however, the direct (no extraction) RIA kits used in some of these latter studies may underestimate total testosterone concentration during late gestation owing to interference from SHBG[77, 96] or may overestimate testosterone concentration owing to cross-reacting steroids, and it is not clear that the specificity of these direct RIA methods has been carefully studied under pregnancy conditions.

The rise in SHBG concentration may contribute to mildly decreased bioavailable testosterone levels early in pregnancy; however, an elevation in free testosterone concentration occurs later in gestation, suggesting either increased testosterone production or further reduction in metabolic clearance.[9, 63] Free or bioavailable testosterone levels provide a more accurate measure of functional maternal testosterone concentrations and should be used as the primary method of monitoring androgen status throughout pregnancy. Free testosterone levels should be determined by equilibrium dialysis in a reliable hormone reference laboratory rather than by direct RIA with an analogue ligand (these commercial kits are now used by many clinical laboratories), which does not provide an accurate index of free testosterone concentration.[121] The serum concentration of 17-hydroxyprogesterone rises dramatically during gestation[20, 21, 96, 116] and is not a useful measure of the effect of glucocorticoid therapy on adrenal androgen suppression.

There are no clear guidelines concerning the extent to which adrenal androgen production should be suppressed during pregnancy in women with virilizing CAH, particularly later in gestation when there is increased capacity for placental aromatization of maternal androgens. The authors recommend targeting free testosterone concentrations to the high normal range expected for the pregnancy trimester, although management for each patient should be individualized. As an example, at Endocrine Sciences (Calabasas Hills, CA), an upper total testosterone level of 209 ng/dL and free testosterone level of 6.9 pg/mL have been reported during the second and third trimester of normal pregnancy.[74] Fetal gender determination by ultrasonography may be helpful in guiding treatment goals in cases in which adrenal androgens remain elevated because maternal androgen excess will have minimal effects on a male fetus. As placental aromatase activity provides a relatively large margin of safety, it is important to avoid the untoward effects of excessive glucocorticoid therapy in the pregnant woman and fetus.[74] Higher glucocorticoid doses increase the risk of fluid overload, excessive weight gain, cushingoid features, hypertension, glucose intolerance, and hyperglycemia, along with other undesirable maternal effects, and these clinical indices should be assessed during gestation. Although the authors do not recommend the use of dexamethasone or betamethasone (which are not inactivated by placental 11β-hydroxysteroid dehydrogenase type II) during pregnancy, if these steroids are used, measurement of maternal serum or urinary estriol levels during the second and third trimesters may be useful in assessing secondary fetal adrenal suppression.

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Labor and Delivery

Stress doses of glucocorticoid therapy are necessary during labor and delivery. The authors recommend administration of hydrocortisone, 50 to 100 mg intravenously every 8 hours, at the start of active labor with continuation until after delivery, followed by a rapid taper to previous maintenance doses. Elective cesarean section should be considered for pregnant women with virilizing CAH, especially for those who have had reconstructive surgery of the external genitalia.[74] Vaginal laceration, injury to the urethra and rectum, and difficulty with postpartum repair of the perineum are significant concerns in women with prior vaginal reconstruction owing to scarring and alteration of normal anatomy.[113] In addition, many patients have historically required delivery by cesarean section for cephalopelvic disproportion, presumably owing to android pelvic characteristics.[60, 91] An android pelvis would be less likely in women with CAH who have received effective glucocorticoid treatment since early infancy and childhood.[113]

Evaluation of the Infant

Transient adrenal unresponsiveness in the infant is not expected to occur following maintenance treatment during gestation with glucocorticoids that are metabolized by the placenta. Indeed, adrenal suppression in the infant is exceedingly rare under these conditions, except perhaps at high doses. Nevertheless, the infant should be evaluated for clinical signs of adrenal suppression (e.g., hypotension, hypoglycemia), particularly in cases in which dexamethasone was administered during pregnancy. Furthermore, it is important to examine the infant for ambiguous external genitalia. Female pseudohermaphroditism may be a consequence of either maternal hyperandrogenism or, if the father is a carrier, of fetal 21-hydroxylase deficiency. As discussed previously, for the pregnant woman with 21-hydroxylase deficiency, the risk of having an affected female infant is approximately 1 in 240 births if the paternal carrier status is unknown. If the external genitalia are ambiguous, appropriate laboratory studies should be carried out in the infant to exclude 21-hydroxylase deficiency. Under these conditions, the male infant may have enlargement and increased pigmentation of the external genitalia, although the clinical features of CAH can be subtle in the absence of a salt-wasting crisis.[123] The authors routinely determine plasma 17-hydroxyprogesterone levels at 48 to 72 hours after delivery in infants born to mothers with classic virilizing CAH.

PREGNANCY OUTCOMES IN WOMEN WITH OTHER FORMS OF CLASSIC CONGENITAL ADRENAL HYPERPLASIA

There have been few reports of pregnancy outcomes in women with CAH owing to other steroidogenic defects, although it is possible that

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historical reports of virilizing CAH and pregnancy might have included a patient with 11β-hydroxylase deficiency.[66] Overall, classic 11β-hydroxylase deficiency occurs in 5% to 8% of cases,[133] although this frequency is much higher in Israel among individuals of Moroccan Jewish descent.[107] The considerations for gestational management in women with classic 11β-hydroxylase deficiency or 21-hydroxylase deficiency are similar, except that the former group are frequently hypertensive owing to excess circulating deoxycorticosterone, and careful monitoring of blood pressure, weight, and signs of fluid retention is advisable. Classic CAH owing to 3β-hydroxysteroid dehydrogenase type II is rare, and, to the authors' knowledge, there have been no published reports of pregnancy outcome in affected women.

Women with 17α-hydroxylase deficiency manifest primary hypogonadism and sexual infantilism owing to deficient production of sex steroids. These patients are infertile and may exhibit ovarian pathology, including abnormal ovarian follicular development and cystic ovaries presumably secondary to excess gonadotropin stimulation.[16] Nonetheless, successful ovum harvest and in vitro fertilization with primary embryonic cleavage (subsequent treatment cycle was aborted) have been reported in a patient with 17α-hydroxylase deficiency.[101] In a second patient, the induction of artificial endometrial cycles followed by transfer of in vitro fertilized donor eggs led to a viable pregnancy[12]; however, adequate endometrial maturation depends on several factors and may not be achievable in all cases.[78] These preliminary data indicate the future possibility of potential fertility in affected patients with assisted reproductive techniques.

PREGNANCY IN WOMEN WITH NONCLASSIC CONGENITAL ADRENAL HYPERPLASIA

Women with nonclassic CAH owing to CYP21 mutations comprise a heterogeneous group of patients with variable biochemical and clinical features. The need for glucocorticoid therapy in these individuals is less clear and is dependent on the degree of hyperandrogenism because there is generally no clinical evidence of hypocortisolism.[5, 36] Women with nonclassic 21-hydroxylase deficiency do not manifest genital ambiguity at birth but may present with evidence of androgen excess during childhood, puberty, or early adulthood, or remain clinically asymptomatic.[5, 18] These patients inherit either two mild CYP21 mutations or, in the case of a compound heterozygote, one mild and one severe CYP21 mutation.[6, 123] Often, the diagnosis of late-onset or nonclassic 21-hydroxylase deficiency is made during endocrine evaluation for hirsutism, menstrual irregularity, or primary infertility. Nevertheless, some women have become pregnant without specific treatment and even before the diagnosis of nonclassic CAH was recognized.[35] The relative hypofertility in female patients with nonclassic 21-hydroxylase deficiency is primarily caused by androgen excess and related hormonal factors that are largely

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correctable with glucocorticoid therapy.[5, 35] For patients who remain anovulatory (often as a result of secondary ovarian dysfunction) despite glucocorticoid treatment and a reduction in circulating androgen levels, ovulation induction with clomiphene citrate can be effective.[5, 35]

For women who have nonclassic CAH and are compound heterozygotes with one severe CYP21 mutation, preconception counseling regarding the risk of an affected female child with classic CAH is advisable, especially if the father is from a population with a high carrier frequency for CYP21 deficiency. If the father is a known carrier of the severe form of CYP21 deficiency and the mother is a compound heterozygote with one severe CYP21 mutation, the risk of having an affected infant with classic CAH would be 1 in 4 pregnancies (or 1 in 8 pregnancies for an affected female infant). In such cases, early-onset prenatal dexamethasone therapy to prevent masculinization of the female fetus owing to fetal CAH might be considered under an institutional review board–approved research protocol, but this recommendation remains a subject of debate because seven of eight fetuses would be treated unnecessarily.[86, 112] Among a large multicenter cohort of women with nonclassic CAH, there has been one report of a woman giving birth to an infant (male) affected with classic CAH.[88] In that case, the original proband was the child. The mother was identified through subsequent biochemical testing (17-hydroxyprogesterone level post-ACTH stimulation >10 ng/mL), although it is not clear that genetic studies were performed to confirm that she was a compound heterozygote with a mild and severe CYP21 mutation. Overall, in the absence of data regarding the molecular status of the CYP21 genes of the parents, the risk of a woman with nonclassic CAH having an affected infant with classic CAH remains low (estimated at 1.7 to 2.3 cases per 1000 births), although consideration should be given to the ethnicity/race and rate of consanguinity of the parents.[5, 6, 123]

For women with nonclassic 21-hydroxylase deficiency who become pregnant without glucocorticoid treatment, the mild degree of hyperandrogenism generally does not warrant the initiation of glucocorticoid therapy, owing to the large capacity for placental aromatization of circulating maternal androgens.[5, 123] Serum total and free testosterone levels should be measured early in the first trimester to assess the degree of androgen excess. For women already receiving maintenance glucocorticoid therapy at the time of pregnancy, it is advisable to continue treatment because of potential hypothalamic-pituitary-adrenal axis suppression and to select a glucocorticoid that is metabolized by placental 11β-hydroxysteroid dehydrogenase type II. These pregnancy considerations are similar for women with nonclassic 11β-hydroxylase deficiency. At least one successful pregnancy has been reported in a normotensive woman with mild virilization owing to nonclassic 11β-hydroxylase deficiency who delivered a healthy female infant.[115] Pregnancy has also been reported in a patient with long-term infertility with putative nonclassic 3β-hydroxylase deficiency who conceived following glucocorticoid administration and in vitro fertilization therapy.[105]

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SUMMARY

Although low fertility rates have traditionally been reported among women with classic CAH and especially among women with the salt-wasting variant, more recent data suggest that fertility rates are significantly improved, largely owing to earlier treatment of CAH, improved compliance with therapy, and surgical advances in genital reconstruction. Furthermore, ovulation induction and assisted reproductive techniques are now available to women who remain infertile despite effective adrenal androgen suppression. Although the pregnancy experience in women with classic CAH remains limited, it is apparent that, once pregnant, these women have a high probability of successful outcome. Key issues should be emphasized in the management of CAH during gestation, including the need for assessing adrenal steroid replacement and adrenal androgen suppression, particularly in light of the interplay between maternal hyperandrogenism and the protective effect of placental aromatase activity, which provides a relatively large margin of safety for the female fetus. Maternal hormone levels should be evaluated in the context of laboratory-specific reference ranges for pregnancy. The infant should be examined for ambiguous genitalia and monitored for evidence of adrenal insufficiency. Although an affected female infant with classic CAH has not been reported as a pregnancy outcome of a mother with classic virilizing CAH, these concerns should be discussed during preconception counseling. Patients should also be aware of the importance of medication compliance and careful hormonal monitoring during the entire pregnancy. In most cases, successful gestational management requires the close coordination of care between the obstetrician and endocrinologist.

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NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA

Bradford L. Therrell, PhD

Newborn screening is the terminology used to refer to the process commonly occurring within the first few days after birth whereby a newborn infant is subjected to the collection of a blood sample for laboratory testing. In some cases, newborn screening may also refer to more comprehensive testing including hearing studies. The comprehensiveness of newborn screening programs varies within and between countries and may include tests for as many as 30 different disorders. In traditional newborn screening, the protocol usually involves absorption of blood from a heelstick onto a specially manufactured absorbent filter paper. In the United States, this "filter paper form" is actually a Food and Drug Administration–approved medical device having a defined expiration date, and a national standard for newborn blood collection exists.[22] Once collected, the blood spot sample is sent to a newborn screening laboratory that has been specified by the newborn screening program. Program oversight is usually maintained by a public health department. The laboratory is usually a public health laboratory, but some programs may contract with another health laboratory or with a commercial laboratory. For example, the Oregon Public Health Laboratory provides testing services for Oregon, Alaska, Hawaii, Idaho, and Nevada, whereas Pennsylvania and the District of Columbia contract with a commercial laboratory in Pennsylvania. Samples are usually transported by mail; only rarely do programs use courier services. Labo-

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ratory testing is usually performed within 1 to 3 working days of receipt at the testing laboratory, and written results are returned within 10 to 14 days of sample submission. Telephone results may be sooner. Recently published newborn screening guidelines[31] suggest that programs should not exceed 7 days from the receipt of sample to the return of a result. Abnormal test results indicating the high probability of a disorder in a newborn are usually reported by a telephone call to minimize the time to treatment. When the results do not clearly indicate the absence of a disorder, and when a high probability of the disorder is not indicated, a follow-up letter may be sent requesting a second filter paper test.

Newborn screening originated in the early 1960s with the work of Guthrie[12] and was initially concerned with detecting inborn metabolic errors, such as phenylketonuria (PKU) that resulted in mental retardation. With early detection and dietary adjustments, many metabolic disorders could be controlled so that normal or near-normal developmental outcomes resulted as the child grew to adulthood. The more prevalent of these disorders were targeted for newborn screening. As newborn screening for inborn errors of metabolism became widespread in developed public health systems in the 1960s, it became necessary to create guidelines concerning the way in which disorders should be viewed for inclusion or exclusion from a newborn screening system. Criteria were proposed by the National Academy of Sciences,[11] including considerations such as population frequency, morbidity and mortality resulting from delayed diagnosis and treatment, treatability, detectability, and cost. From 1961 to the present, many disorders have been considered for newborn screening based on these criteria.

Following a 1969 report of increased risk of congenital adrenal hyperplasia (CAH) in the native Alaskan population[13] and the development of a sensitive and specific filter paper test for CAH in 1977,[27] a pilot CAH newborn screening program was undertaken in Alaska.[28] The results of this program confirmed the feasibility of adding filter paper CAH screening to newborn screening systems and the likelihood that case finding by this technique would significantly improve case detection and decrease associated morbidity and mortality. Additionally, the screening results increased the likelihood of identifying missed sex assignments in severely virilized females. Of particular benefit was the early identification of asymptomatic males at increased risk of not being detected.

Subsequent to the early work by Pang resulting in the filter paper assay for 17-hydroxyprogesterone and the Alaskan pilot studies, other testing methodologies were reported along with additional data from pilot screening studies.[6, 8, 10, 19, 35, 40, 42] These early programs primarily used inexpensive radioimmunoassay techniques, with the exception of Japan, where an enzyme-linked immunosorbent assay was used. Samples were usually collected around the third day of life; however, in some programs, collection occurred as late as day 5. Patients were generally presumed to have salt-wasting CAH if there were clinical and serum biochemical evidence. Patients exhibiting no symptoms nor

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biochemical abnormalities were presumed to have simple-virilizing CAH if their 17-hydroxyprogesterone elevations were confirmed through serum testing.

During the first 8 years of newborn screening for CAH, approximately 1 million newborns were tested in six countries (United States, France, Italy, New Zealand, Japan, and Scotland), with 77 cases of classical CAH reported, yielding an estimated worldwide birth prevalence of approximately 1 in 14,000. For salt-wasting CAH, the prevalence was approximately 1 in 18,850 and for the simple-virilizing form, approximately 1 in 57,500.[29] By 1991, 29 programs in 13 countries (now expanded to Brazil, Switzerland, Sweden, Germany, Portugal, Canada, and Spain) had reported screening almost 6.5 million newborns, demonstrating a consistent overall prevalence of classic CAH of 1 in 15,000 to 1 in 16,000 live births. Salt-wasting CAH accounted for 66% of the cases reported and simple-virilizing CAH accounted for 32%.[25]

Some newborn screening programs require two screening tests. The second sample is obtained between 1 and 2 weeks of age or when the newborn is slightly older. Although originally intended to act as a safety net for detecting cases of PKU that may have been missed as a result of the collection of a screening sample before biologic elevations of phenylalanine, this second test revealed interesting CAH screening results in a population of 1.9 million systematically studied newborns in Texas.[37] Using a testing protocol that resulted in approximately 0.5% patient recall, none of the 88 cases of salt-wasting CAH detected were missed on initial screening in the Texas study, whereas 17 of 28 cases (61%) of simple-virilizing CAH detected through screening were detected on the second screen following a normal first screen (overall, 19% of all classic CAH cases reported). This second sample testing protocol also led to the detection of 47 of 54 cases (87%) classified as nonclassic CAH by virtue of persistent slight elevations of 17-hydroxyprogesterone, a lack of symptoms, and adrenocorticotropic hormone (ACTH) stimulation testing results. The screening results in Texas also pointed to the diagnostic/treatment difficulties arising from detecting newborns in whom the severity of non–salt-wasting CAH cannot be clearly distinguished biochemically. Should all newborns with non–salt-wasting CAH be treated? If not, how should the decision to treat be made? Despite an agreed-upon classification protocol among program subspecialty advisors that distinguished simple-virilizing from nonclassic CAH using biochemical testing and corticotropin stimulation, diagnostic/treatment issues remained when slight-to-moderate elevations of 17-hydroxyprogesterone were reported from newborn screening and symptoms were absent.

In a comprehensive review of newborn screening for CAH in 1993, Pang[25] reported that the incidence of classic CAH obtained from newborn screening uniformly exceeded the incidence from clinical ascertainment in all programs where sufficient comparative data existed. The reported screening incidence in Europe ranged from 1 in 10,000 to 1 in 14,000, similar to the reported incidence results in North America of 1 in 15,000 to 1 in 16,000. Japanese programs reported slightly lower

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incidences, and higher incidences were noted in Brazil (1 in 7500), La Reunion (1 in 4000), and Alaska (1 in 288 in Yupik Eskimos and 1 in 800 in native Alaskans). Recent studies in the Philippines (C.D. Padilla, MD, personal communication, April 2000) indicate an increased CAH incidence of approximately 1 in 7000. A comparison of these screening data worldwide with reported findings from clinical studies shows an overall twofold increase in cases detected in areas with newborn screening. In Japan, the data suggest an even greater impact, with disease prevalence increasing from approximately 1 in 44,000 before screening in 1981 to 1 in 19,000 after 4 million screens.[34] Overall, nearly 70% of cases reported during the first 15 years of screening (6 million newborns) were not suspected clinically, including approximately 20% of females and 99% of males. Similar findings were reported in the nearly 2 million newborns studied in Texas.[37] Programs often reported that, even when genital ambiguity or other symptoms of CAH were present, a diagnosis of CAH had often not been made by the time that newborn screening results were reported. In some instances, screening results were critical to the proper treatment of newborns already in crisis but without an appropriate diagnosis when the results were reported.

Clearly, the speed at which newborn screening programs operate is critical in testing newborns and detecting cases of CAH. Speed is of particular concern in newborn screening systems when screening samples are not routinely collected until the newborn is several days old, sample transport to the testing laboratory is slow, or laboratory analysis is slow. Sample transport and laboratory analysis over weekends or during holiday periods present special challenges.

TESTING METHODS

During the 1980s, a growing number of studies evaluated the efficacy of newborn screening for CAH and explored various laboratory methodologies. Although the early extraction technique reported by Pang is a suitable screening procedure, commercial test kits have routinely avoided the organic extraction step. Lack of extraction of cross-reacting maternal antibodies may contribute to less sensitive test results, which can lead to increased numbers of false-positive results requiring unnecessary follow-up.[17, 32] Production and selection of the best antibodies for kit use remain a challenge for kit vendors, and only a limited number of commercial kits specifically approved for newborn screening are available in the United States. Similar commercial kit limitations exist worldwide. A summary of various United States laboratory testing protocols has been reported,[26] and specific procedural details are available from various commercial sources and kit inserts.

Because of the limited amount of blood collected on the filter paper card and limitations in the manner in which automated paper-punching systems operate, most laboratories prefer the use of a 3-mm disk of blood for testing. Analytic procedures must be capable of accurately

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testing extracted serum samples of approximately 1.5 μL final serum volume. The least expensive laboratory testing methodology continues to be radioimmunoassay, and the largest number of testing laboratories use this procedure. Two general radioimmunoassay procedures are commercially available—^{125}I-labeled coated tube assays and double-antibody precipitation assays.[26] Nonradiolabeled enzyme immunoassay systems are also available. Of increasing popularity is the time-resolved fluoroimmunoassay procedure using europium-labeled 17-hydroxyprogesterone as a tracer. This testing methodology has been widely used outside the United States, especially where the use of radioactive isotopes is problematic, and is now expanding within the United States. One study of this commercial fluorescent immunoassay suggests that a test deficiency contributes to high 17-hydroxyprogesterone values in preterm (<1500 g) infants.[32]

Newborn screening programs generally function alike, but there are many differences in the details of operational procedures. A newborn screening multidisciplinary advisory committee is recommended for all screening programs, and larger screening systems may have the luxury of a separate endocrine advisory group.[38] In the United States, newborn screening programs are fee based in all but six states, with fees ranging from $10 to $60 (J. Simmank, personal communication, December 1999). There are many variations in program complexity, expenses, and fee collection mechanisms. Fees collected for screening may not necessarily go to direct support of the screening program. Fees usually involve either advance payment for newborn screening collection forms or direct billing to hospitals after the testing is completed. Some newborn screening programs in the United States and other countries are government supported in their entirety. In most programs, financial support for screening does not usually include treatment or confirmatory testing.

A validated cost-effectiveness study of newborn CAH screening has not been reported; however, a detailed cost analysis of 15 newborns with classic CAH detected in Texas in 1994 (among 325,521 screened) indicated a cost of approximately $115,000 per case diagnosed on first screen and $243,000 per case diagnosed on second testing.[5] That study did not include cases of nonclassic CAH as part of the cost considerations. The cost of laboratory testing was calculated at $1.62, including all related costs (reagents, supplies, personnel, overhead, equipment), and specimen collection at the hospital was estimated to cost about $1.14.

The American Academy of Pediatrics has recommended testing all newborns before hospital discharge but no later than 7 days after birth, including newborns in intensive care.[3] Samples may be collected by nurses or laboratory phlebotomists and are usually transported by mail to the central testing laboratory. Demographic data accompany the sample and may be electronically transmitted in some programs. Currently, newborn screening for CAH includes tests for elevations of 17-hydroxyprogesterone; therefore, screening is technically restricted to detection of 21-hydroxylase–deficient CAH (and perhaps to CAH resulting from 3β-hydroxysteroid dehydrogenase deficiency[7]). For this reason, physicians

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encountering virilized infants without elevated 17-hydroxyprogesterone levels on newborn screening are cautioned to consider other forms of CAH in their differential diagnosis.[33] Routine newborn genotyping is not yet available, although it has been suggested to be a desired confirmatory test to clarify treatment issues.[4, 9, 20, 24, 37]

Analytic ranges in newborn screening laboratories are usually determined either by adhering to the recommendations of the kit's manufacturer or by establishing individual laboratory ranges through pilot testing. For a variety of reasons, including testing specificity, testing sensitivity, the filter paper blood matrix, and the desire to eliminate false negatives, programs may establish expected normal ranges based on the ability of the program to follow up potentially positive cases adequately (recall rate). Programs may routinely design their screening protocols to produce a certain number of false-positive results to eliminate (if possible) false negatives. Factored into determining cut-off levels for CAH is the fact that premature infants exhibit higher levels of 17-hydroxyprogesterone[2]; therefore, the ranges will account for a disproportionate percentage of false-positive cases of premature infants unless they are judged against a different (higher) normal range. To counter the effect of higher 17-hydroxyprogesterone results in preterm newborns, programs usually establish separate normal ranges for full-term and preterm newborns. These ranges may be based on birth weight[1] or gestational age[41] depending on availability and reliability, and the program may develop algorithms using percentile or numeric results to evaluate screening results so that a specific recall rate is not exceeded (usually, between 0.5% and 1.0%). The recent finding[16] that test protocols used in screening for CAH in the United States had the lowest positive predictive value (translating to 200 positive screens per case detected nationally) of five disorders studied (PKU, galactosemia, biotinidase deficiency, congenital hypothyroidism, and CAH) is not surprising given the nonuniformity of procedures for establishing patient recall and the desire to eliminate all false-negative results. Because of the continuum of clinical presentations of CAH, a small but finite number of false negatives will always exist. The underlying principle, but not the only goal, of newborn screening for CAH must be to ensure detection of all severe salt-losing cases.

Limited uniformity exists in the way newborn screening test results for CAH are reported. Originally, results were reported in picograms per disk. Today, the more common unit is nanograms per milliliter (whole blood). Some programs use algorithms that avoid reporting numeric results in favor of interpretations (normal or abnormal) of laboratory findings owing to the fact that adult serum values of 17-hydroxyprogesterone, with which physicians are more familiar, differ significantly in magnitude from the results obtained from newborn filter paper blood spots. Newborn screening laboratory testing procedures are different from routine serum tests, and there is often more variability in test values as a result of the filter paper testing environment. A laboratory-interpreted report of "within expected range" or "outside of expected range" (or some variation of this) may be used to reduce or

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eliminate the opportunity for misinterpretation of laboratory values by persons not knowledgeable in a particular laboratory's method of filter paper testing. Because of protocol variability, 17-hydroxyprogesterone testing kit differences, and the lack of a recognized gold standard in newborn screening laboratory testing for CAH, it is critical that qualified specialists be available to the screening program to advise on procedural questions and laboratory interpretations. The procedure for reporting results must be acceptable to the physician community and have the support of the program's advisory committee (which should include an endocrinologist).

General standards of good laboratory practice are required for laboratory licensure as defined by the Clinical Laboratory Improvement Amendments of 1988 (CLIA 1988). Because a CLIA 1988–approved proficiency testing program for newborn screening samples collected on filter paper is not currently available, the Centers for Disease Control and Prevention (CDC) provides a Newborn Screening Quality Assurance Program (NSQAP) as a service for laboratories. Quarterly 17-hydroxyprogesterone challenges are provided to testing laboratories using the blood spot filter paper matrix. Additional proficiency testing programs for filter paper 17-hydroxyprogesterone testing are available outside the United States in New Zealand and Japan. The NSQAP cooperates with these other quality assurance programs in an effort to help standardize testing results internationally. Kit manufacturers are also participants in the CDC-NSQAP and work with the CDC to develop uniform result standardization among the commercial 17-hydroxyprogesterone kits.

THE SCREENING SYSTEM

The general approach used in newborn screening systems is shown in Figure 1. In accordance with accepted newborn screening practices, a filter paper sample is collected within a few days of birth, transported to the designated newborn screening laboratory, and analyzed for 17-hydroxyprogesterone. As a quality control check, samples testing positive are reaccessed, repunched, and reanalyzed. The final test results are transmitted to the newborn's primary care physician and usually to a designated follow-up coordinator associated with the screening program. The program's follow-up coordinator is responsible for ensuring rapid follow-up of abnormal screening results, including telephone notification of the newborn's physician. In many programs, an endocrine subspecialist may also be contacted to assist in the final diagnosis. In some programs, laboratory results may be available through 24-hour automated voice response systems with faxing capability. The follow-up protocol includes ensuring linkage to a medical home for appropriate diagnosis, treatment, and ancillary services. In places where testing for CAH is not currently mandated as part of a newborn screening program, physicians and birthing centers should make themselves aware of the availability of testing services from other sources. The timeline indicated in Figure 1 is typical for screening programs and serves to emphasize

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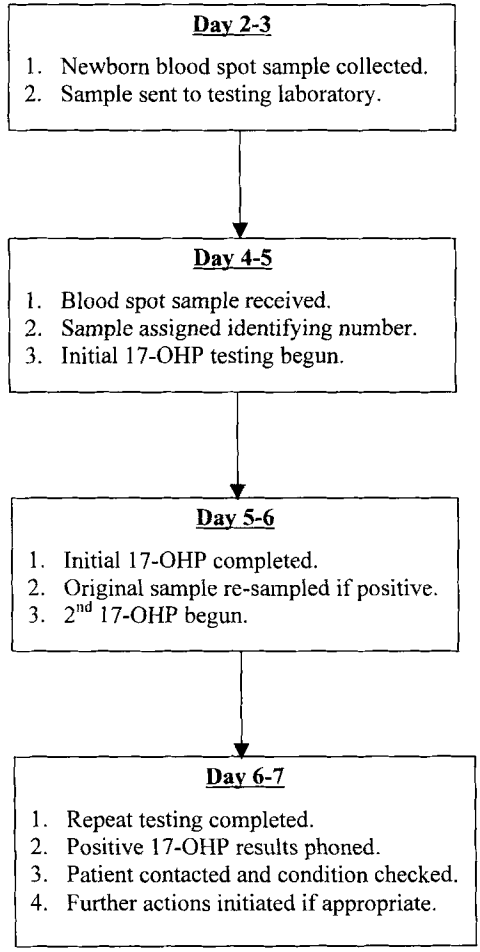

Figure 1. Typical newborn screening sample flow. OHP = hydroxyprogesterone.

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the timing problems faced by CAH screening laboratories. Slow sample transport, laboratory operations on less than a 7-day-per-week basis, and laboratory down-time on holidays can produce testing results too slow to be of use in preventing salt-losing crises.

In areas where large-scale newborn screening has been implemented, diagnostic questions have increased owing to the continuum nature of the disorder. Pediatric endocrinologists advising the Texas newborn screening program outlined a diagnostic protocol in an attempt to resolve treatment issues for newborns who consistently displayed elevated 17-hydroxyprogesterone concentrations with no physical symptoms.[37] When newborns were identified biochemically without symptoms, it was particularly difficult to differentiate simple-virilizing from nonclassic CAH in boys with moderately elevated 17-hydroxyprogesterone concentrations and in girls with moderately elevated 17-hydroxyprogesterone concentrations and only slight clitoral enlargement. Even though agreement was reached regarding routine diagnostic criteria using biochemical markers and ACTH stimulation testing results, consistent application of diagnostic categorization and treatment did not occur. Similarly, in newborns classified with nonclassic CAH in Texas, treatment is mixed, with approximately 50% of infants receiving some form of therapeutic management. Recently published guidance from the Council of Regional Networks for Genetic Services (CORN) has attempted to clarify the interpretation of results arising from screening and possible diagnostic follow-up actions for the general medical community.[31] Care is taken to include endocrinology subspecialists in the final diagnostic phase of the screening algorithm.

With appropriate long-term data collection, it will be possible to evaluate the impact of treatment versus nontreatment in the nonclassic Texas cases. Nevertheless, the manner in which follow-up newborn screening data for CAH is kept by most newborn screening programs is of concern. Because these programs are generally regarded as part of public health service, long-term outcome data are often collected only anecdotally, without appropriate validation, or not collected at all. Two data collection forms were established at the beginning of the Texas CAH screening program and later modified slightly to be brief and informative.[36] The forms were developed as potential research tools with the cooperation of the program's pediatric endocrinology consultants. A two-sided questionnaire (Fig. 2) is completed at the initial patient visit, including Prader genital rating of girls, and a simpler follow-up form (Fig. 3) is completed at each subsequent patient visit. Data from these forms are collected and evaluated periodically as an aid to making program changes and as a means of learning more about CAH. Accumulation and evaluation of these or similar data are critical to the evaluation of any CAH newborn screening system and greatly aid in understanding CAH and CAH-related sequelae. Newborn screening systems can provide a substantial service by ensuring that the collection of outcome data is a part of the overall screening system. If similar data are collected

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TEXAS DEPARTMENT OF HEALTH
NEWBORN SCREENING PROGRAM – CONGENITAL ADRENAL HYPERPLASIA

Initial CAH Medical Information at Diagnosis

Date: _____ Case No. (from TDH): _____

Name: _____ DOB: _____

Sex (check): ☐ M ☐ F How determined (check): ☐ chromosome ☐ descended testes _____ (method) other

Gestational age: _____ wks Birthweight: _____ g Head circumference _____ cm

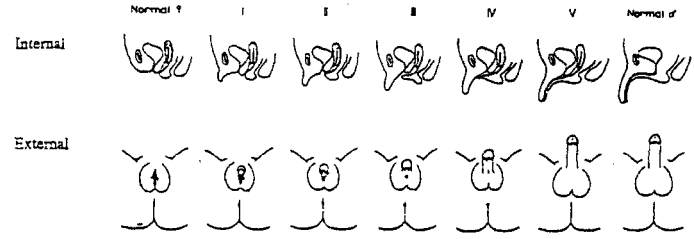
Type (check): ☐ Salt-loser ☐ Simple-virilizer ☐ Non-classical ☐ Uncertain ☐ not 21-OH _____ (defect)

Decision Criteria: _____
(briefly)

Siblings: ages of brothers _____ ages of sisters _____
(please circle ages of sibs above with CAH)
sib deaths in newborn period: # males _____ # females _____

I. Genitalia

Degree of virilation, Prader scale (rate-06): internal _____ external _____



Configuration of the genitalia of normal females (left) and males (right) along with the various stages of masculinizations seen in female pseudohermaphroditism caused by congenital adrenal hyperplasia. In type I, there is only an enlargement of the clitoris. In type II, III and IV, there are various degrees of labial scrotal fusion with formation of urogenital sinus. In type V, there is a penile urethra. (From Pediatric Endocrinology, Eds. Collu, R., et. al., Raven Press, N.Y., 1981, p. 491).

Anogenital distance: _____ mm Clitoral/penile size: _____ mm Genital pigmentation? (check) ☐ Y ☐ N

Females only: Amount of labial fusion (check): ☐ 0% ☐ 25% ☐ 50% ☐ 75% ☐ 100%

Urogenital sinus? (check) ☐ Y ☐ N

Will genital surgery be necessary? (check) ☐ Y ☐ N

Figure 2. *See legend on opposite page*

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II. *Laboratory*

Newborn screen 17-OHP: first screen: date _____ result _____
second screen: date _____ result _____

Confirmatory serum tests: Lab conducting assay: _____
Date of collection: _____ time of day of collection: ☐ AM ☐ PM

17-OHP (serum): _____ ng/dL	rennin:	_____ ng/dL/hr	testosterone:	_____ ng/dL
Sodium: _____ mEq/L	DHEA:	_____ ng/dL	cortisol:	_____ ng/dL
Potassium: _____ mEq/L	androstenedione:	_____ ng/dL	other:	_____
Aldosterone: _____ ng/dL	17-OHP-preg.:	_____ ng/dL	other:	_____

ACTH Stimulation: Baseline 17-OHP: _____ ng/dL 60 min. 17-OHP: _____ ng/dL

III. *Initial Medications*

		Type	Total Daily Dose	Schedule	How given? (e.g. po, im.)
Glucocorticoid:	☐ Cortef				
	☐ Other				
Mineralocorticoid:	☐ Florinef				
	☐ Other				

Other Medication:

Reason	Type	Dose	Schedule	How given? (e.g. po, im)

IV. *Clinical Symptoms* (check all that apply)

Ambiguous genitalia	☐	Poor feeding	☐	Hyponatremia	☐	Other ☐	(list below)
Vomiting	☐	Poor wt. gain	☐	Hyperkalemia	☐		
Diarrhea	☐	Dehydration	☐	Apathy	☐		

V. *Hospitalization*

Date: _____ Date: _____

Cause: _____ Cause: _____

Consultant signature: _____

Figure 2. Initial encounter congenital adrenal hyperplasia (CAH) data sheet. (Reprinted with permission of the Texas Department of Health.)

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TEXAS DEPARTMENT OF HEALTH
NEWBORN SCREENING PROGRAM – CONGENITAL ADRENAL HYPERPLASIA

UPDATED CAH MEDICAL INFORMATION

Name (last. First): _____

Date of Birth: _____ Case No.: _____

CAH Type: ☐ Salt-loser ☐ Simple-virilizer ☐ Non-classical
☐ Uncertain ☐ not 21-OH

Date of Visit: _____ Height: _____ cm _____ %ile Weight: _____ % _____ %ile

Any new sibling: (check) ☐ Y ☐ N Sex? (check) ☐ M ☐ F New sibling with CAH? (check) ☐ Y ☐ N

I. Genitalia

Current degree of virilization, Prader scale (rate 0-6): Internal _____ External _____

Current Tanner stage (check): ☐ I ☐ II ☐ III ☐ IV ☐ V

Recent genital surgery ☐ Y ☐ N Surgeon: _____

Date and type _____

_____

II. Laboratory

Date _____ Lab Name _____

17-OHP (serum):	_____ ng/dL	rennin:	_____ ng/dL/hr	testosterone:	_____ ng/dL
Sodium:	_____ mEq/L	DHEA:	_____ ng/dL	cortisol:	_____ ng/dL
Potassium:	_____ mEq/L	androstenedione:	_____ ng/dL	other:	_____
Aldosterone:	_____ ng/dL	17-OHP-preg.:	_____ ng/dL	other:	_____

ACTH Stimulation: Baseline 17-OHP: _____ ng/dL 60 min. 17-OHP: _____ ng/dL

III. Medication prescribed at this visit

		Type	Total Daily Dose	Schedule	How given? (e.g. po, im.)
Glucocorticoid:	☐ Cortef	_____	_____	_____	_____
	☐ Other	_____	_____	_____	_____
Mineralocorticoid:	☐ Florinef	_____	_____	_____	_____
	☐ Other	_____	_____	_____	_____

Figure 3. *See legend on opposite page*

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Other Medication:

Reason	Type	Dose	Schedule	How given? (e.g. po, im)

IV. ***Compliance***

Estimate of compliance with medication from last visit (check): ☐ poor ☐ fair ☐ good

V. ***Control*** (since last visit)

Number of times medication missed: _____ Number of times parent increased medication: _____

Overall estimate of quality of disease control (check): ☐ poor ☐ fair ☐ good

VI. ***Recent hospitalizations***

Date: _____ Cause: _____

VII. ***Other Comments*** _____

_____

_____

Consultant signature: _____ Date: _____

Return completed form to: Newborn Screening Coordinator
Texas Department of Health
1100 West 49th Street
Austin, Texas 78756
Telefax: (512) 458-7421

Figure 3. Follow-up encounter congenital adrenal hyperlasia (CAH) data sheet. (Reprinted with permission of the Texas Department of Health.)

in all programs, future comparisons will be facilitated. A detailed listing of items that should be collected by newborn screening programs for each disorder has also been outlined in the recent CORN guidelines.[31]

SUMMARY

Classic CAH (salt-wasting and simple virilizing) meets all of the recommended criteria for newborn screening. There are reliable and efficient newborn screening tests,[14, 26, 27] the disorder results in high morbidity and mortality if left undetected,[21, 23] there is effective treatment that reduces negative outcomes,[21, 23] and there is a relatively high incidence.[25, 29] When compared with the case findings without the benefit of screening, the data from screening programs show reduced adrenal crises, reduced incorrect sex assignments, and reduced deaths.[18, 23, 34, 39]

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Racial/ethnic prevalence differences are present in newborn screening program data. The Texas data indicate a lower disease frequency in African-Americans when compared with Caucasians, and international data indicate higher frequencies in native Yupik Eskimos, Brazilians, residents of La Reunion, and Filipinos. When worldwide clinical ascertainment data are compared with newborn screening data, it is clear that newborns with CAH (especially males) die when screening is not done.[25] To be effective in reducing mortality, newborn screening must be performed soon after birth, and the results must be available quickly so that early salt-wasting crises can be averted. It is preferable that newborn screening laboratories be operational 7 days a week, and that sample delivery from the collection site to testing laboratory be as efficient as possible, including weekends and holidays, so that undue testing delays are not encountered. These two requirements pose major challenges for most programs, but they are critical to optimal screening outcome.

Based on the studies in Texas, with second screening samples collected at approximately 2 weeks of age, some newborns with simple-virilizing CAH are missed on initial screening using current testing protocols. There is a need to set a screening cut-off such that the false-positive rate does not oversaturate the follow-up system, in part owing to the insensitivity of current kit methodologies and the biochemical manifestations of CAH. With advances in genetic testing procedures and improved automation techniques, it may soon be possible for CAH screening programs to include genotyping as a second-tier confirmation as a part of the newborn screening protocol. Despite the fact that CAH is a continuum of disorders, the correlation between genotype and phenotype is fairly consistent in most cases.[30] For the purpose of screening, genotyping will likely be useful only for differential diagnoses of non–salt wasters, given the necessary time constraints and expense of obtaining genotypes and the need for immediate diagnosis/treatment of salt wasters. It is hoped that newborn screening programs will begin to provide answers to some of these questions in addition to their primary function of reducing the morbidity and mortality resulting from CAH.

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CONGENITAL ADRENAL HYPERPLASIA OWING TO 21-HYDROXYLASE DEFICIENCY

Phyllis W. Speiser, MD

Congenital adrenal hyperplasia (CAH) owing to 21-hydroxylase deficiency is the most common cause of genital ambiguity in the newborn and is present in about 1 in 15,000 live births worldwide. The disease is further characterized in its classic salt-wasting form (~75% of cases) by potentially lethal adrenal insufficiency. A non–salt-wasting form of classic CAH with 21-hydroxylase deficiency is also recognized by genital ambiguity in affected females and by signs of androgen excess in later childhood in males. Nonclassic CAH with 21-hydroxylase deficiency may be detected in 1% to 3% of populations and is often mistaken for idiopathic precocious pubarche in children or polycystic ovary syndrome in young women. This article presents an overview of clinical and genetic aspects of the various forms of CAH with 21-hydroxylase deficiency. Other articles in this issue provide a detailed discussion of special topics, including prenatal diagnosis and treatment, neonatal screening, growth, and cognitive, psychosexual, reproductive, and novel treatment aspects of this disease. Table 1 (see Appendix) summarizes the genetic, hormonal, and clinical features of the steroidogenic defects discussed in this issue.

PATHOPHYSIOLOGY

The primary defect among patients with CAH owing to 21-hydroxylase deficiency is that the adrenal cortex cannot synthesize adequate

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Table 1. CHARACTERISTICS OF DIFFERENT FORMS OF CONGENITAL ADRENAL HYPERPLASIA

Characteristics	21-Hydroxylase Deficiency	11β-Hydroxylase Deficiency	Aldosterone Synthase Deficiency	17α-Hydroxylase Deficiency	3β-Hydroxysteroid Dehydrogenase Deficiency	Lipoid Hyperplasia
Defective gene	*CYP21*	*CYP11B1*	*CYP11B2*	*CYP17*	*HSD3B3*	*STAR*
Alias	P450c21	P450c11	P450aldo	P450c17	3β-HSD	
Chromosomal location	6p21.3	8q24.3	8q24.3	10q24.3	1p13.1	8p11.2
Ambiguous genitalia	+In females	+In females	No	+In males No puberty in females	+In males Mild in females	+In males No puberty in females
Addisonian crisis	+	Rare	Salt wasting only	No	+	++
Incidence (general population)	1:10,000–18,000	1:100,000	Rare	Rare	Rare	Rare
Hormones						
Glucocorticoids	↓	↓	Normal	Corticosterone normal	↓	↓
Mineralocorticoids	↓	↑	↓	↑	↓	↓
Androgens	↑	↑	Normal	↓	↓ In males ↑ In females	↓
Estrogens	Relatively ↓ in females	Relatively ↓ in females	Normal	↓	↓	↓
Physiology						
Blood pressure	↓	↑	↓	↑	↓	↓
Na balance	↓	↑	↓	↑	↓	↓
K balance	↑	↓	↑	↓	↑	↑
Acidosis	+	±Alkalosis	+	±Alkalosis	+	+
Elevated metabolites	17-OHP	DOC, 11-deoxycortisol	Corticosterone, ±18-hydroxy-corticosterone	DOC, corticosterone	DHEA, 17Δ⁵Preg	None

17-OHP = 17-hydroxyprogesterone; DOC = deoxycorticosterone; DHEA = dehydroepiandrosterone; 17Δ⁵Preg = 17-Δ⁵-pregnenolone; + = present; ++ = frequent; ↓ = decreased; ↑ = increased; ± = may or may not be present.

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amounts of the vital hormone cortisol. Inefficient cortisol synthesis leads to excessive secretion by the hypothalamus and pituitary of corticotropin-releasing hormone and corticotropin (ACTH), respectively. Chronic tropic hormone stimulation causes the adrenal glands to become hyperplastic. Similar pathophysiology applies in CAH owing to deficiency of several other enzymes crucial for cortisol synthesis, namely, 3β-hydroxysteroid dehydrogenase (3β-HSD), 11β-hydroxylase, and 17α-hydroxylase.

Because the adrenal cortex affected with CAH owing to 21-hydroxylase deficiency cannot produce cortisol, it instead produces excess sex hormone precursors that do not require 21-hydroxylation. These hormones are further metabolized outside the adrenal gland to more active androgens—testosterone and dihydrotestosterone, and to a lesser extent to estrogens—estrone and estradiol. In CAH owing to 21-hydroxylase deficiency, large quantities of adrenal-derived sex hormones are produced. Salient features associated with this inappropriate hormone secretion include androgen-induced virilization and disruption of normal ovarian function, estrogen-induced rapid epiphyseal maturation, and progestin interference with hypothalamic-pituitary-gonadal dynamics.

Most patients with CAH owing to 21-hydroxylase deficiency also have inadequate aldosterone; therefore, they cannot maintain sodium balance. If the condition is not recognized and diagnosed promptly, potentially life-threatening hyponatremic dehydration and shock occur. Approximately 25% of patients have sufficient aldosterone levels and no salt-wasting yet show prenatal virilization or markedly increased production of hormonal precursors of 21-hydroxylase (e.g., 17-hydroxyprogesterone). These individuals are referred to as *simple virilizers*. Girls with the milder nonclassic form of the disorder are distinguished by little or no virilization at birth.

STEROID BIOSYNTHESIS

The details of adrenal steroid synthesis relating to other enzymatic defects are discussed elsewhere in this issue. A brief overview of the biochemical consequences of 21-hydroxylase deficiency is given here. Pregnenolone is the common precursor of all three classes of adrenal cortical products, that is, mineralocorticoids, glucocorticoids, and sex hormones (Fig. 1). Aldosterone, the principal mineralocorticoid, is synthesized in the zona glomerulosa. The first several enzymatic reactions are held in common within the zona fasciculata as it synthesizes cortisol from 17-hydroxylated substrates, with divergence occurring only at the terminal enzymatic step. Pregnenolone is converted to progesterone by 3β-HSD in the endoplasmic reticulum and mitochondria.[61] Progesterone is in turn 21-hydroxylated in the endoplasmic reticulum by CYP21 (P450c21, 21-hydroxylase) to produce deoxycorticosterone (DOC). 11β-Hydroxylation of DOC produces corticosterone, which is then 18-hydroxylated and 18-oxidized to aldosterone. A single mitochondrial P450

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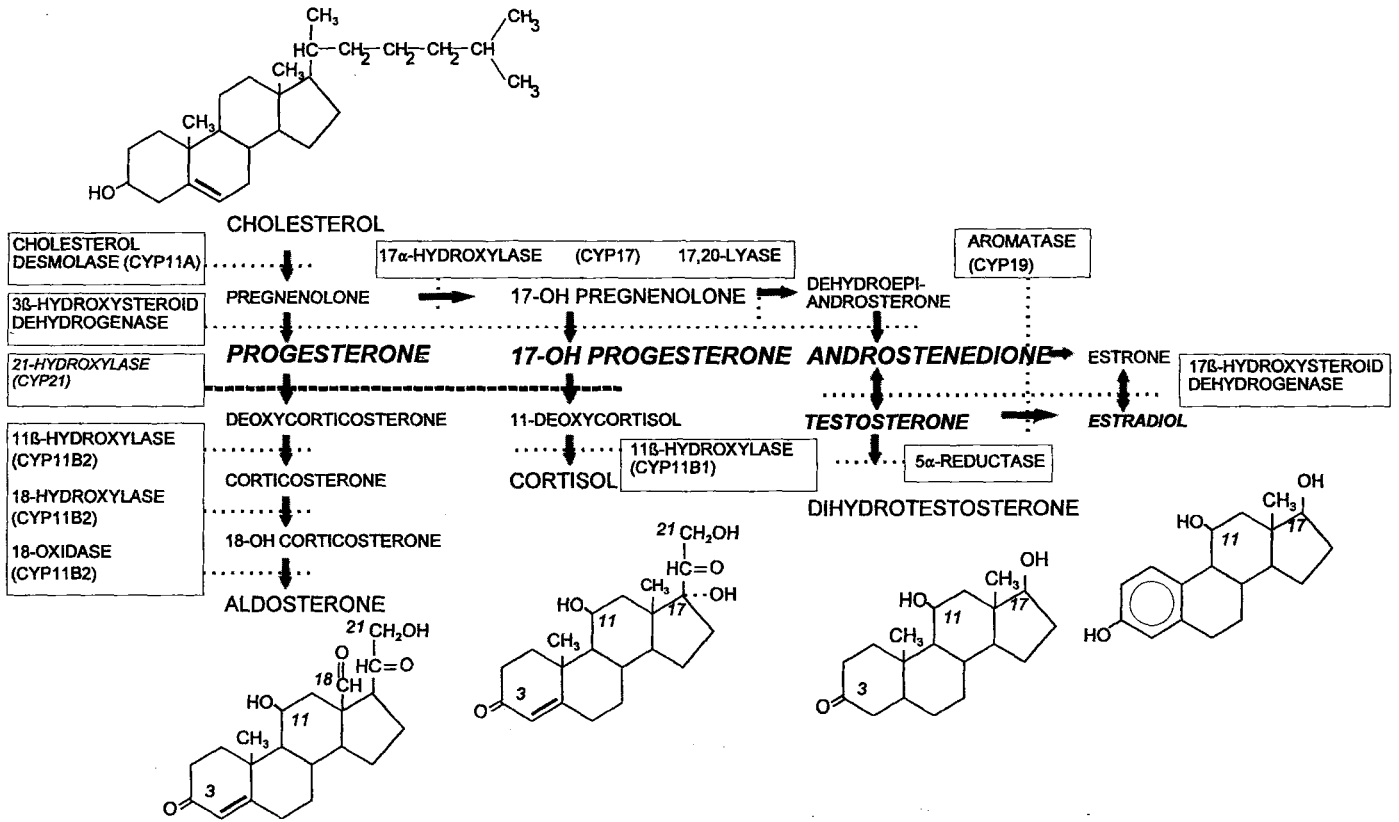

Figure 1. *See legend on opposite page.*

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enzyme expressed exclusively in glomerulosa, CYP 11B2 (P450aldo, aldosterone sythase, reviewed in the article by White) possesses 11-hydroxylase, 18-hydroxylase, and 18-oxidase activities.[82]

To produce cortisol, the major glucocorticoid in humans, pregnenolone is converted to 17α-hydroxypregnenolone by CYP17 (P450c17, 17α-hydroxylase/17,20 lyase).[94] In the next step, 17α-hydroxypregnenolone is a substrate for 3β-HSD in the zona fasciculata, producing 17α-hydroxyprogesterone. The latter is 21-hydroxylated by CYP21 to form 11-deoxycortisol, the immediate precursor of cortisol through 11β-hydroxylation (by CYP11B1 in mitochondria). In severe cases of CAH owing to 21-hydroxylase deficiency, neither aldosterone nor cortisol can be synthesized effectively, leading to shunting of precursors into the sex hormone pathways of the zona reticularis.

In adrenal cortical zona reticularis and in the gonads, the 17,20-lyase activity of CYP17 converts 17α-hydroxypregnenolone to dehydroepiandrosterone (DHEA, a C_{19} steroid and sex hormone precursor). DHEA is further converted by 3β-HSD to androstenedione. In the gonads, androstenedione is converted to testosterone and estrone by 17β-hydroxysteroid dehydrogenase and aromatase (CYP19, P450c19), respectively.[64] Testosterone may be further metabolized to dihydrotestosterone by steroid 5α-reductase[90] in androgen target tissues or to estradiol by aromatase.

GENETICS OF STEROID 21-HYDROXYLASE

The genes encoding human *CYP21* and a pseudogene, *CYP21P*, are located in the major histocompatibility complex on chromosome 6p21.3 approximately 30 kb apart, adjacent to and alternating with the *C4B* and *C4A* genes encoding the fourth component of serum complement (Fig. 2*A*).[83] Several other genes of uncertain function have been mapped to this region, most notably one encoding a putative extracellular matrix protein, tenascin-X, *TNX*, which is defective in some cases of Ehlers-Danlos syndrome.[14] *CYP21* and *CYP21P* each contain 10 exons spaced over 3.1 kb. Their nucleotide sequences are 98% identical in exons and

Figure 1. Pathways of steroid biosynthesis. The pathways for synthesis of progesterone and mineralocorticoids (aldosterone), glucocorticoids (cortisol), androgens (testosterone and dihydrotestosterone), and estrogens (estradiol) are arranged from left to right. The enzymatic activities catalyzing each bioconversion are written in boxes. For those activities mediated by specific cytochromes P450, the systematic name of the enzyme (CYP followed by a number) is listed in parentheses. CYP11B2 and CYP17 have multiple activities. CYP21 is depicted in smaller italic letters, and selected steroids are large, bold, and italicized, to indicate the consequences of congenital adrenal hyperplasia caused by 21-hydroxylase deficiency. The planar structures of cholesterol, aldosterone, cortisol, dihydrotestosterone, and estradiol are placed near the corresponding labels. 17-OH = 17-hydroxyprogesterone. (*Modified from* White PC, Speiser PW: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21:245–291, 2000; with permission.)

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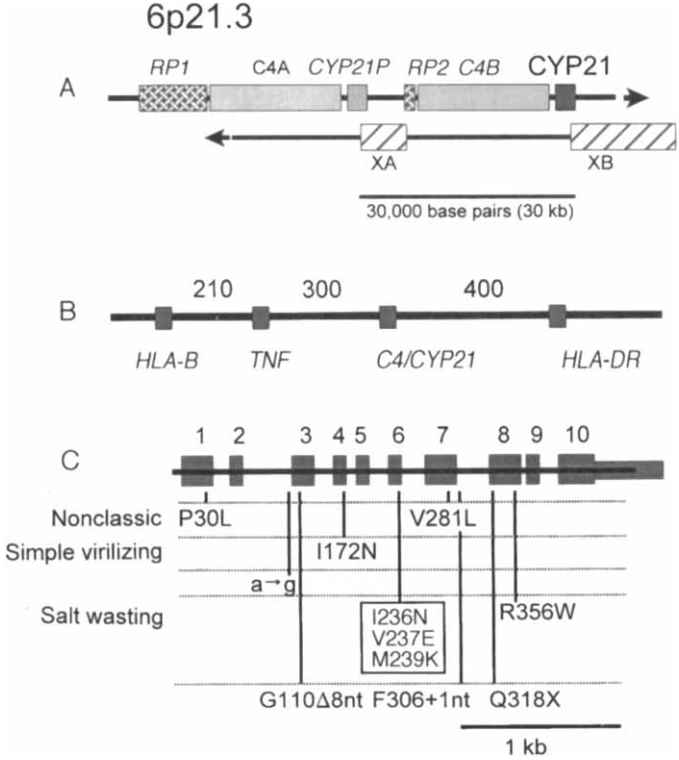

Figure 2. *A*, Map of the genetic region around the 21-hydroxylase (*CYP21*) gene. Arrows denote direction of transcription. *CYP21P* = 21-hydroxylase pseudogene; *C4A* and *C4B* = genes encoding the fourth component of serum complement; *RP1* = gene encoding a putative nuclear protein of unknown function; *RP2* = truncated copy of this gene. *TNXB* (XB, or tenascin-X gene) and *TNXA* (XA, or a truncated copy of this gene) are on the opposite chromosomal strand overlapping the 3′ end of each *CYP21* gene. The 30-kb scale bar is positioned to show the region involved in the tandem duplication location of the *CYP21* genes within the *HLA* major histocompatibility complex on chromosome 6p21.3 XA = Tenascin-X truncated gene; XB = Tenascin-X active gene. *B*, An extended view of the short arm of chromosome 6. Numbers denote distances between genes in kilobasepairs (kb). The *HLA-B* and *HLA-DR* histocompatibility genes flank the *CYP21* gene. The centromere is nearest *HLA-DR*. *TNF* (tumor necrosis factor) (actually two genes, *TNF A* and *B*) is situated between the *C4/CYP21* region and *HLA-B*. There are many other genes in this region with functions as yet unknown. *C*, The location and functional significance of the nine most common mutations (other than deletion) found in patients with congenital adrenal hyperplasia caused by 21-hydroxylase deficiency. Numbered boxes represent exons. (*Modified from* White PC, Speiser PW: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21:245–291, 2000; with permission.)

approximately 96% identical in introns.[31, 85] An important difference is that *CYP21P* transcripts do not contain a long open reading frame and cannot be translated into a functional enzyme.

Hormonal regulation of *CYP21* expression in the adrenal cortex is

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principally governed by ACTH.[24] ACTH action is mediated mainly through cyclic adenosine monophosphate (cAMP) and protein kinase A. Other stimuli, such as angiotensin II, protein kinase C, insulin, and insulin-like growth factor 1 (IGF-1), may induce *CYP21* transcription in cultured human adrenocortical carcinoma cells.[9]

Constitutive basal expression of *CYP21* depends on several transcription factors. Binding sites for Sp1, adrenal-specific protein (ASP), and steroidogenic factor 1 (SF-1) have been identified in the 5′ flanking sequence of the gene within minus 175 nucleotides from the translational start site.[31] More distal *cis*-acting regulatory elements may act as adrenal-specific enhancers for *CYP21* and *TNX*.[74]

Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency is inherited as a monogenic autosomal recessive trait closely linked to the *HLA* complex (Fig. 2*B*). No other form of CAH is linked to *HLA*. Siblings who have 21-hydroxylase deficiency are almost always *HLA* identical,[23] and these *HLA* markers were a major tool in prenatal diagnosis before cloning of *CYP21*.[57] Specific clinical forms of 21-hydroxylase deficiency are in genetic linkage disequilibrium with particular *HLA* haplotypes. Northern European patients with the salt-wasting form of the disease associated with *CYP21* deletion often carry *HLA-A3, Bw47, DR7*, whereas Ashkenazi Jewish patients with the nonclassic form of 21-hydroxylase deficiency associated with the conservative Val281Leu mutation carry *HLA-B14, DR1*. The associations between different clinical forms of CAH with particular HLA types vary according to ethnic group.[66]

To date, more than 40 *CYP21* mutations have been found in association with CAH owing to 21-hydroxylase deficiency (Table 2). Most of the commonly found mutations (Fig. 2*C*) arise from one of two types of recombinations between *CYP21* and the *CYP21P* pseudogene: (1) unequal crossing-over during meiosis resulting in deletion of *C4B* and *CYP21*,[84] or (2) apparent gene conversion events that transfer deleterious mutations normally present in *CYP21P* to *CYP21*.[22] Interestingly, *CYP21* has the highest rate of single nucleotide polymorphisms among over 100 human genes tested.[16] The high frequency of neutral polymorphisms and mutations may reflect a tendency to generate sequence diversity within the major histocompatibility complex, possibly conferring potential survival benefits.

The most common deleterious mutations in *CYP21* are a deletion spanning approximately 30 kb on chromosome 6p (~20% of affected haplotypes)[87] and an A→G substitution 13 nucleotides (nt) before the end of intron 2 that results in aberrant splicing of pre-mRNA (~20% to 25% of affected alleles).[30] Large gene conversions, in which multiple mutations are transferred from *CYP21P* to *CYP21*, may also be detected and account for approximately 10% of alleles in classic 21-hydroxylase deficiency.[67] Other commonly identified severe mutations include an 8 nt deletion in exon 3 and a 1 nt insertion in exon 7, each of which shifts the reading frame of translation. A cluster of three mutations in exon 6 introduces three nonconservative amino acid substitutions. A nonsense

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Table 2. MUTATIONS CAUSING 21-HYDROXYLASE DEFICIENCY

Name	Mutation/nt‡	E/I	Activity (% nl)§	Effect	Clinical Severity
Deletion*	—	—	0	No enzyme	SW
Conversion*	—	—	0	No enzyme	SW
+L9*	28+CTG	E1	100	Normal polymorphism	Normal
W22X†	66 G→A	E1	0	Nonsense	SW
W22+1nt	64+T	E1	0	Frameshift	SW
P30L*	89 C→T	E1	30–60	? Orientation in ER	NC
P30Q	89 C→A	E1	0	—	SW
Y47Δ1nt	138 ΔT	E1	0	Frameshift	SW
Intron 1 splice acceptor	295 A→G	I1		Abnormal splicing	SW
G90V	366 G→T	E2	0	—	SV
Intron 2 splice donor	387 G→A	I2		Abnormal splicing	—
Intron 2 "G"*	656 A/C→G	I2	<5	Abnormal splicing	SV-SW
Y97X	670 C→A	E3	0	Nonsense	
K102R	684 A→G	E3		Normal polymorphism	Normal
P105L	693 C→T	E3	60	—	NC
G110Δ8nt*	Δ708–715	E3	0	Frameshft	SW
C168Δ1nt	991–992 TG→A	E4	0	Frameshift	SW
I172N*	1001 T→A	E4	1	? Insertion in ER	SV
G178A	1019 G→C	E5	0–19	—	SW
D183E	1123 C→G	E5	100	Normal polymorphism	Normal
ΔE196	Δ1160–1162	E5	6–23	Unstable enzyme	—
I236N*	1382 T→A	E6	0	? Substrate binding	SW
V237E*	1385 T→A	E6			
M239K*	1391 T→A	E6			
S268T	1647 G→C	E7	100	Normal polymorphism	Normal
V281L*	1685 G→T	E7	20–50	? Insertion in ER ? Heme binding	NC
G291S	1715 G→A	E7	0.8	? Proton transfer from H_2O to heme	SW

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G291C	1715 G→T	E7	—	—	SW
W302X	1750 G→A	E7	0	Nonsense	SW
F306+1nt*	1759+T	E7	0	Frameshift	SW
Intron 7 splice donor	1781 G→C	I7	—	Abnormal splicing	SW
Intron 7 splice donor	1782 T→G	I7	—	Abnormal splicing	—
R316X	1990 C→T	E8	0	Nonsense	SW
Q318X*	1996 C→T	E8	0	Nonsense	SW
S330Δ10nt	Δ2032–2041	E8	0	Frameshift	SW
R339H	2060 G→A	E8	20–50	—	NC
R354H	2105 G→A	E8	0	Interactions with reductase	—
R356W*	2110 C→T	E8	0	Interactions with reductase	SW
R356P	2111 G→C	E8	0.2	Interactions with reductase	—
R356Q	2111 G→A	E8	1	Interactions with reductase	—
E380D	2267 G→T	E9	30	Decreased heme binding	—
V397+16nt	Duplication, 2303–2318	E9	0	Frameshift	SW
W405X	2341 G→A	E9	0	Nonsense	SW
G424S	2494 G→A	E10	—	—	SV
P453S	2580 C→T	E10	20–50	—	NC
P475Δ1nt	Δ2649	E10	0	Frameshift	—
R483P	2672 G→C	E10	1–2	Unstable enzyme	—
R483Δ1nt	2672–2273, GG→C	E10	0	Frameshift	SW
N493S	2702 A→G	E10	100	Normal polymorphism	Normal

Δ = deletion; + = insertion; nt = nucleotide; single-letter amino acid codes: A = alanine; C = cysteine; D = aspartic acid; E = glutamic acid; F = phenylalanine; G = glycine; H = histidine; I = isoleucine; K = lysine; L = leucine; M = methionine; N = asparagine; P = proline; Q = glutamine; R = arginine; S = serine; T = threonine; V = valine; W = tryptophan; Y = tyrosine; X = stop codon; E = exon; I = intron; ER = endoplasmic reticulum; SW = salt-wasting; SV = simple virilizing; NC = nonclassic.

*Mutations generated by intergenic recombinations between *CYP21* and *CYP21P*.

†As an example of mutation terminology, WZZX is a nonsense mutation of tryptophan-22.

‡Nucleotides are numbered beginning with the A in the initial ATG of the coding sequence. Introns are included. Numbering is based on a consensus of sequences. A, C, G, T are nucleotides and are not to be confused with single-letter amino acid codes.

§Activity when the mutant enzyme is expressed in cultured cells. When two numbers are present, the higher and lower numbers denote activity using 17-hydroxyprogesterone and progesterone as substrates, respectively.

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mutation at position 318 of exon 8 truncates the protein, rendering it nonfunctional, and Arg356Trp, also in exon 8, severely reduces enzymatic activity. The aforementioned mutations are usually associated with the classic salt-wasting form of CAH if the patient is homozygous or a compound heterozygote carrying each of two different, but equally deleterious, mutations on each allele. Prominent missense mutations associated with other forms of 21-hydroxylase deficiency include the simple virilizing mutation Ile172Asn in exon 4 and nonclassic mutations Val281Leu in exon 7 (~70% of nonclassic alleles) and Pro30Leu in exon 1. Together, these 10 mutations account for 95% of all affected alleles in most large genotyping studies of 21-hydroxylase–deficient patients.[86]

Correlation of Genotype with Phenotype

The functional effects of various mutations have been assessed in vitro,[29, 77] and the degree of enzymatic compromise has been correlated with the different clinical forms of 21-hydroxylase deficiency. Deletions and large conversions are most often found in salt-wasting patients; the intron 2 nt656G mutation is found in salt-wasting and simple virilizing patients; I172N is characteristically seen in simple virilizing patients; and V281L and P30L are found in nonclassic patients.[50] This distribution is consistent with the compromise in enzymatic activity conferred by each mutation. Because patients are often compound heterozygotes for different mutations, a simplistic model based on in vitro observations of mutant enzyme activity has reduced predictive value. Empiric observation indicates that the phenotype of each patient is likely to reflect his or her less severely impaired *CYP21* allele.

Mutations may be classified by the expected degree of enzymatic compromise as severe (class A), moderate (class B), or mild (class C). One might hypothesize that salt-wasting patients would have severe/severe genotypes, simple virilizing patients severe/moderate or moderate/moderate genotypes, and nonclassic patients severe/mild, moderate/mild, or mild/mild genotypes. In one study of 88 families,[67] these three predictions were correct in 90%, 67%, and 59% of cases, respectively. The overall correct classification rate was 79%. An expanded follow-up study of the same population[91] yielded even better results, with 177 of 197 patients (88%) correctly classified in this manner. Similar results were obtained in other studies using the same approach.[35, 81] These findings suggest an excellent correlation between genotype and phenotype in the vast majority of patients with CAH.

Categorical distinctions of salt-wasting, simple virilizing, and nonclassic CAH are qualitative in nature, sometimes based on the subjective impressions of clinicians with different criteria for diagnosis (Table 3). The distinction between simple virilizing and nonclassic disease is especially difficult in males in whom signs of androgen excess cannot readily be detected. In an attempt to correlate genotype with objective diagnostic criteria, quantitative measures of disease severity, such as basal and

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Table 3. FEATURES OF DIFFERENT CLINICAL FORMS OF CONGENITAL ADRENAL HYPERPLASIA OWING TO 21-HYDROXYLASE DEFICIENCY*

	Phenotype		
Feature	***Classic Salt Wasting***	***Classic Simple Virilizing***	***Nonclassic***
Age at diagnosis	Males, newborn to 6 months Females, newborn to 1 month	Males, 1.5 to 4 years Females, newborn to 2 years	Males, child to adult Females, child to adult
Genitalia	Males, normal Females, ambiguous	Males, normal Females, ambiguous	Males, normal Females ± ↑ clitoris
Aldosterone	↓	Normal	Normal
Renin	↑	May be ↑	Normal
Cortisol	↓	↓	Normal
17-OH-progesterone	>20,000 ng/dL	>10,000–20,000 ng/dL	1,500–10,000 ng/dL (ACTH-stimulated)
Testosterone	Males, ↑ in prepuberty only Females, ↑	Males, ↑ in prepuberty only Females, ↑	Males, variably ↑ in prepuberty only Females, variably ↑
Treatment	Glucocorticoid + mineralocorticoid (+ sodium)	Glucocorticoid (+ mineralocorticoid)	Glucocorticoid, if symptomatic
Somatic growth	− 2 to 3 SD, husky-obese	− 1 to 2 SD	nl to − 1 SD
Disease incidence†	1/15,000–20,000	1/50,000–60,000	1/1000
Typical mutations	Deletion Large conversion nt 656g ("intron 2 g") G110Δ8nt 1236N/V237E/M239K Q318X R356W	I172N nt 656g	V281L P30L
Percent enzymatic activity	0	1	20–50

SD = standard deviation scores; ↓ = decrease; ↑ = increase; ± = with or without.

†Incidence for classic CAH in a general population from neonatal screening in various populations.[56]

*Although distinct categories are assigned to the three major phenotypic forms of CAH, the true disease spectrum is better represented as a continuum with indistinct borders.

ACTH-stimulated 17-hydroxyprogesterone levels, plasma renin/urinary aldosterone ratios, and Prader genital virilization scores, have been used to classify patients. Interestingly, this analysis yielded similar correlations with genotype as when the broader clinical categories were used. In general, there is excellent discrimination in these quantitative measures of in vivo 21-hydroxylase activity between severe and mild genotypes but a high degree of phenotypic variability among patients with moderate genotypes (i.e., those carrying the Ile172Asn mutation).[67]

The most obvious explanation for genotype/phenotype discordance is that the apparent severity of the disease is part of a continuum. Patients with disease severity near the borders of the various classifica-

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tions may easily fall on either side of these borders. Several mutations are prone to this problem. First, although the intron 2 nt656G mutation is classified as *severe*, it is clearly *leaky* and may yield enough normally spliced mRNA to ameliorate the enzymatic deficiency in some patients. Second, the Ile172Asn mutant has marginal enzymatic function (1% of normal), which may not always be sufficient to prevent salt-wasting. These two explanations accounted for 12 of 20 examples of apparent discordance between genotype and phenotype in one study.[91] Significant phenotypic variation was observed within a single kindred in which all five affected individuals spanning two generations were compound heterozygotes for these two mutations.[17] Third, because many patients who are discordant for genotype and phenotype are compound heterozygotes for mutations that compromise enzymatic activity to different extents, in vivo enzymatic activities are actually intermediate between those seen in patients who are homozygous for each mutation. Consistent with this idea, presumed compound heterozygotes for a classic and nonclassic allele as a group have higher stimulated 17-hydroxyprogesterone levels than presumed homozygotes for nonclassic alleles.[69] Fourth, in studies relying on screening limited to known mutations, additional novel mutations within *CYP21* might not be detected and might further decrease enzyme activity.

One must also consider that genetic or environmental factors other than 21-hydroxylase activity influence phenotype. The degree of salt-wasting tends to improve with time, even in subjects who are genetically predicted to have no 21-hydroxylase activity,[65] and genetically identical siblings are occasionally discordant for the severity of salt-wasting.[73] These observations may reflect expression of additional 21-hydroxylase activities not encoded by *CYP21*.[48, 92] The timing and severity of a salt-wasting crisis may additionally reflect the individual's reserve to cope with sodium and fluid depletion. Ancillary factors important in these homeostatic mechanisms include the concentration and transcriptional activity of mineralocorticoid receptors and receptor coactivators or corepressors, the ability to increase vasopressin, and the ability to decrease atrial natriuretic factor.

Similarly, genetically based variations in androgen biosynthesis or sensitivity to androgens would be expected to influence expression of signs of androgen excess. Girls with CAH, even siblings, do not manifest the same degree of genital ambiguity; thus, the physical signs of androgen excess are not exclusively dependent on levels of adrenal androgen precursors. Presumably, the efficiency with which such hormones are converted to more potent dihydrotestosterone by peripheral enzymes such as 5α-reductase[90] and the concentration and transcriptional activity of androgen receptors influenced by a highly polymorphic CAG repeat sequence within the coding region[18] have a role in determining genital phenotype. Although not reported to date, it is reasonable to speculate that variations in the biologic response to androgens may be attributable to polymorphisms in sex hormone receptor coactivators and corepressors.

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Applied Genetics

Knowing an established patient's *CYP21* genotype does not necessarily aid in making treatment decisions. Nevertheless, genotyping is helpful in performing prenatal diagnosis when there is a known proband in the family (see the article by New). Errors in prenatal diagnosis can be minimized by genotyping both parents whenever possible and by the concomitant use of linked microsatellite markers. Two large-scale studies have shown the utility of *CYP21* genotyping as an adjunct to hormonal measurements in newborn screening programs.[27, 53]

CLINICAL PRESENTATION: CLASSIC CONGENITAL ADRENAL HYPERPLASIA OWING TO 21-HYDROXYLASE DEFICIENCY

Physical Stigmata in Newborns

Although anecdotal observations suggest there may be some phallic enlargement and scrotal hyperpigmentation in affected infant boys, many newborn boys with 21-hydroxylase deficiency show no overt signs of adrenal androgen excess. Male sexual differentiation is normal. In contrast, in newborn girls with virilizing CAH, high levels of circulating adrenal androgens beginning at about 7 weeks' gestation prevent the formation of separate vaginal and urethral canals, leaving these girls with a common urogenital sinus. Clitoral enlargement occurs as adrenal-derived androgens interact with genital skin androgen receptors. In severe cases, there may be partial or complete fusion and rugation of the labial folds and rostral migration of the common urethral/vaginal orifice. Although the external genitalia may be virilized, 46,XX patients with CAH do not usually develop internal wolffian structures such as the prostate gland and spermatic ducts, presumably because development of the wolffian ducts requires much higher local concentrations of testosterone.

The typical girl with classic CAH has ambiguous or male-appearing external genitalia with perineal hypospadias, chordee, and undescended testes. The severity of virilization is often described using a 5-point scale developed by Prader, with stage I representing minimal clitoral enlargement and stage V resembling a cryptorchid male with a penile urethra.[58]

Postnatal Signs of Abnormal Sex Hormone Production

Ongoing adrenal sex steroid production in the untreated or incompletely treated patient causes several problems. Boys show inappropriately rapid somatic growth accompanied by marked advancement of epiphyseal maturation after about 18 months.[76] Pubic hair and apocrine

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body odor develop, and penile size increases without testicular enlargement. Girls may show similar signs of sex steroid excess and progressive clitoral enlargement. In adolescence, girls with poorly controlled disease manifest acne, hirsutism, and ovarian dysfunction.

Although children with CAH grow too rapidly, adult height is often below average in severely affected patients when compared with the general population and with parentally determined target height[51] (see the article by Migeon and Wisniewski for a discussion of growth in CAH). Balancing medical treatment is not simple in CAH. Untreated or inadequately treated patients grow rapidly and may not reach their height potential, whereas patients treated with excessive doses of glucocorticoids inevitably have growth retardation.[39] Sensitivity to exogenously administered glucocorticoids may be determined in part by interindividual variations in drug metabolism and polymorphisms in the glucocorticoid receptor affecting in vivo action[33] (see the article by Migeon and Wisniewski for a discussion of growth and the article by Merke and Cutler for a discussion of new treatment regimens for CAH).

Reproductive Function in Girls with Congenital Adrenal Hyperplasia

The presence of ambiguous genitalia is strongly associated with this disease in girls and has far-reaching implications regarding psychosexual and reproductive function. On a more subtle level, hypothalamic-pituitary-gonadal function may be perturbed in the setting of adrenal sex hormone excess. For instance, infant girls with CAH have higher than typical luteinizing hormone (LH) levels, similar to levels observed in healthy infant boys.[7] Women with well-controlled classic CAH, but not women with nonclassic CAH, have exaggerated LH responses to gonadotropin-releasing hormone (GnRH) and increased production of ovarian androgens, consistent with imprinting by prenatal exposure to androgens or progestins.[5] These tests are not pathognomonic for CAH.

Reproductive problems for women with CAH become apparent in adolescence. The average age at which menarche occurs in inadequately treated girls is late when these patients are compared with healthy peers.[28] Such girls and women with CAH often have a clinical presentation similar to polycystic ovarian syndrome with one or more problems, including sonographic evidence of multiple cysts, anovulation, irregular bleeding, and hyperandrogenic symptoms. Moreover, a significant reduction in insulin sensitivity, although not frank diabetes, is found among nonobese young women with nonclassic CAH when compared with controls of similar age and weight.[71] Insulin resistance is also associated with functional ovarian hyperandrogenism independent of obesity, although the precise pathophysiology in both disease states is uncertain.

Several hypotheses have been proposed to explain reproductive dysfunction in CAH. The aromatization of excess adrenal androgen to

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estrogen[12] or excess adrenal progesterone could inhibit normal hypothalamic-pituitary cyclicity.[28] Alternatively, elevated sex steroids could induce abnormal ovarian function by programming the hypothalamus early in development.[5] Direct gonadal damage might be inflicted by androgen excess or by displacement of normal gonadal parenchyma by adrenal rest tissue.

The majority of women eventually undergo menarche. In general, the regularity of menses depends on the adequacy of treatment. A small proportion of women do not undergo menarche and are unable to suppress progesterone levels even when 17-hydroxyprogesterone is adequately suppressed.[32] Adrenalectomy is sometimes considered in this setting when medical therapies are unsuccessful in achieving adrenal suppression.[79]

Breast development is often suboptimal in women with CAH. Evidence from animal studies suggests that testosterone exposure in utero may inhibit growth of the breast anlage, resulting in poor breast development at adolescence.[34]

Pregnancy outcome in women affected with classic CAH is reviewed elsewhere in this issue. Interestingly, despite elevated maternal testosterone of 400 to 600 ng/dL, unaffected female offspring have no genital virilization.[47] Placental aromatase effectively prevents maternal androgens from reaching the fetus. Elevated maternal sex hormone–binding globulin and androgen antagonism by progesterone also contribute to restricting transplacental passage of androgens.

Reproductive Function in Men with Congenital Adrenal Hyperplasia

Impaired gonadal function is less frequent among men with classic CAH when compared with affected women. Most men with CAH can father children or at least have normal sperm counts.[78] Oligospermia, observed in classic and nonclassic CAH, does not preclude fertility.[59] Among simple virilizing patients, testicular integrity may be normal even in the absence of treatment.[89] A prominent complication in men with CAH is the development of testicular adrenal rests.[63]

Other Problems

Anatomic adrenocortical hyperplasia is not seen consistently in CAH. The diagnostic utility of adrenal ultrasound may be enhanced in newborn infants by examining not only the size but the shape, surface contours, and echogenicity of the adrenal.[1] Steroid treatment can reverse such structural anomalies.

The incidence of adrenal masses increases with age and is higher in patients with CAH and heterozygotes than in the general population.[36] Steroid-responsive hyperplastic adrenal nodules can be the first pre-

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senting sign in previously undiagnosed patients late in life and can be confused with virilizing adrenal adenomas.[60] Rarely, virilizing adrenal carcinoma has been found in adult patients with CAH[6]; most adrenal masses in children with CAH are benign.[46]

Testicular adrenal rests (also referred to as *testicular tumors of adrenogenital syndrome*), sometimes nearly obliterating normal testis parenchyma, may occur in boys with CAH, especially if they are inadequately treated salt wasters.[3, 12] Testicular masses have been detected in boys with classic CAH as young as 3 years old,[72] prompting the recommendation that boys undergo careful testicular examinations and a baseline testicular sonogram by adolescence. Testicular tumors in boys with CAH, although most often benign, may have a rock-hard consistency, prompting extreme concern leading to biopsy and sometimes even orchiectomy. The preferred mode of treatment is effective long-term adrenal suppression with dexamethasone because many of these tumors are ACTH responsive. When they do not respond to dexamethasone, testis-sparing surgery may be performed after imaging of the tumor by sonography or MR imaging.[80] Adrenalectomy would not be expected to alleviate problems caused by gonadal adrenal rests.

Clinically significant pituitary tumors have not been observed in patients with CAH, despite the fact that glucocorticoid replacement doses do not fully suppress elevated corticotropin-releasing hormone and ACTH. In one study, four of seven CAH patients showed incidental pituitary abnormalities on MR imaging (three patients had apparent microadenomata and one an empty sella).[68] White matter changes seen on brain MR imaging in some young adult patients with CAH do not seem to be associated with any neurologic deficits. To date, there is no demonstrated association between androgen excess in girls with CAH and structural brain differences. Although girls with CAH often lack maternal feelings and have tomboyish behavior, heterosexual orientation is most common in adult women with CAH.[21] Cognitive impairment is known to be associated with severe or frequent salt-wasting episodes, but most patients with CAH have a normal overall intelligence quotient.[8] A detailed discussion of these topics can be found in articles by Berenbaum and Meyer-Bahlburg elsewhere in this issue.

Hormonal Profile

The characteristic hormonal abnormality of classic 21-hydroxylase deficiency is a markedly elevated serum level of 17-hydroxyprogesterone, the main substrate for the enzyme (Fig. 3). Basal 17-hydroxyprogesterone values usually exceed 10,000 ng/dL in affected infants, whereas normal newborn levels are below 100 ng/dL. At least 10% of severely affected infants have low initial levels in the newborn period,[19] especially if levels are obtained on the day of birth.[75] Conversely, most sick or premature infants have elevated 17-hydroxyprogesterone levels without having inborn errors in steroid biosynthesis.[27] Repeat hormone measure-

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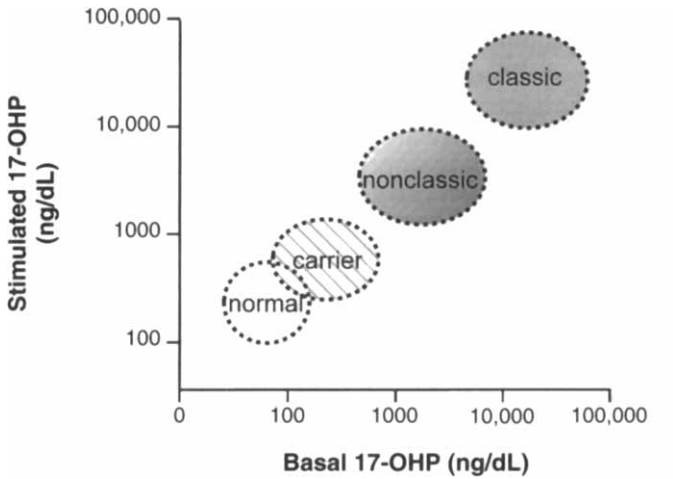

Figure 3. Nomogram for comparing 17-hydroxyprogesterone (17-OHP) levels before and 60 minutes after a 0.25-mg intravenous bolus of cosyntropin in subjects with or without 21-hydroxylase deficiency. Note that the values for normals and heterozygotes (carriers) overlap. (*From* White PC, Speiser PW: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21:245–291, 2000; with permission.)

ments usually help in determining the diagnosis. The advantage of ACTH (cosyntropin) stimulation is that one can differentiate the various forms of CAH by comparing precursor-to-product ratios after stimulation. The findings are clinically relevant for several reasons. First, unaffected stressed newborns with transient elevations of 17-hydroxyprogesterone need not be treated with steroids. On the other hand, if 21-hydroxylase deficiency is erroneously diagnosed instead of 3β-HSD or 11β-hydroxylase deficiency, inappropriate genetic counseling will inevitably be given. Furthermore, 11β-hydroxylase patients are often hypertensive and require additional treatment for this problem.

Assays for serum adrenocortical steroid hormones should be performed in laboratories with high standards for quality control, including pediatric reference standards and the ability to measure accurately most or all of these hormones in small volumes. The assay should include preliminary chromatography to avoid problems of cross-reactivity when some hormone levels are extremely high. The laboratory should be informed that CAH is suspected, and that hormonal levels may be beyond the standard range of measurement, necessitating sample dilutions. The laboratory of choice can be a serious concern when working in a managed care environment.

The severity of abnormalities observed in the hormonal profile depends on the type of CAH. Patients with salt-wasting disease have the highest levels of 17-hydroxyprogesterone (up to 100,000 ng/dL after ACTH stimulation), followed by simple virilizing patients, who tend to

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have somewhat lower levels, although the range overlaps that seen in salt-wasting patients. Patients with the nonclassic form of CAH have more modestly elevated hormone levels, especially in the newborn period.[52] Random measurements of basal serum 17-hydroxyprogesterone are often normal in nonclassic patients (unless performed in the early morning before or at 8 AM), and the diagnosis is most reliably made by the response to ACTH stimulation. A newer fluoroimmunoassay may improve the sensitivity of the random 17-hydroxyprogesterone measurement.[13] The author uses a uniform dose of 0.25 mg of cosyntropin, providing a pharmacologic stimulus to the adrenal cortex. Testing can be performed regardless of time of day. This diagnostic test should be distinguished from the low-dose ACTH stimulation test now becoming increasingly popular for evaluating the integrity of the hypothalamic-pituitary-adrenal axis.[95]

Other hormones that are elevated in 21-hydroxylase deficiency include progesterone, androstenedione, and, to a lesser extent, testosterone. An atypical steroid, 21-deoxycortisol, is also characteristically elevated.[26] DHEA, the main adrenocortical product, is not a particularly good marker of 21-hydroxylase activity. DHEA-sulfate binds with high affinity to albumin, has a long plasma half-life, and is not responsive to acute change or to ACTH stimulation. Finding elevated 24-hour urinary levels of pregnanetriol (a metabolite of 17-hydroxyprogesterone) is also of diagnostic value.

Salt-Wasting

Approximately 75% of patients with classic CAH have severely impaired 21-hydroxylation of progesterone and cannot synthesize adequate amounts of aldosterone. Ideally, measurement of serum or plasma and urinary aldosterone should be correlated with plasma renin activity and with sodium balance for an accurate assessment of phenotype. In interpreting levels of plasma renin activity, it should be kept in mind that they are normally higher in neonates than in older children, and that age-specific reference values for plasma renin vary by laboratory. Because aldosterone is essential for normal sodium homeostasis, individuals with inadequate reserve have sodium loss through the kidney, colon, and sweat glands and are termed *salt wasters*. Concomitant severe cortisol deficiency exacerbates the systemic effects of aldosterone deficiency because glucocorticoids contribute to cardiac contractility, cardiac output, and regulation of vascular response to catecholamines and other pressors.[44] Hyponatremic dehydration and shock (adrenal crisis) are common in untreated or inadequately treated salt-wasting 21-hydroxylase deficiency. Recent data suggest that catechol secretion is deficient in CAH, potentially contributing to inadequate response to stress.[48a]

Among the 25% of patients who have adequate aldosterone production, accumulated steroid precursors may directly antagonize aldosterone's action at the mineralocorticoid receptor.[42] Progesterone is well known to have antimineralocorticoid effects,[54] and that steroid or a

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metabolite are likely culprits in this phenomenon. There is no evidence that 17-hydroxyprogesterone has direct or indirect antimineralocorticoid effects.

Symptoms of impending adrenal crisis owing to salt-wasting may include poor appetite, vomiting, lethargy, and failure to gain weight. These rather nonspecific manifestations of disease may be mistaken for formula intolerance, colic, sepsis, renal tubular acidosis, pyloric stenosis, or other common diseases of the newborn. Severely affected patients with CAH usually come to medical attention by 1 to 4 weeks of age but, occasionally, not until several months of age.[40] Physical examination shows a lethargic, gray, or mottled infant with a rapid pulse and low or unobtainable blood pressure. Treatment should include immediate administration of one or more boluses (20 mL/kg) of isotonic intravenous fluids and pressor support if required. Initial laboratory studies show hyponatremia and hyperkalemia; plasma renin activity is markedly elevated. These adrenal crises may prove fatal if proper medical care is not delivered. If CAH is suspected in a patient, one should obtain blood for hormonal measurements and then treat immediately with a 100 mg/m^2 dose of hydrocortisone by the parenteral route (usually, 25 mg in infants). Continuing hydrocortisone treatment may then be given in divided doses every 6 hours until the child is stable. This regimen of intravenous fluids and hydrocortisone provides adequate sodium and mineralocorticoid replacement. Maintenance treatment is discussed in more detail in a later section.

Epidemiology

Overall, about 1 in 15,000 newborn infants are affected with classic CAH. Morbidity and mortality are particularly high in affected infant boys[56] who have no genital ambiguity to alert physicians to the diagnosis before the onset of dehydration and shock.[88] There are no accurate records regarding exact mortality rates for CAH. Many states in the United States (approximately 22 and increasing) and several countries (e.g., Switzerland, Japan, New Zealand) have adopted newborn screening for CAH (discussed in the article by Therrell).

NONCLASSIC CONGENITAL ADRENAL HYPERPLASIA OWING TO 21-HYDROXYLASE DEFICIENCY

Clinical Findings

The nonclassic condition is a mild, more common allelic variant of severe classic CAH owing to 21-hydroxylase deficiency.[70] Patients with the nonclassic form of 21-hydroxylase deficiency may have any of the signs of postnatal androgen excess listed previously, but affected female infants are born with nonambiguous (normal or with mild clitoromegaly) external genitalia. The severity of the associated mild androgen

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excess varies widely, and many affected individuals never come to medical attention. Premature adrenarche with mildly elevated DHEA-sulfate and moderately elevated 17-hydroxyprogesterone is the most frequent manifestation of untreated nonclassic CAH in young children. The most common presenting signs in adolescents and young adults are cystic acne, hirsutism, and oligomenorrhea.[41] Nonclassic adolescent or young adult male patients sometimes present with acne or infertility[2] but are most often diagnosed in the course of family studies and are asymptomatic. Infertility and testicular adrenal rest rumors are uncommon in these male individuals.

The explanations for the less frequent and milder reproductive problems observed in nonclassic CAH are thought to be similar to those proposed for classic CAH. Data regarding reproductive function in nonclassic CAH come mainly from studies of an inherently biased referral population who have sought treatment for symptoms and signs of hyperandrogenism with or without infertility. In one study, 39% of women presented with hirsutism, 39% with oligomenorrhea or other signs of polycystic ovaries, and 22% with no obvious signs of androgen excess.[20] Based on the information derived from individuals detected through family studies, it is clear that many cases of mild 21-hydroxylase deficiency remain undiagnosed because of a lack of clinically important symptoms. French investigators found that approximately 50% of their patients became pregnant before the diagnosis of nonclassic CAH was made. The other patients who desired pregnancy conceived during hydrocortisone treatment, and only 1 of 20 women required additional treatment with clomiphene citrate to conceive.[25] To the best of this author's knowledge, there have been no cases of virilized infant girls (an infant unaffected with CAH) born to affected mothers.

In contrast to studies of height in classic CAH, height standard deviation scores (SDS) in adults with nonclassic CAH are reasonably good. One study demonstrated that the height SDS were lower in comparison with those in the general population (-0.99 $\pm$ 0.98) but not when compared with midparent heights (0.43 $\pm$ 0.77).[51] Similarly, other investigators found no differences between nonclassic patients and their unaffected siblings.[15]

Hormonal Profile

Because of the circadian variability of adrenocortical hormones, the diagnosis may be missed by measuring only baseline serum 17-hydroxyprogesterone after 8 AM; however, an early morning basal 17-hydroxyprogesterone below 200 ng/dL is seldom associated with nonclassic 21-hydroxylase deficiency. Depending on the laboratory, affected individuals have serum 17-hydroxyprogesterone levels of greater than 1000 or 1500 ng/dL 60 minutes after an intravenous bolus of cosyntropin (ACTH 1-24).

Aldosterone synthesis and sodium balance are not significantly im-

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paired in patients with nonclassic 21-hydroxylase deficiency,[37] although under stress conditions, subtle abnormalities may be elicited. Cortisol synthesis during stress is similarly intact, and there have been no deaths from adrenal insufficiency reported with this condition.

Prevalence

Signs of androgen excess in nonclassic 21-hydroxylase deficiency can be difficult to detect, especially in men; therefore, case reports are not helpful in determining prevalence. Estimates of allele and disease frequencies have been derived from ascertainment of affected individuals in the course of pedigree analysis, that is, classic and nonclassic 21-hydroxylase deficiency coexist within the same kindred.[66] The disease frequency is estimated at 0.1% of the general population, 1% to 2% of Hispanics and Yugoslavs, and 3% to 4% of Ashkenazi (Eastern European) Jews. Similar frequencies have been estimated from a small screening study of a mixed ethnic asymptomatic adult group of volunteers using morning salivary 17-hydroxyprogesterone levels.[96] Molecular genetic screening of normal newborns in New Zealand showed that 5% were carriers for mutations in the 21-hydroxylase gene (*CYP21*) associated with either classic (2.8%) or nonclassic (2%) 21-hydroxylase deficiency.[27] This method, more sensitive for the detection of heterozygotes, implies a disease frequency for nonclassic 21-hydroxylase deficiency of 0.06%, slightly higher than estimates based on case ascertainment and hormone measurements in other populations.

Although it has been suggested that nonclassic 21-hydroxylase deficiency is among the most frequent autosomal recessive genetic disorders in humans,[66] the proportion of affected individuals who have problems attributable to androgen excess is not known. There has been no prospective study of symptomatology in any nonclassic 21-hydroxylase deficiency patient population, although the frequency of symptomatology will eventually become apparent as more cases are detected through newborn screening. In light of the stigma and anxiety that may be associated with the diagnosis of a *genetic disease*, it is suggested that nonclassic 21-hydroxylase deficiency initially be considered a *genetic polymorphism* and treated as a disease only if clear signs of androgen excess develop.

Only a small percentage of individuals presenting with signs of androgen excess prove to be affected with nonclassic 21-hydroxylase deficiency. Among the children referred for precocious pubarche, 4% to 7% have nonclassic 21-hydroxylase deficiency.[4] In the largest study of hyperandrogenic women, 6% of 400 hirsute French women had hormonal profiles compatible with the diagnosis of late-onset CAH.[43] These statistics have been confirmed in other large clinic population samples. Variations in the frequency of nonclassic alleles among different ethnic groups may account for some of the discrepancies noted. New York City has a high proportion of Ashkenazi Jews. This group has the highest

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frequency of nonclassic CAH associated with the typical *CYP21* allele, valine-to-leucine 281 (V281L), and with duplication of the 21-hydroxylase pseudogene and *HLA-B14, DR1*.

Heterozygotes

Carriers of a single mutant allele have slightly elevated 17-hydroxyprogesterone levels after ACTH stimulation. The range of the 17-hydroxyprogesterone response of most heterozygotes at 60 minutes following cosyntropin stimulation is approximately 200 to 1000 ng/dL, overlapping the general population to some extent.[91] In one study, only 50% of obligate heterozygotes had 17-hydroxyprogesterone measurements after ACTH stimulation that differed significantly from those of genotypically normal individuals.[93] Other investigators found that approximately 70% of women with hyperandrogenic symptoms and hormonal evidence suspicious for CAH heterozygosity were actually found to be heterozygous for *CYP21* mutations.[10] Heterozygotes can be more reliably identified hormonally by examining the ratio of 17-hydroxyprogesterone to cortisol[45]; however, genotyping is the gold standard for purposes of genetic counseling, especially when the proband's mutation is known. With estimated nonclassic and classic heterozygote frequencies of 10% and 1.5% in the general population, respectively,[66] it is unlikely that heterozygosity confers any reproductive disadvantage. In fact, screening of subjects referred for evaluation of infertility has not revealed a high prevalence of nonclassic 21-hydroxylase deficiency or heterozygotes,[55] suggesting that heterozygotes for CAH alleles do not have any significant hormone imbalance and do not require hormonal replacement therapy.

CONVENTIONAL HORMONAL REPLACEMENT THERAPY

Glucocorticoids

Patients with classic 21-hydroxylase deficiency and symptomatic patients with nonclassic disease require glucocorticoid treatment. Glucocorticoids inhibit excessive secretion of corticotropin-releasing hormone and ACTH by the hypothalamus and pituitary and reduce the elevated levels of adrenal sex steroids. In children, the preferred cortisol replacement is hydrocortisone (i.e., cortisol itself) in doses of 10 to 20 mg/m^2/d in two or three divided doses. These doses exceed physiologic levels of cortisol secretion, which are 6 to 7 mg/m^2/day in children and adolescents.[38] Although cortisol secretion is normally only slightly higher in neonates (7–9 mg/m^2/d[49]), infants with CAH are usually given a minimum of 6 mg/d in three divided doses. Supraphysiologic doses

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seem to be required to suppress adrenal androgens adequately in CAH patients.

The short half-life of hydrocortisone minimizes growth suppression and other adverse side effects of longer-acting, more potent glucocorticoids, such as prednisone and dexamethasone. On the other hand, a single daily dose of a short-acting glucocorticoid is ineffective in controlling adrenocortical hormone secretion. Cortisone acetate is not a first choice for CAH therapy. It has only 80% of the bioavailability of hydrocortisone and approximately two thirds of its potency. Moreover, cortisone must be converted to cortisol to be biologically active.

Liquid hydrocortisone preparations may not be homogeneously mixed, resulting in erratic hormonal control; crushed and weighed tablets may be substituted. Children who are compliant but nonetheless difficult to control with hydrocortisone alone may otherwise benefit from morning hydrocortisone combined with a nighttime dose of prednisone to suppress more effectively the overnight rise in ACTH. Older adolescents and adults can be treated with prednisone (e.g., 5–7.5 mg daily in two divided doses) or dexamethasone (total of 0.25–0.5 mg given as one or two daily doses). Patients should be monitored carefully for signs of iatrogenic Cushing's syndrome, such as rapid weight gain, hypertension, pigmented striae, and osteopenia. Men with testicular adrenal rests may require higher dexamethasone doses to suppress ACTH.

Treatment efficacy is assessed by monitoring 17-hydroxyprogesterone and androstenedione levels. Testosterone levels can also be a useful parameter in girls and prepubertal boys. Because of the adverse effects of overtreatment, it is not desirable to suppress endogenous adrenal corticosteroid secretion completely. A target 17-hydroxyprogesterone range might be 100 to 1000 ng/dL with commensurate age and gender-appropriate androgen levels.[11] Hormones should be measured at a consistent time in relation to medication dosing, preferably at 8 AM at the physiologic peak of ACTH secretion, or at least at the nadir of hydrocortisone blood levels just before the next dose is to be given. Children should also have an annual bone age radiographic study and careful monitoring of linear growth.

Stress Dosing

Patients with classic CAH cannot mount a sufficient cortisol response to stress and require pharmacologic doses of hydrocortisone in situations such as febrile illness and surgery under general anesthesia. Such treatment should ideally approximate typical endogenous adrenal secretion in critically ill and perioperative patients,[44] although common practice is to err on the side of giving larger doses of glucocorticoids. Traditional dose guidelines include doubling or tripling the maintenance dose of oral hydrocortisone (administered in three divided doses) in minor febrile illnesses. If a patient is unable to tolerate oral medication,

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intramuscular hydrocortisone sodium succinate (Solu-Cortef) can be given at home, but medical advice should be promptly sought concerning the need for intravenous hydration. Patients and their parents should receive instructions for these types of emergency contingencies, and patients should carry or wear identification with information about their medical condition. For major surgery, the administration of hydrocortisone (empiric doses of 100 mg/m^2/d) divided in four intravenous doses is warranted for at least 24 hours perioperatively and postoperatively before tapering over several days to a maintenance dose. Intravenous hydrocortisone is preferred over equivalent glucocorticoid doses of methylprednisolone (Solu-Medrol) or dexamethasone because (when it is administered in high doses) its mineralocorticoid activity can substitute for oral fludrocortisone.

Patients with nonclassic CAH do not require stress doses of hydrocortisone for surgery unless they have iatrogenically been rendered hypoadrenal by earlier chronic administration of glucocorticoids. If given adequate advance notice, one could discontinue treatment and test the integrity of the hypothalamic-pituitary-adrenal axis with a low-dose ACTH stimulation test.[95] There are no reports of fatal adrenal insufficiency among patients with nonclassic CAH.

Mineralocorticoids

Infants with the salt-wasting form of 21-hydroxylase deficiency require mineralocorticoid (fludrocortisone, usually 0.1 to 0.2 mg but occasionally up to 0.4 mg daily) and sodium chloride supplements (1 to 2 g daily, with each gram of sodium chloride containing 17 mEq of sodium) in addition to glucocorticoid treatment. The sodium content of breast milk and most infant formulas (about 8 mEq/L) is insufficient to supply sodium requirements, except in healthy term infants. Older children acquire a taste for salty food, do not require daily supplements of sodium chloride tablets, and often have lower fludrocortisone dose requirements.

Although patients with the simple virilizing form of the disease by definition secrete adequate amounts of aldosterone, they are nevertheless often treated with fludrocortisone. This treatment can aid in adrenocortical suppression, reducing the dose of glucocorticoid required to maintain acceptable 17-hydroxyprogesterone levels.[62] Plasma renin activity can be measured to monitor mineralocorticoid and sodium replacement. Hypertension, edema, tachycardia, and suppressed plasma renin activity are clinical signs of overtreatment with mineralocorticoids. Excessive increases in fludrocortisone dosage may also retard growth.

Experimental medical regimens for the treatment of CAH and the approach to surgical treatment for genital anomalies are discussed elsewhere in this issue.

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STEROID 11β-HYDROXYLASE DEFICIENCY AND RELATED DISORDERS

Perrin C. White, MD

Congenital adrenal hyperplasia, the inherited inability to synthesize cortisol, usually presents with signs of androgen excess such as masculinization of female external genitalia. In many patients, signs and symptoms of aldosterone deficiency develop, including hyponatremia, hyperkalemia, and hypovolemia. By the early 1950s it was recognized that, in a small percentage of patients with congenital adrenal hyperplasia, hypertension develops rather than mineralocorticoid deficiency. The hypertension in such patients responds to glucocorticoid replacement. Most of these patients have a distinct metabolic defect, steroid 11β-hydroxylase deficiency,[10] whereas patients without hypertension usually have 21-hydroxylase deficiency. In most populations, 11β-hydroxylase deficiency accounts for approximately 5% to 8% of cases of congenital adrenal hyperplasia, occurring in approximately 1 in 100,000 births.[56] A large number of cases of 11β-hydroxylase deficiency have been reported in Israel among Jewish immigrants from Morocco, a relatively inbred population. The incidence in this group is currently estimated to be 1 in 5000 to 1 in 7000 births, with a gene frequency of 1.2% to 1.4%.[48]

This article reviews biochemical, clinical, and genetic studies of 11β-hydroxylase deficiency and two genetically related disorders, aldoste-

This work was supported by grants R37 DK37867 and R01 DK54408 from the National Institutes of Health, Bethesda, Maryland.

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Table 1. CLINICAL, BIOCHEMICAL, AND GENETIC CHARACTERISTICS OF DISEASES INVOLVING 11β-HYDROXYLASE ISOZYMES

Characteristics	11β-Hydroxylase Deficiency	Aldosterone Synthase Deficiency	Glucocorticoid-Suppressible Hyperaldosteronism
Gene	*CYP11B1*	*CYP11B2*	*CYP11B1/CYP11B2* crossover
Alias	P450c11	P450aldo	
Incidence, general population	1:100,000	Rare	Rare
Group with increased prevalence	Moroccan Jews	Iranian Jews	Anglo-Irish (?)
Hormones			
Glucocorticoids	↓		
Mineralocorticoids	↑	↓	↑
Renin	↓	↑	↓
Androgens	↑		
↑ Metabolites	Deoxycorticosterone, 11-deoxycortisol	Corticosterone (CMO I), 18-hydroxycorticosterone (CMO II)	18-Oxocortisol
Clinical signs			
Ambiguous genitalia	Females		
Salt-wasting crisis	Rarely, after treating	Yes	
Hypertension	Yes		Yes
Na balance	↑	↓	↑
K balance	↓	↑	↓

↓ = diminished quantity; ↑ = increased quantity; P450 = cytochrome P450; CMO = corticosterone methyloxidase.

rone synthase deficiency and glucocorticoid-suppressible hyperaldosteronism (Table 1).

BIOCHEMISTRY OF ADRENAL STEROID BIOSYNTHESIS

Cortisol is synthesized from cholesterol in the zona fasciculata of the adrenal cortex. This process requires five enzymatic conversions (Fig. 1): cleavage of the cholesterol side chain to yield pregnenolone, 17α-hydroxylation and 3β-dehydrogenation to 17-hydroxyprogesterone, and successive hydroxylations at the 21 and 11β positions. A *17-deoxy* pathway is also active in the zona fasciculata in which 17α-hydroxylation does not occur, and the final product is normally corticosterone.

The same 17-deoxy pathway is active in the adrenal zona glomerulosa, which contains no 17α-hydroxylase activity; however, corticosterone is not the final product. Corticosterone is successively hydroxylated and oxidized at the 18 position to yield aldosterone.

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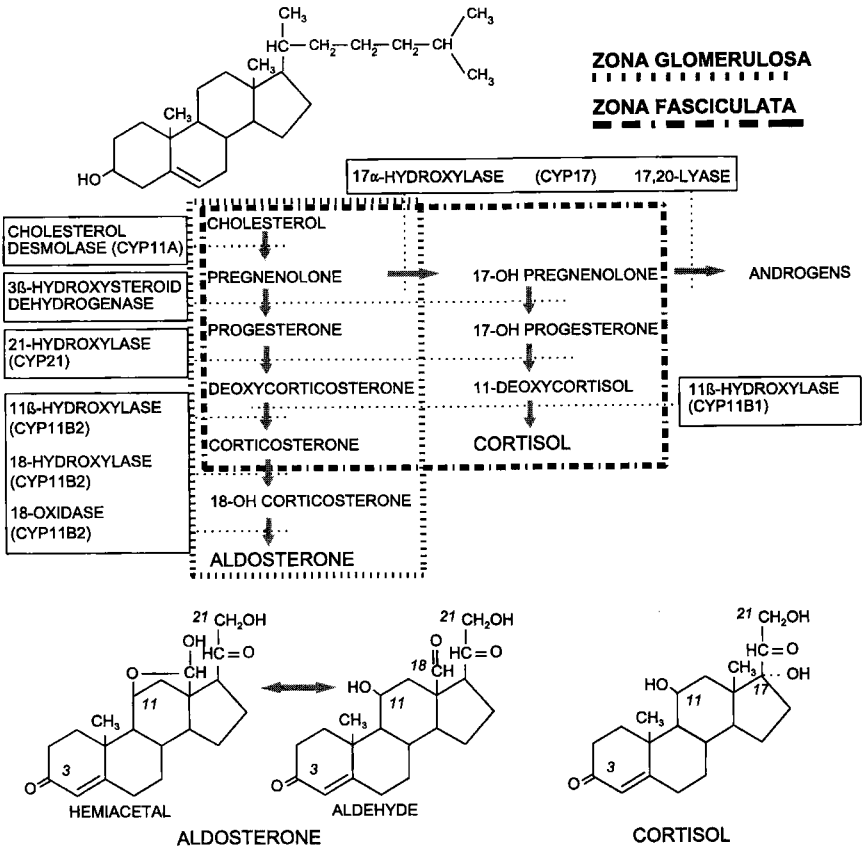

Figure 1. Pathways of adrenal steroid biosynthesis. The pathways of biosynthesis of aldosterone and cortisol from cholesterol are shown with the planar structures of these substances respectively at the bottom and top of the figure. Aldosterone exists in two conformations (18-aldehyde and hemiacetal) that are freely interconvertible; the hemiacetal predominates under physiologic conditions. The enzymes responsible for each biosynthetic step are listed in boxes on the left; the last three steps of aldosterone biosynthesis are mediated by a single enzyme, aldosterone synthase (CYP11B2). The conversions taking place within the zonae glomerulosa and fasciculata are marked by broken rectangles.

Humans have two 11β-hydroxylase isozymes that are responsible for cortisol and aldosterone biosynthesis, respectively, CYP11B1 (also termed 11β-hydroxylase, P450c11, and P450XIB1, with the CYP designation currently the standard nomenclature[34]) and CYP11B2 (aldosterone synthase, P450aldo, P450c18, P450cmo, and P450XIB2). These isozymes are mitochondrial P450 cytochromes located in the inner membrane on the matrix side. Each isozyme is synthesized with 503 amino acid residues, but a signal peptide targeting the protein to mitochondria is cleaved to yield the mature protein of 479 residues.[58] The sequences of the proteins are 93% identical.[30]

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The CYP11B1 isozyme 11β-hydroxylates 11-deoxycortisol to cortisol, as determined by expressing the corresponding cDNA in cultured cells[5, 22] and after actual purification from aldosterone-secreting tumors.[36] It can also convert 11-deoxycorticosterone to either corticosterone (the predominant product) or 18-hydroxy, 11-deoxycorticosterone, but it 18-hydroxylates corticosterone poorly. It cannot convert corticosterone into aldosterone. In contrast, CYP11B2 has somewhat weaker 11β-hydroxylase activity than CYP11B2, but it can 18-hydroxylate and then 18-oxidize corticosterone to aldosterone. When deoxycorticosterone is converted to aldosterone, the same steroid molecule probably remains bound to the enzyme for all three conversions without release of the intermediate products. CYP11B2 can also convert 11-deoxycortisol to 18-oxocortisol. This reaction normally does not occur at significant levels in vivo because CYP11B2 is not expressed in the zona fasciculata but becomes important in patients with glucocorticoid-suppressible hyperaldosteronism.

Although the human CYP11B1 and CYP11B2 enzymes differ in 33 amino acid residues, studies of chimeric enzymes from patients with glucocorticoid-suppressible aldosteronism suggest that the residues specific to CYP11B2 that are critical for 18-hydroxylase and 18-oxidase activities are located in the C-terminal half of the enzyme.[39]

Like other P450 enzymes, CYP11B1 and CYP11B2 use molecular oxygen and reducing equivalents (i.e., electrons) provided by reduced nicotinamide-adenine dinucleotide phosphate (NADPH) to catalyze specific hydroxylations. As is true with other mitochondrial P450s, the reducing equivalents are not accepted directly from NADPH but from a series of two accessory electron transport proteins. Adrenodoxin reductase accepts electrons from NADPH and donates them to adrenodoxin, which, in turn, transfers them to the P450. These intermediate proteins are necessary because NADPH donates electrons in pairs, whereas P450s can only accept single electrons.

GENETICS OF STEROID 11β-HYDROXYLASE ISOZYMES

Characteristics of the Human *CYP11B* Genes

In humans, CYP11B1 and CYP11B2 are encoded by two genes[30] on chromosome 8q24.3 (Fig. 2).[50] Each gene contains nine exons spread over approximately 7000 base pairs (7 kb) of DNA. The nucleotide sequences of these genes are 95% identical in coding sequences and approximately 90% identical in introns.

Genomic clones linking the human *CYP11B1* and *CYP11B2* genes have not been isolated. *CYP11B2* is on the left (if the genes are pictured as being transcribed left to right) in the mouse,[9] and studies of crossovers in families with aldosterone synthase deficiency[40] and glucocorticoid-suppressible hyperaldosteronism suggest that the same arrangement exists in humans. Based on the resolution of large restriction fragments

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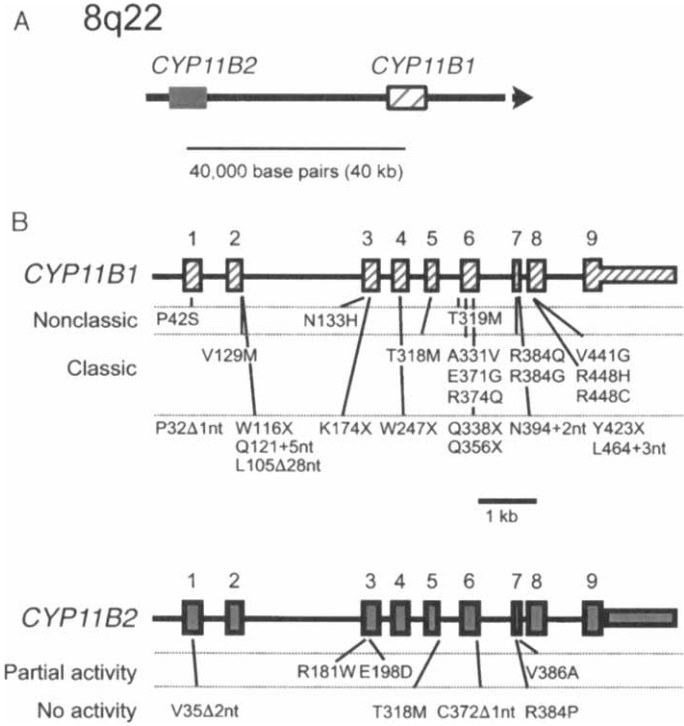

Figure 2. Mutations involving the 11β-hydroxylase (*CYP11B1*) and aldosterone synthase (*CYP11B2*) genes. *A*, The arrangement of genes. The arrow indicates direction of transcription. *B*, Mutations in *CYP11B1* cause nonclassic or classic 11β-hydroxylase deficiency, and mutations in *CYP11B2* cause aldosterone synthase deficiency. The mutations are arranged so that those causing increasing enzymatic compromise are arrayed from top to bottom. *Dotted lines* divide mutants into groups with similar activities. As an example of mutation terminology, P42S is Proline-42 to Serine. Δ = deletion; + = insertion. Amino acids: A = alanine; C = cysteine; D = aspartic acid; E = glutamic acid; F = phenylalanine; G = glycine; H = histidine; I = isoleucine; K = lysine; L = leucine; M = methionine; N = asparagine; P = proline; Q = glutamine; R = arginine; S = serine; T = threonine; V = valine; W = tryptophan; Y = tyrosine.

Although there are two types of aldosterone synthase deficiencies, they do not correspond exactly to in vitro levels of enzymatic activity. R181W and V386A do not cause disease separately, but double homozygosity causes CMO II type aldosterone synthase deficiency. Other combinations with this phenotype include T318M + V386A and R181W + C372Δ1nt. Homozygosity for either V35Δ2nt and R384P, or double homozygosity for E198D + V386A, has been associated with CMO I type deficiency.

using pulsed field gel electrophoresis, the human genes are located approximately 40 kb apart.[24, 39]

CYP11B1 is expressed at high levels in normal adrenal glands.[30] *CYP11B2* transcripts are present at low levels in normal adrenal RNA[5, 30] but at increased levels in aldosterone-secreting tumors.[5, 21] Using in situ hybridization, expression of *CYP11B2* transcripts seems to be limited to the zona glomerulosa.[41]

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The *CYP11B1* and *CYP11B2* genes are regulated in a manner consistent with their respective roles in cortisol and aldosterone biosynthesis. *CYP11B1* is regulated primarily by corticotropin (ACTH), which acts through a specific G protein–coupled receptor on the surface of cells of the adrenal cortex[31] to increase levels of cAMP (cyclic adenosine 3′, 5′ monophosphate). Cyclic AMP preferentially increases *CYP11B1* mRNA expression over that of *CYP11B2*.[5,6]

CYP11B2 expression is regulated mainly by the renin-angiotensin system and by potassium levels, with ACTH having only a short-term effect.[44] Angiotensin II occupies a G protein–coupled receptor, activating phospholipase C. The latter protein hydrolyzes phosphatidylinositol bisphosphate to produce inositol triphosphate and diacylglycerol, which raise intracellular calcium levels. Similarly, potassium signaling in glomerulosa cells involves membrane depolarization, leading to an influx of calcium through T- and L-type channels. Increased intracellular calcium increases expression of *CYP11B2*,[3] probably by activating a calmodulin-dependent protein kinase that phosphorylates one or more transcription factors. Increased expression of *CYP11B2* increases synthesis of aldosterone.

STEROID 11β-HYDROXYLASE DEFICIENCY

Clinical Presentation

In 11β-hydroxylase deficiency, 11-deoxycortisol and deoxycorticosterone are not efficiently converted to cortisol and corticosterone, respectively. Decreased production of glucocorticoids reduces their feedback inhibition on the hypothalamus and anterior pituitary, increasing secretion of ACTH, which, in turn, stimulates the zona fasciculata of the adrenal cortex to overproduce steroid precursors proximal to the blocked 11β-hydroxylase step. 11β-Hydroxylase deficiency can be diagnosed by high basal or ACTH-stimulated levels of deoxycorticosterone or 11-deoxycortisol in the serum, or by increased excretion of the tetrahydrometabolites of these compounds in a 24-hour urine collection. Obligate heterozygous carriers of 11β-hydroxylase deficiency alleles (e.g., parents) have no consistent biochemical abnormalities detectable even after stimulation of the adrenal cortex with intravenous ACTH,[38] consistent with an autosomal recessive mode of inheritance.

Approximately two thirds of patients with the severe classic form of 11β-hydroxylase deficiency have high blood pressure,[48] often beginning in the first few years of life.[28] Although the hypertension is usually of mild-to-moderate severity, left ventricular hypertrophy with or without retinopathy has been observed in as many as one third of patients, and deaths from cerebrovascular accidents have been reported. Other signs of mineralocorticoid excess, such as hypokalemia and muscle weakness or cramping, occur in a minority of patients and are not well correlated with blood pressure. Plasma renin activity is usually

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suppressed in older children, and levels of aldosterone are consequently low even though the ability to synthesize aldosterone is actually unimpaired.

The cause of hypertension in 11β-hydroxylase deficiency is not well understood. Despite assumptions that it is caused by elevated serum levels of deoxycorticosterone, blood pressure and deoxycorticosterone levels are poorly correlated in patients. In addition, this steroid has only weak mineralocorticoid activity when administered to humans or other animals. Other metabolites of deoxycorticosterone may be responsible for the development of hypertension. The 18-hydroxy and 19-nor metabolites of deoxycorticosterone are thought to be more potent mineralocorticoids,[14] but consistent elevation of these steroids in 11β-hydroxylase deficiency has not been documented. Moreover, synthesis of these steroids requires hydroxylations within the adrenal (19-nor-deoxycorticosterone is synthesized through 19-hydroxy and 19-oic intermediates) that are probably mediated primarily by CYP11B1.[37] This reaction is unlikely to take place efficiently in 11β-hydroxylase deficiency.

In addition to hypertension, patients with 11β-hydroxylase deficiency often exhibit signs of androgen excess similar to those seen in 21-hydroxylase deficiency. Accumulated cortisol precursors in the adrenal cortex are shunted (through the activity of 17α-hydroxylase/17,20-lyase) into the pathway of androgen biosynthesis, which is active in the human adrenal in both sexes. Affected girls are born with some degree of masculinization of their external genitalia, including clitoromegaly and partial or complete fusion of the labioscrotal folds. Such ambiguous genitalia in 46XX girls can be difficult to distinguish from the genitalia of a 46XY cryptorchid boy.[48] In contrast to the external genitalia, the gonads and the internal genital structures (fallopian tubes, uterus, and cervix) arising from the Müllerian ducts are normal, and affected girls have intact reproductive potential if their external genital abnormalities are corrected surgically.

Other signs of androgen excess that occur postnatally in both sexes include rapid somatic growth in childhood and accelerated skeletal maturation leading to premature closure of the epiphyses and short adult stature. Additionally, patients may have premature development of sexual and body hair (premature adrenarche) and acne. Androgens may affect the hypothalamic-pituitary-gonadal axis, leading to amenorrhea or oligomenorrhea in girls and true precocious puberty or, conversely, poor spermatogenesis in boys.[17]

A mild nonclassic form of 11β-hydroxylase has been detected among children with mild virilization or precocious pubarche; such patients are not characteristically hypertensive.[59] This disorder seems to be rare as compared with nonclassic 21-hydroxylase deficiency, and no mutations in the *CYP11B1* gene have been detected among women who are referred to reproductive endocrinology clinics for signs of androgen excess.[1, 19] ACTH-stimulated levels of 11-deoxycortisol are at least five times the upper limit of normal in patients with genuine 11β-hydroxylase deficiency.[19]

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Treatment

Glucocorticoid administration (usually hydrocortisone) replaces deficient cortisol and reduces ACTH secretion, suppressing excessive adrenal androgen production and preventing further virilization. Such therapy should also suppress ACTH-dependent production of mineralocorticoid agonists and ameliorate hypertension. If the hypertension has been long-standing before treatment, additional antihypertensive drugs may be required to lower blood pressure into the normal range. These drugs may include potassium-sparing diuretics such as spironolactone or amiloride with or without a calcium channel blocker such as nifedipine.[32] Because the renin-angiotensin system is suppressed in these patients, angiotensin-converting enzyme inhibitors are unlikely to be effective. Thiazide diuretics should not be used except in combination with a potassium-sparing diuretic because they will cause hypokalemia in patients with mineralocorticoid excess.

Genetic Analysis

Deficiency of 11β-hydroxylase results from mutations in *CYP11B1* (Fig. 2). Thus far, 20 mutations have been identified in patients with classic 11β-hydroxylase deficiency.[4,12,16,33] Among Moroccan Jews, a group that has a high prevalence of 11β-hydroxylase deficiency, almost all affected alleles carry the same mutation, Arg-448 to His (R448H).[57] This abnormality probably represents a founder effect, but this mutation has also occurred independently in other ethnic groups, and another mutation of the same residue (R448C) has been reported.[12] This apparent mutational *hotspot* contains a CpG dinucleotide. Such dinucleotides are prone to methylation of the cytosine followed by deamidation to TpG. Several other mutations in *CYP11B1* (T318M, R374Q, R384Q) are of this type.

These defects and almost all other missense mutations identified thus far are in regions of known functional importance[43,45] and abolish enzymatic activity.[4] For example, Arg-448 is adjacent to Cys-450, which is a ligand of the heme iron atom of this cytochrome P450 enzyme. T318M modifies an absolutely conserved residue that is thought to be critical for proton transfer to the bound oxygen molecule.[45] E371G and R374Q also mutate highly conserved residues and may affect binding of adrenodoxin. R384Q is in a region that may form part of the substrate-binding pocket.[45] Almost all P450s have a basic residue (H or R) at this position or the immediately adjacent position. V441G is adjacent to the highly conserved heme-binding region, and this mutation may change the secondary structure of the protein.

Other mutations found in patients with the classic form of the disease are nonsense or frameshift mutations that also abolish enzymatic activity. One, a nonsense mutation of Trp-247 (W247X), has been identi-

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fied in several unrelated kindreds in Austria and also probably represents a founder effect.[12]

Each patient with mild nonclassic disease carries at least one mutation that reduces but does not destroy activity; the other mutation may be either mild or severe.[19]

Although patients with classic disease apparently completely lack 11β-hydroxylase activity, they differ significantly in the severity of the various signs and symptoms of disease. There is no strong correlation between the severity of hypertension and biochemical parameters, such as plasma levels of the 11β-hydroxylase substrates deoxycortisol and deoxycorticosterone, and urinary excretion of tetrahydrodeoxycortisol.[48,57] Moreover, there is no consistent correlation between the severity of hypertension and the degree of virilization. These phenotypic variations must be governed by factors outside the *CYP11B1* locus.

Prenatal Diagnosis and Treatment

The ambiguous genitalia seen in affected girls can be ameliorated by prenatal administration of dexamethasone to the mother, as performed in 21-hydroxylase deficiency (discussed elsewhere in this issue). Prompt and accurate prenatal diagnosis of at-risk pregnancies is required to avoid unnecessary treatment of unaffected fetuses. Prenatal diagnosis of this disorder was first accomplished by measuring 11-deoxycortisol levels in amniotic fluid[49]; however, that approach requires interruption of prenatal treatment. Molecular genetic diagnosis is preferable. In most populations, identification of actual disease-causing mutations is more difficult than in 21-hydroxylase deficiency because ten mutations account for 95% of mutant alleles in the latter disorder, whereas many different mutations cause 11β-hydroxylase deficiency. Identification of mutations is an effective strategy if the mutations in a family are already known,[2] or if populations such as Moroccan Jews are known to carry a specific mutation. Alternatively, if a DNA sample is available from another affected child in the family, highly polymorphic *microsatellite* DNA markers near the *CYP11B1* gene[50] can be used to determine whether the fetus carries the same alleles as the known affected child.

ALDOSTERONE SYNTHASE (CORTICOSTERONE METHYLOXIDASE) DEFICIENCY

Clinical Presentation

By far the most frequent defect of aldosterone biosynthesis is congenital adrenal hyperplasia owing to steroid 21-hydroxylase deficiency (see the article by Speiser). Two thirds of patients with classic 21-hydroxylase deficiency are unable to synthesize adequate amounts of

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aldosterone and are said to have the *salt-wasting* form of the disorder. Signs of androgen excess are prominent in such patients.

In contrast, rare patients have isolated aldosterone deficiency with entirely normal cortisol and sex steroid synthesis; this disorder is caused by deficient activity of CYP11B2 (aldosterone synthase). The largest number of cases have been identified among Iranian Jews from the city of Isfahan,[47] but the disease also has been documented throughout Europe and North America.[54]

Mineralocorticoid deficiency leads to excessive sodium excretion and potassium retention in the renal distal tubule and cortical collecting duct, causing hyponatremia and hyperkalemia. In untreated infants with aldosterone synthase deficiency, serum sodium is usually in the range of 120 to 130 mEq/L, whereas serum potassium ranges from 6 to 8.5 mEq/L. Children older than 3 to 4 years usually have normal serum electrolytes even if untreated. Plasma renin activity is markedly elevated (up to 100 times normal) in affected infants and young children but may be normal in adults.

Two forms of aldosterone synthase deficiency are recognized—corticosterone methyloxidase (i.e., aldosterone synthase) deficiency types I and II.[52] These syndromes have identical clinical features but differ in profiles of secreted steroids. Levels of deoxycorticosterone are increased and urinary excretion of corticosterone metabolites elevated in type I and type II deficiencies relative to excretion of cortisol metabolites. Excretion of 18-hydroxycorticosterone is mildly decreased in type I deficiency, whereas urinary and serum levels of this steroid are dramatically increased in patients with type II deficiency. Aldosterone and its metabolites may be undetectable in patients with type I deficiency, whereas urinary excretion is mildly decreased in type II deficiency, and serum levels of aldosterone are usually within normal limits. Type II deficiency may be readily diagnosed by a marked (often 100-fold) elevation of the ratio of 18-hydroxycorticosterone to aldosterone in either urine or serum; the ratio does not vary with age in affected individuals and may be the sole biochemical abnormality in adults.[53] Levels of cortisol may be normal or elevated, and levels of adrenal androgens and their precursors are normal.

The clinical presentation of aldosterone synthase deficiency varies with age.[47, 53] Infants may show signs and symptoms of mineralocorticoid deficiency at a few days to weeks of age. These signs and symptoms include vomiting and dehydration leading to hypovolemia that may cause cyanosis, tachycardia, hypotension, acidosis, and prerenal azotemia. As discussed previously, hyponatremia and hyperkalemia are also characteristic of aldosterone deficiency. These problems may end in circulatory collapse. Although fatalities have occurred, the morbidity of aldosterone synthase deficiency is usually not as severe as that engendered by the salt-wasting form of congenital adrenal hyperplasia. This difference presumably reflects normal synthesis of deoxycorticosterone, corticosterone, and cortisol in aldosterone synthase deficiency, which ameliorate the development of shock.

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Some children are diagnosed in early childhood with failure to thrive, anorexia, mild dehydration, and electrolyte abnormalities. Although electrolytes usually normalize by 4 years of age (even with a low-sodium diet), growth retardation may persist throughout childhood. Adults are usually asymptomatic but occasionally tolerate severe salt loss (e.g., from gastroenteritis) less well than do unaffected individuals. Asymptomatic adults with type II deficiency are occasionally ascertained through family studies by the persistently elevated ratio of 18-hydroxycorticosterone to aldosterone.[47]

It is difficult to distinguish variations in clinical severity between individuals from the marked improvement that occurs with age in all patients. Because affected individuals from the Iranian Jewish community have identical mutations, any individual variations in severity that exist cannot reflect allelic variation; instead, they must represent effects of other genetic loci or nongenetic factors.

Although severely symptomatic infants require intravenous fluids, most infants and children are treated with oral sodium supplementation (1 to 2 g/d of NaCl alone or in combination with $NaHCO_3$) and fludrocortisone (0.1–0.3 mg/d). Electrolyte abnormalities are quickly resolved, but plasma renin activity and levels of steroid precursors may not return to normal for several months. In children in whom aldosterone synthase deficiency has resulted in failure to thrive, therapy may lead to dramatic catch-up growth.

Oral sodium supplements can be discontinued once plasma renin activity has decreased to normal, but mineralocorticoid replacement therapy should be maintained throughout childhood until growth is complete. It is prudent to evaluate sodium balance on discontinuing therapy.

Genetic Analysis

Mutations in *CYP11B2* cause aldosterone synthase deficiency (Fig. 2). Iranian Jewish patients with corticosterone methyloxidase II deficiency are all homozygous for two mutations, R181W and V386A. Owing to an intragenic recombination segregating in these kindreds, it was determined that homozygosity for either mutation alone was insufficient to cause disease. When these mutants were expressed in cultured cells, V386A alone had a minimal effect on activity, whereas R181W and the R181W/V386A double mutant both had intact 11β-hydroxylase activity, markedly decreased 18-hydroxylase activity, and undetectable 18-oxidase activity.[40] When expressed in a different cell line at higher levels of activity that were easier to quantitate, the R181W, V386A, and double mutants had 0.4%, 33%, or 0.2% of wild-type 18-oxidase activity, respectively.[60] These findings suggest that 0.4% of normal 18-oxidase activity is not rate limiting for aldosterone biosynthesis but 0.2% is. This observation is consistent with previous studies of congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Patients hemizygous for mutants

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with approximately 1% of wild-type 21-hydroxylase activity are able to synthesize normal amounts of aldosterone.[51]

It might be assumed that corticosterone methyloxidase I deficiency would be associated with null mutations in *CYP11B2*. Indeed, one kindred with corticosterone methyloxidase I deficiency carried a frameshift mutation,[29] and one carried a missense mutation, R384P, that destroyed enzymatic activity when expressed in cultured cells.[11] Nevertheless, corticosterone methyloxidase I deficiency can also be caused by double homozygosity for mutations E198D and V386A, although the double mutant enzyme retains 11β-hydroxylase activity in a manner similar to the R181W/V386A double mutant.[42]

Conversely, one patient with corticosterone methyloxidase II deficiency carried mutations on each chromosome that completely inactivated the enzyme. One was a frameshift mutation, and the other was a missense mutation, T318M, affecting a residue crucial for catalysis (interestingly, each occurred in conjunction with one of the previously reported corticosterone methyloxidase II mutations, the frameshift on the same chromosome as R181W and T318M on the same chromosome as V386A).[60] Corticosterone methyloxidase I and II deficiencies are not simple allelic variants. The development of corticosterone methyloxidase I or II deficiency must depend on factors outside *CYP11B2*. There might be polymorphisms in the ability of CYP11B1 to synthesize 18-hydroxycorticosterone. Coding sequence polymorphisms of this nature have been documented in the *CYP11B1* gene in rats, where they are associated with salt-resistant hypertension.[26]

GLUCOCORTICOID-SUPPRESSIBLE HYPERALDOSTERONISM

Clinical Presentation

Glucocorticoid-suppressible hyperaldosteronism (also called dexamethasone-suppressible hyperaldosteronism or glucocorticoid-remediable aldosteronism) is a form of hypertension inherited in an autosomal dominant manner with high penetrance. It is characterized by moderate hypersecretion of aldosterone, suppressed plasma renin activity, and rapid reversal of these abnormalities after administration of glucocorticoids. It is a rare disorder. Until several years ago, the absence of reliable biochemical or genetic markers made it difficult to ascertain.

Hypokalemia is usually mild and may be absent. Absolute levels of aldosterone secretion are usually moderately elevated in the untreated state but may be within normal limits. Because plasma renin activity is strongly suppressed, the ratio of aldosterone secretion to renin activity is always abnormally high. Levels of 18-hydroxycortisol and 18-oxocortisol are elevated to 20 to 30 times normal. The ratio of urinary excretion of tetrahydrometabolites of 18-oxocortisol to those of aldosterone exceeds 2, whereas this ratio averages 0.2 in normal individuals.[46] Elevation of 18-

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oxocortisol is the most consistent and reliable biochemical marker of the disease, although it may also be elevated in cases of primary aldosteronism. This steroid may be of pathophysiologic significance; it is an agonist for the mineralocorticoid receptor and has been shown to raise blood pressure in animal studies.[15]

Commonly available biochemical screening tests for glucocorticoid-suppressible hyperaldosteronism include a plasma aldosterone to plasma renin activity ratio greater than 30,[8] and a postdexamethasone plasma aldosterone level less than 4 ng/dL.[25] Once an affected individual has been identified in a kindred, additional cases can be ascertained within that kindred using 18-oxocortisol levels[46] or genetic markers. It is apparent from these studies that affected individuals have blood pressures that are markedly elevated as compared with unaffected individuals in the same kindred, although some patients may, in fact, have normal blood pressures. Even young children typically have blood pressures greater than the 95th percentile for age, and most are frankly hypertensive before the age of 20 years. The hypertension is often of moderate severity, and blood pressures exceeding 180/120 mm Hg are unusual. Associated signs of hypertension are frequent, including left ventricular hypertrophy on the electrocardiogram and retinopathy. Some affected kindreds have remarkable histories of early death from stroke (before age 45) in many family members.[46] Because steroid biosynthesis is otherwise normal, affected individuals have normal growth and sexual development.

Most laboratory and clinical abnormalities are suppressed by treatment with glucocorticoids, whereas infusion of ACTH exacerbates these problems.[35] These observations suggest that aldosterone is inappropriately synthesized in the zona fasciculata and is regulated by ACTH. Moreover, 18-hydroxycortisol and 18-oxocortisol, the steroids that are characteristically elevated in this disorder, are 17α-hydroxylated analogues of 18-hydroxycorticosterone and aldosterone, respectively. Because 17α-hydroxylase is not expressed in the zona glomerulosa, the presence of large amounts of a 17α-hydroxy, 18-oxo-steroid suggests that an enzyme with 18-oxidase activity (i.e., aldosterone synthase, CYP11B2) is abnormally expressed in the zona fasciculata.[55]

The initial treatment of choice in adults is dexamethasone (~1 mg/d). Children with this condition should be treated cautiously because of potential adverse effects of glucocorticoid therapy on growth. If therapy is indicated, children should be treated with the lowest effective dose of hydrocortisone. If hypertension is long-standing, it may not completely respond to glucocorticoids. This problem is similar to the difficulty observed in patients with 11β-hydroxylase deficiency, and the choice of adjunctive therapy is governed by the same considerations. Patients with this disorder usually respond poorly to conventional antihypertensive medications unless they are also treated with glucocorticoids.

Glucocorticoid-suppressible hyperaldosteronism must be distinguished from aldosterone-producing adenomas, considering that the

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latter condition is best treated by surgical removal of the affected adrenal gland.[27] Secretion of 18-hydroxycortisol and 18-oxocortisol may be increased in patients with adenomas, but the ratio of urinary excretion of tetrahydrometabolites of 18-oxocortisol and aldosterone is rarely greater than 1.0. Suppression of aldosterone secretion with glucocorticoids and familial aggregation[13] are unusual findings in patients with adenomas but have been reported. Presentation of an adenoma during childhood is exceedingly rare.

Genetic Analysis

All patients with glucocorticoid-suppressible hyperaldosteronism have the same type of mutation, a chromosome that carries three *CYP11B* genes instead of the normal two (Fig. 2).[23, 24, 39] The middle gene on this chromosome is a chimera with 5′ and 3′ ends corresponding to *CYP11B1* and *CYP11B2*, respectively. The chimeric disease-associated gene is flanked by presumably normal *CYP11B2* and *CYP11B1* genes. The chimeric gene is regulated like *CYP11B1* (expressed at high levels in the zona fasciculata and regulated primarily by ACTH) because it has transcriptional regulatory sequences identical to those of *CYP11B1*. If the chimeric gene has enzymatic activity similar to that of *CYP11B2*, a single copy of such an abnormally regulated gene should be sufficient to cause the disorder, consistent with the known autosomal dominant mode of inheritance of this syndrome (Fig. 3). Abnormal expression of the chimeric gene in the zona fasciculata was directly demonstrated by in situ hybridization studies of an adrenal gland from a patient with this disorder.[41]

The chromosomes carrying chimeric genes are presumably generated by unequal crossing over. The high homology and proximity of the *CYP11B1* and *CYP11B2* genes make it possible for them to become misaligned during meiosis. If this event occurs, crossing over between the misaligned genes creates two chromosomes, one of which carries one *CYP11B* gene (i.e., a deletion), whereas the other carries three *CYP11B* genes. The breakpoints (the points of transition between *CYP11B1* and *CYP11B2* sequences) are not identical in different kindreds, but all are located between intron 2 and exon 4. A disproportionate number of ascertained kindreds are of Anglo-Irish extraction. The reason for this pattern is not known.[24]

The chimeric genes causing glucocorticoid-suppressible hyperaldosteronism can be readily detected by hybridization to Southern blots of genomic DNA or can be specifically amplified using the polymerase chain reaction.[20] As these techniques are widely used in molecular genetics laboratories, direct molecular genetic diagnosis may be more practical in many cases than assays of 18-oxocortisol levels, which are not routinely available.[7]

The limited region in which crossover breakpoints have been observed in glucocorticoid-suppressible hyperaldosteronism alleles suggests that there are functional constraints on the structures of chimeric

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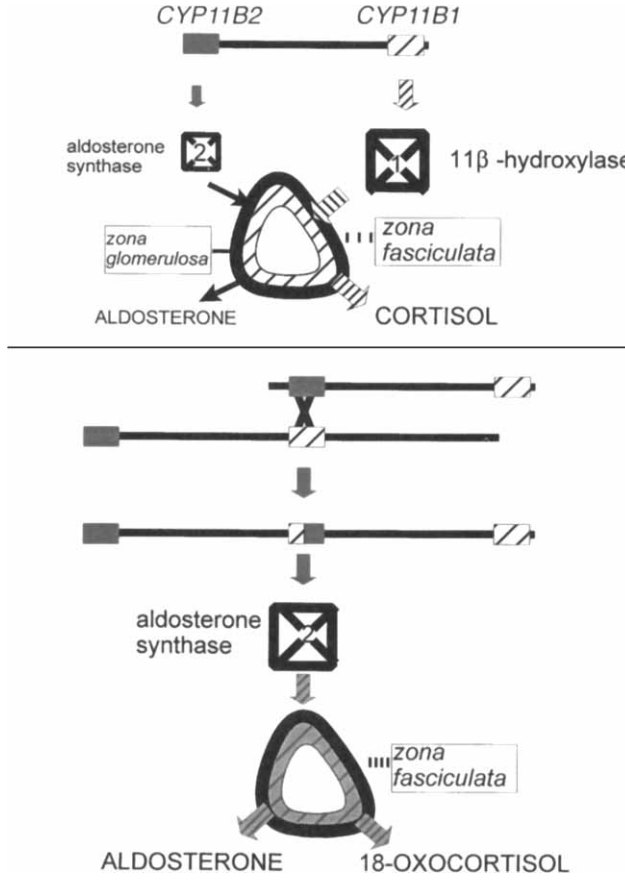

Figure 3. *A, CYP11B1* and *CYP11B2* genes, showing patterns of expression of each. *B,* Unequal crossing over generating a chimeric *CYP11B1/2* gene that has aldosterone synthase activity but is expressed in the zona fasciculata, causing glucocorticoid-suppressible hyperaldosteronism.

genes able to cause this disorder. One obvious constraint is that sufficient *CYP11B2* coding sequences must be present in the chimeric gene so that the encoded enzyme actually has aldosterone synthase (i.e., 18-hydroxylase and 18-oxidase) activity. As determined by expressing chimeric cDNAs in cultured cells, chimeric enzymes with amino termini from *CYP11B1* and carboxyl termini from *CYP11B2* have 18-oxidase activity only if at least the region encoded by exons 5 to 9 corresponds to *CYP11B2*. If the sequence of exon 5 instead corresponds to *CYP11B1,* the enzyme has 11β-hydroxylase but no 18-oxidase activity.[39] This finding is entirely consistent with the observation that no breakpoints in glucocorticoid-suppressible hyperaldosteronism alleles occur after exon 4. The chimeric enzymes either have strong 18-oxidase activity or none detect-

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able, and there does not seem to be any location of crossover that yields an enzyme with an intermediate level of 18-oxidase activity. There is no evidence for allelic variation in this disorder (i.e., variations in clinical severity are unlikely to be the result of different crossover locations).

Other factors, such as kallikrein levels, may affect the development of hypertension in this disorder.[7] One study found that blood pressure in persons with glucocorticoid-suppressible hyperaldosteronism was higher when the disease was inherited from the mother than when it was paternally inherited.[18] Although it is theoretically possible that the gene is imprinted (i.e., the maternal and paternal copies are expressed differently), it seems more likely that exposure of the fetus to elevated levels of maternal aldosterone subsequently exacerbates the hypertension.

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CONGENITAL ADRENAL HYPERPLASIA OWING TO 3β-HYDROXYSTEROID DEHYDROGENASE DEFICIENCY

Songya Pang, MD

3β-Hydroxysteroid dehydrogenase/Δ5→Δ 4 isomerase (3β-HSD) catalyzes the conversion of Δ5-3β-hydroxysteroid to Δ4-3β-ketosteroids in the adrenals and gonads and is essential for the formation of progesterone, the precursor hormone for aldosterone, and 17-hydroxyprogesterone (17-OHP), the precursor hormone for cortisol, in the adrenal cortex (Fig. 1). It is also essential for the formation of androstenedione (Δ4-A), testosterone, and estrogens in the adrenal cortex and gonads (Fig. 1). 3β-HSD activity is essential in placental steroidogenesis[56] and has been demonstrated in the liver,[22] kidney,[10] skin,[11] and mammary gland.[1]

In humans, 3β-HSD expression in the adrenals and gonads is under the control of a single gene and differs from the genetic control of 3β-HSD expression in extra-adrenal and extragonadal tissues.[19, 28] Disorders involving altered 3β-HSD activity in the adrenals and gonads have long been recognized in humans. Severe 3β-HSD deficiency in adrenals and gonads results in unequivocal clinical, biochemical, and metabolic manifestations of adrenal and gonadal failure associated with anatomic evidence of congenital adrenal hyperplasia (CAH). The pathophysiology and hormonal abnormalities of the severe form of inherited 3β-HSD deficiency CAH are well understood, whereas the biochemical basis

This work was supported in part by grant HD 36399 from United States Public Health Service.

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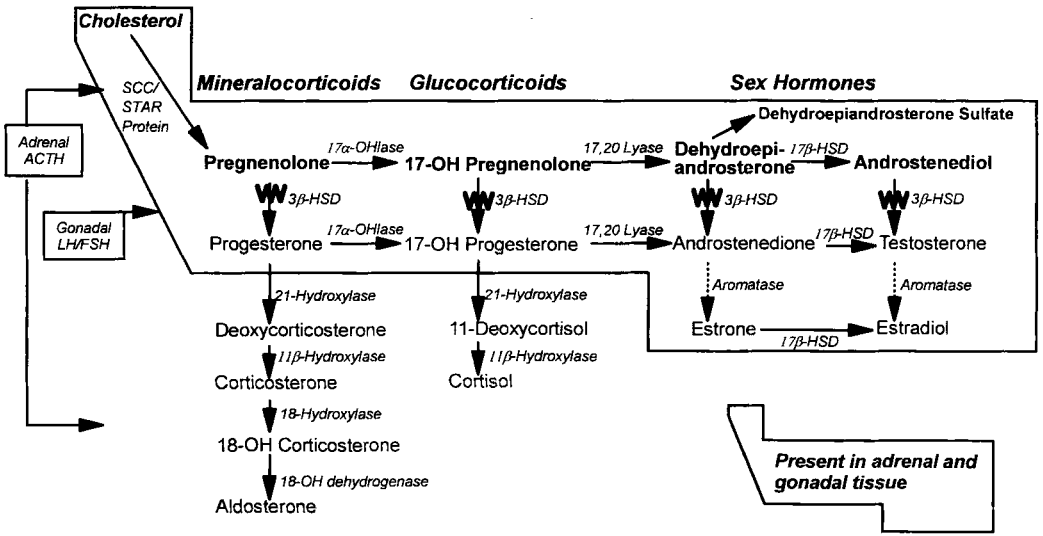

Figure 1. Adrenal and gonadal steroidogenesis. Impairment in *3β-HSD* activity is indicated by a W. Solid line = major pathway; dotted line = minor pathway in adrenals and major pathway in ovaries; 3β-HSD = 3β-hydroxysteroid dehydrogenase; SCC = P450 cholesterol side chain cleavage enzyme; STAR = steroidogenic acute regulatory protein; ACTH = adrenocorticotropic hormone; LH/FSH = luteinizing hormone and follicle-stimulating hormone.

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of the milder nonclassic form of 3β-HSD deficiency disorder reported primarily in young women with hirsutism, with or without menstrual disorder, and in young children with premature sexual hair growth is controversial and is not universally accepted. Recent molecular advances in understanding the family of 3β-HSD genes have helped to explain the genetic basis of 3β-HSD deficiency disorder. This article addresses the molecular, clinical, and hormonal findings related to 3β-HSD deficiency disorder.

PATHOPHYSIOLOGY OF 3β-HYDROXYSTEROID DEHYDROGENASE DEFICIENCY AND CONGENITAL ADRENAL HYPERPLASIA

Inherited 3β-HSD deficiency in humans is concomitantly present in adrenal and gonadal tissue and is transmitted by an autosomal recessive trait. A single gene encodes adrenal and gonadal 3β-HSD.[7, 17, 29, 32, 33, 44–51, 53–55, 61] Adrenocortical 3β-HSD deficiency results in cortisol deficiency with or without aldosterone deficiency. Low cortisol leads to increased corticotropin (ACTH) secretion through a feedback mechanism, which, in turn, results in increased production of Δ5 precursor steroids, such as pregnenolone (Δ5-P), 17-hydroxypregnenolone (Δ5-17P), dehydroepiandrosterone (DHEA), and androstenediol from the adrenal cortex.[38, 39, 62] Aldosterone deficiency causes the salt-wasting disorder, and combined cortisol and aldosterone deficiencies cause adrenal crisis if not treated in time in affected subjects.[4, 37–39] In genetic girls, excess fetal production of extra-adrenal metabolites of the weak adrenal androgen precursors DHEA and androstenediol causes mild virilization of the external genitalia[4, 59] or, in some cases, no apparent virilization at birth.[33, 46] In boys, 3β-HSD deficiency in testes impairs testosterone biosynthesis from early fetal life, thereby causing undervirilization of male genitalia and genital ambiguity[4, 34, 52] (Fig. 1).

CLINICAL AND BIOCHEMICAL SPECTRA

Severe 3β-HSD deficiency CAH manifested by salt-wasting and ambiguous genitalia in male and female infants was first described in 1962.[4] Since then, numerous cases have been reported.[37–39] The clinical spectrum of 3β-HSD deficiency CAH ranges from salt-wasting to non–salt-wasting forms in boys affected with pseudohermaphroditism[4, 34, 52] and girls with normal[34, 47] or slightly virilized genitalia, such as clitoromegaly, at birth.[4, 59] Non–salt-wasting CAH owing to 3β-HSD deficiency causes premature acne, premature sexual hair growth, and mild growth acceleration in children.[33] In girls, clitoromegaly may be present.[33] During adolescence and adulthood, 3β-HSD deficiency CAH results in varying degrees of hypogonadism in men[5, 7, 44, 52, 63] and in hirsutism, menstrual disorder, and polycystic ovaries in women.[5, 47]

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During the last 15 years, nonclassic (also termed mild or late-onset) forms of 3β-HSD deficiency disorder have been diagnosed in a substantial number of hirsute women[13, 14, 15, 35] and in children with premature pubarche[14, 56] based on mildly elevated ACTH-stimulated Δ5 precursor steroid levels, such as Δ5-17P and DHEA levels, and mildly elevated Δ5 precursor to Δ4 product ratios, such as Δ5-17P/17-OHP, Δ5-17P/cortisol, and DHEA/Δ4-A. The validity of these hormonal criteria used for diagnosing mild 3β-HSD deficiency is now in question owing to the absence of genetic evidence to prove that such mild Δ5 steroid abnormalities resulted from mutations of the 3β-HSD gene. This review addresses the molecular findings in subjects previously diagnosed as having mild nonclassic 3β-HSD deficiency CAH.

Biochemically, deficient 3β-HSD activity in patients with the salt-wasting or non–salt-wasting form is present in the adrenals and gonads and not in the extra-adrenal or extragonadal tissues.[34, 52] This evidence of independent genetic regulation of the 3β-HSD enzyme in the intra- and extra-adrenal and intra- and extra-gonadal tissues is now proven by the advent of molecular information on 3β-HSD genes.[19, 28] The presence[4, 9, 16] or absence[31, 34, 47] of aldosterone deficiency in patients with 3β-HSD deficiency CAH also predicts varying genotype between the salt-wasting and non–salt-wasting forms of the disorder. It is now apparent that a family of closely related genes encodes for 3β-HSD isozymes. This information has led to a better understanding of the genetic regulation of intra- and extra-adrenal and gonadal 3β-HSD. Additionally, the relationship between structure and function of the gene encoding adrenal and gonadal 3β-HSD and the relationship between genotype and phenotype in 3β-HSD deficiency CAH have been elucidated.

GENES AND PROTEINS

In humans, two types of genes (type I and II) encode the 3β-HSD protein (Fig. 2). The loci of both genes are assigned to the chromosome I p11-13 region.[3, 23] Both genes are 7.84 to 7.88 kb long and consist of four exons and three introns. The type I and II 3β-HSD proteins are 93.5% homologous in amino acid sequence.[19, 23, 25, 28] These genes belong to the aldo-keto reductase family rather than the cytochrome P450 gene family typified by several other steroidogenic genes encoding the enzymes cholesterol desmolase *(CYP11A)*, 17α-hydroxylase/ 17,20-desmolase *(CYP17)*, 21-hydroxylase *(CYP21)*, 11β-hydroxylase (*CYP11B1* and *B2*), and aromatase *(CYP19)*. The type I gene is primarily and abundantly expressed in the placenta, mammary gland, and skin.[11, 19, 20, 21, 23] The type II gene is primarily and abundantly expressed in the adrenals and gonads.[43] A low level of type I gene expression is detected in the gonads and a low level of type II gene expression, in the mammary gland.[19]

The 3β-HSD protein is a membrane-bound protein in the endoplasmic reticulum and mitochondria.[19, 26, 28] The putative functional domain of the type I and II genes includes two predicted membrane-spanning

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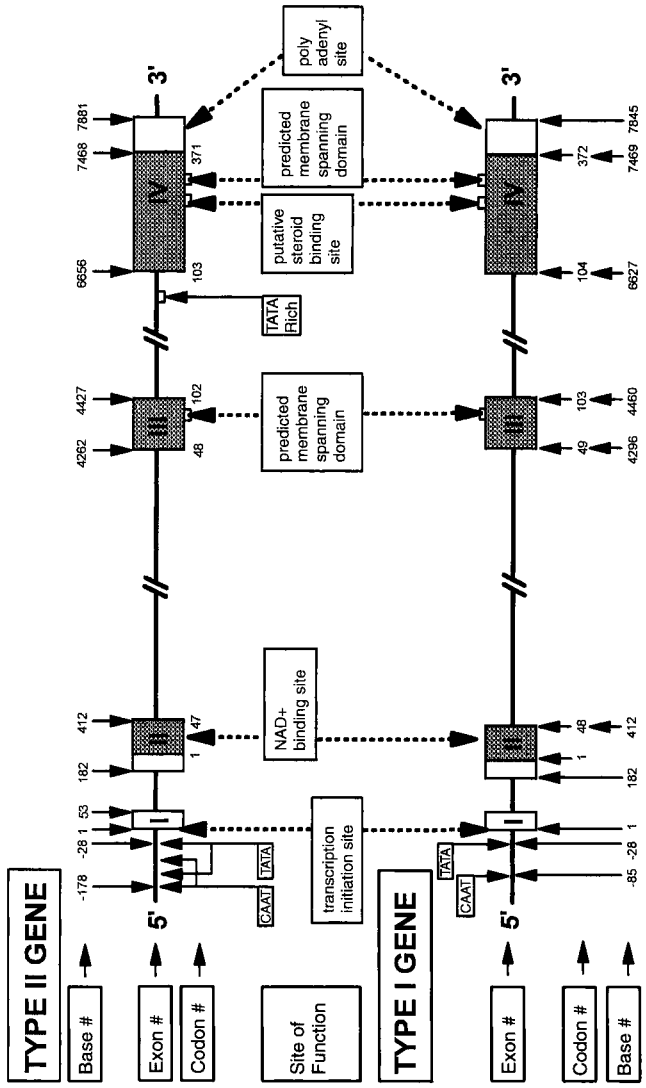

Figure 2. Schematics of the human *3β-HSD* gene structure. NAD = nicotinamide-adenine denucleotide. (*Data from* references 12, 18, 19, 21, 23, 42, and 46.)

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segments, one in exon III (codons 73 to 90) and the other in exon IV (codons 286 to 305),[12, 18, 42] and two additional suggested membrane-spanning segments in the 5′ and middle region of exon IV.[12, 42] In the type I gene, an additional suggested membrane-spanning segment is present in exon II.[42] The putative steroid-binding site in codon 250 of exon IV[58] and the cofactor NAD+ binding site in codon 15 of exon II[46] are present in type I and II genes. These predicted and suggested functional domains of the gene are expected to be critical sites of the structure for gene expression.

In vitro kinetics of human types I and II 3β-HSD proteins translated by cells transfected with the respective genes reveal that the type I 3β-HSD enzyme activity is greater than that of the type II enzyme (Table 1).[11, 23, 43] The K_m values for Δ5-P, DHEA, and dihydrotestosterone of the type I 3β-HSD gene are approximately tenfold, sixfold, and tenfold lower than the K_m values for the type II 3β-HSD gene for all three substrates, respectively. The V_{max}/K_m values for Δ5-P, DHEA, and dihydrotestosterone of the type I 3β-HSD gene are approximately 5.9-fold, 4.5-fold, and 2.8-fold greater than the V_{max}/K_m values for the type II 3β-HSD gene for the substrates, respectively (Table 1).[11, 23, 43] The type I enzyme is predicted to be very efficient in converting the predictably low Δ5 steroid concentrations in the peripheral tissues (extra-adrenal and extragonadal tissues). Additional 3β-HSD isozymes in the liver, kidney, lung, brain, and adipose tissue in humans are not yet characterized; however, three possible 3β-HSD pseudogenes or related genes in humans have been identified by screening a human leukocyte genomic DNA library.[27] These pseudogenes contain stop codons or deletions in their coding regions, and it has been suggested that these pseudogenes diverged from the type I gene millions of years ago.[27]

In other species, several types of 3β-HSD genes have been identified.[19, 28] In rats and mice, the type I gene encodes adrenal and gonadal 3β-HSD, the type II gene encodes the liver isozyme, the type III gene

Table 1. IN VITRO (CELL HOMOGENATES) KINETIC PARAMETERS OF HUMAN TYPE I AND II 3β-HYDROXYSTEROID (HSD) GENES

	Substrate→Product					
	Δ5P→P		*DHEA→Δ4-A*		*DHT→5α-Andiol*	
Gene	*K_m (μM)*	*V_{max}/K_m (fold)*	*K_m (μM)*	*V_{max}/K_m (fold)*	*K_m (μM)*	*V_{max}/K_m (fold)*
Type I 3β-HSD	0.24 <1	5.9	0.24 <1	4.5	0.26	2.8
Type II 3β-HSD	1.24 1–2	1	1.6 1–2	1	2.7	1

Δ5P = pregnenolone; P = progesterone; DHEA = dehydroepiandrosterone; Δ4-A = androstenedione; DHT = dihydrotestosterone; 5α-Andiol = androstenediol.

Note: Each K_m value represents independent report data. The V_{max}/K_m fold of type I 3β-HSD is based on the unity of the type II 3β-HSD value.

Data from references 11, 23, and 43.

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encodes liver and kidney isozymes, and the type IV gene encodes placental/skin and kidney isozymes. The macaque type I gene encodes gonadal 3β-HSD. The bovine type I gene encodes ovarian 3β-HSD. The regulation of 3β-HSD expression in various tissue sites is under tissue-specific and independent genetic control in humans, rodents, and other mammalian species.

MOLECULAR BASIS OF 3B-HYDROXYSTEROID DEFICIENCY CONGENITAL ADRENAL HYPERPLASIA

3β-HSD deficiency CAH result from a deleterious mutation in the type II 3β-HSD gene encoding adrenal and gonadal 3β-HSD in humans (Fig. 3, Table 2). Analysis of the type II gene from 21 unrelated families of patients of various ethnic backgrounds with salt-wasting 3β-HSD deficiency revealed a premature stop codon or a frameshift and subsequent premature stop codon in the gene owing to a homozygous or compound heterozygous point mutation involving codons 135, 171, 249, 273, 308, and 318, an insertion mutation between codons 186 and 187, or combined mutations at codons 248 and 249.[7, 29, 43, 54, 55, 61] The resulting truncated 3β-HSD protein is predicted to lack any 3β-HSD activity, causing the salt-wasting disorder. Ten alleles from seven unrelated patients had a missense mutation at codon 10, 15, or 108, 142 or 186, 205 or 253, or 259 in one or both alleles.[17, 33, 46, 51, 53, 55] These codon regions involve either functional domains of the gene or conserved regions of the gene throughout species, suggesting that these regions of amino acid residues are predicted to be critical domains for 3β-HSD activity. The mutant 3β-HSD proteins translated by these cells transfected with the respective mutant genes exhibited no or little enzyme activity in vitro, explaining the clinical phenotype of the salt-wasting form of 3β-HSD deficiency.[33, 46, 51, 53, 55, 63] A point mutation involving codon 259 resulting in a substitution of amino acid residue (Thr → Met or Thr → Arg) showed no detectable protein in vitro, suggesting that the mutant 3β-HSD proteins are unstable.[63] This finding suggests a novel mechanism for absent 3β-HSD activity resulting in a severe clinical disorder of 3β-HSD deficiency CAH.[63] Additionally, alleles from four patients belonging to three families with the salt-wasting disorder revealed intron mutations at nucleotide number 558 (ins C), 687 (del 27), and 867 (del G) in one or both alleles, predictably causing frameshift of the gene and undetectable enzyme activity.[33]

Analysis of the type II 3β-HSD gene from 14 patients in 13 unrelated families with classic non–salt-wasting 3β-HSD deficiency CAH revealed primarily missense mutations in coding regions of the gene in all[32, 33, 45, 53, 63] but two alleles[33, 45] (Fig. 3). A few mutant type II 3β-HSD enzymes translated by the respective mutant genes after in vitro mutagenesis revealed 2% to 52.8% of wild-type enzyme activity,[33, 45, 53] sufficient to prevent aldosterone deficiency resulting in the non–salt-wasting disorder (see Table 2). The codon 155, 222, and 254 mutations exhibited no

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Table 2. PREDICTED/PROVEN IN VITRO MUTANT TYPE II 3β-HYDROXYSTEROID (HSD) ACTIVITY VERSUS CLINICAL PHENOTYPE IN 3β-HSD DEFICIENCY IN CONGENITAL ADRENAL HYPERPLASIA

Mutant Allele 1		Mutant Allele 2		Clinical Phenotype					
Genotype	*V_{max}/K_m Δ5→P*	*Genotype*	*V_{max}/K_m Δ5→P*	*SW or NSW*	*AG in Male*	*AG in Female*	*Premature Pubarche*	*Spontaneous Puberty*	Reference
Wild-type	100%		100%	—	—	—	—	—	
135 Stop codon	0%*	Homozygous	0%	SW	—	No	—	—	29
171 Stop codon	0%*	Homozygous	0%	SW	—	No	Yes	No	44
186/Ins/187	0%*	171 Stop codon	0%	SW	Yes	—	No	Yes (gynecomastia)	44
248 Val→Asn/249 Arg→Stop	0%*	Homozygous	0%	SW	Yes	—	No	Yes (gynecomastia)	7
249 Arg→Stop	0%*	Homozygous	0%	SW	Yes	—	—	—	55
273 Frameshift	0%*	Homozygous	0%	SW	Yes	—	—	—	54
308 Thr→ Stop	0%*	Homozygous	0%	SW	Yes	—	—	—	55
318 Frameshift	0%*	273 Frameshift	0%	SW	Yes	Yes	Yes	Yes†	61
253 (Tyr→Asn)	0%‡	186/Ins/187	0%	SW	Yes	—	—	—	53
142 (Glu→Lys)	0%‡	171 Stop codon	0%	SW	Yes	—	—	—	53
15 (Gly→Asp)	0%‡	Homozygous	0%	SW	Yes	—	—	—	46
259 (Thr→Arg)	NP§	Homozygous	NP§	SW	—	No	—	—	33, 55, 63
259 (Thr→Met)	NP§	Homozygous	NP§	SW	Yes	—	—	—	33, 63
222 (Pro→Glu)	0%‡	Homozygous	0%	SW	Yes	—	—	—	33
10 (Ala→Glu)	0%‡	Homozygous	0%	SW	—	No	—	Yes	33
186 (Pro→Leu)	0.2%‡	108 (Leu→Pro)	0.3%	SW	Yes	—	—	—	51
108 (Leu→Pro)	0.3%‡	186 (Pro→Leu)	0.2%	SW	Yes	—	—	—	51
205 (Leu→Pro)	0%‡	Homozygous	0%	SW	Yes	—	—	—	17

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N558 (InsC/559)	0%*	253 (Tyr→Asn)	0%	SW	Yes	—	—	—	33
N687/del 27	0%‡	Homozygous	0%	SW	Yes	—	—	—	33
N867/del G	0%*	259 (Thr→Met)	0%	SW	Yes	—	—	—	33
254 (Thy→Asp)	0%‡	No mutation found		NSW	—	No	No	Yes (1° amenorrhea, hirutism, PCO)	50
155 (Pro→Leu)	0%‡	294 (Gly→Val)	20.5%‡	NSW	Yes	—	—	—	33
222 (Pro→His)	0%‡	129 (Gly→Arg)	11.7%‡	NSW	—	No	Yes	—	33
N867/del G	0%*	236 (Leu→Ser)	100%‡	NSW	Yes	—	—	—	33
10 (Ala→Val)	29.1%‡	Homozygous	29.1%	NSW	Yes	—	—	—	33
129 (Gly→Arg)	2%‡	N6651 intron Mutation	NR	NSW	—	No	Yes	Yes (2° amenorrhea, hirsutism, PCO)	45
				NSW	Yes	—	Yes	Yes (testicular hypertrophy)	45
100 (Asn→Ser)	3%‡	Homozygous	3%	NSW	Yes	—	—	—	30
82 (Ala→Thr)	7.6%‡	Homozygous	7.6%	NSW	Yes	—	Unknown	—	32, 33
				NSW	Yes	—	No	—	
				NSW	—	No	Yes	—	
				NSW	—	No	No	Yes	
6 (Leu→Phe)	NP	Homozygous	NP	NSW	Yes	—	—	—	63
254 (Ala→Pro)	12%‡	Homozygous	12%	NSW	Yes	—	—	—	53
173 (Leu→Arg)	52.8%‡	Homozygous	52.8%	NSW	Yes	—	—	—	33, 48
				NSW	—	No	—	—	

Δ5P = pregnenolone; P = progesterone; SW = salt-wasting; NSW = non–salt wasting; AG = ambiguous genitalia; NR = not reported; PCO = polycystic ovaries.
*Predicted.
†Post publication follow-up data.
‡Proven.
§Kinetic study not possible (NP) owing to unstable protein.

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Figure 3. Molecular basis of 3β-HSD deficiency congenital adrenal hyperplasia (CAH). A schematic of type II *3β-HSD* gene map and reported mutations in the type II *3β-HSD* gene in the alleles of patients with salt-wasting (SW) and nonsalt-wasting (NSW) 3β-HSD deficiency CAH are depicted. The number in the mutant allele box in the exon indicates the codon number and in the intron indicates genomic nucleotide sequence number. Roman numerals indicate exon numbers. The shaded area of the exon indicates the translated region of the gene; Star = alleles demonstrating no detectable enzyme activity in vitro; Plus = alleles reported on both SW and NSW forms. Circle = homozygous genotype; square = predicted/suggested functional domain of the gene. (*Data from* references 7, 17, 29, 30, 32, 33, 43–46, 50, 51, 61, and 63).

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enzyme activity in vitro; therefore, these mutations are predictably deleterious, but the second allele mutation was either mildly deleterious[33] or not identified in the patient.[50] The mildly deleterious or unidentified mutation on the second allele presumably mitigates the severity of the phenotype; thus, the patients are non–salt-wasters.[33, 50] Homozygous mutations in codon 6 (Leu → Phe = L6F) result in decreased enzyme activity to convert Δ5-P and DHEA as compared with wild-type II 3β-HSD protein in intact cells. Inconsistent detection of L6F mutant protein on Western blotting and undetectable enzyme activity during the enzyme kinetic study suggest the relative instability of the L6F mutant enzyme.[63] It is unlikely that L6F affects the putative NAD+ binding site because the reported mutations in the type II 3β-HSD gene involving this domain were associated with only severe salt-wasting 3β-HSD deficiency CAH. Furthermore, a reasonable amount of L6F mutant enzyme activity in intact cells essentially excluded an enzyme with a high NAD K_m.[63]

Sibs have been described with non–salt-wasting 3β-HSD deficiency with CAH owing to a codon 129 missense mutation in one allele, with the second allele having a G to A mutation in intron III (at nucleotide 6651 six bases upstream from the 5′ region of exon IV).[45] The latter defect may create a new splicing junction, altering the normal splicing of the mRNA. In non–salt-wasting 3β-HSD deficiency with CAH, all amino acid substitutions except one are in regions of the gene conserved throughout evolution in all species studied, suggesting that the amino acid residues in these regions are essential to normal enzyme activity. Two sequence variants, S213G and K216E, in the type II 3β-HSD gene from a patient previously reported on by the author[6] were later proven to be polymerase chain reaction artifacts. A recent report of decreased enzyme activity in vitro associated with these sequence variants[33] was also demonstrated in the author's laboratory; however, the clinical consequence of sequence variants of S213G and K216E is unknown and can only be based on theoretic grounds. Type I gene sequences have been normal in both alleles of all patients with severe 3β-HSD deficiency tested to date.[44, 45, 53] A comparison between predicted or proven in vitro type II mutant 3β-HSD activity (genotype) and clinical phenotype of patients with 3β-HSD deficiency CAH indicates a good correlation between genotype and clinical phenotype (see Table 2).

UPDATED HORMONAL CRITERIA FOR CLASSIC AND NONCLASSIC 3β-HYDROXYSTEROID DEFICIENCY CONGENITAL ADRENAL HYPERPLASIA BASED ON TYPE II 3β-HSD GENE STUDY

Mild, late-onset, or nonclassic 3β-HSD deficiency has been diagnosed during the past 15 years in 1.5% to 13% of children with premature pubarche[15, 56] and in 3% to 60% of girls with pubertal- or postpubertal-onset hirsutism with or without menstrual disorders based solely on the hormonal findings without any genotypic proof. Previously published

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hormonal criteria for the nonclassic variant of 3β-HSD deficiency were based on ACTH-stimulated Δ5-17P and DHEA levels and ratios of Δ5-17P/17-OHP, Δ5-17P/cortisol, or DHEA/Δ4-A greater than 2 standard deviations above pubertal stage-matched normal mean values.[13, 14, 15, 35]

One would expect that a mild variant of 3β-HSD deficiency CAH results from a mildly deleterious mutation in the type II 3β-HSD gene; however, a recent study by the author demonstrated normal type II 3β-HSD gene sequences in 17 children with premature pubarche and in seven hirsute women with ACTH-stimulated Δ5-17P level and Δ5-17P to cortisol ratio meeting the previously published hormonal criteria of mild, late-onset 3β-HSD deficiency[8, 49] (Fig. 4). Reported ACTH-stimulated Δ5-17P levels and Δ5-17P to cortisol ratios in bona fide patients with 3β-HSD deficiency CAH[34, 45, 47, 50] were exceedingly higher than the findings in patients without the gene mutation[8, 37–39, 49] (Fig. 4). The lowest Δ5-17P to cortisol ratio in affected children with premature pubarche was +36 standard deviations compared with levels in healthy Tanner II to III children,[49] and a reported Δ5-17P to cortisol ratio in hirsute women with bona fide 3β-HSD deficiency CAH was +572 standard deviations[47] compared with reported values for normal women.[8, 37–39] The ACTH-stimulated DHEA level and DHEA to Δ4-A ratio were not consistently distinguishable in patients with and without the type II gene mutation.[36–39] The type I 3β-HSD gene sequence was also normal in patients with mild hormone abnormalities.[49] The author's 3β-HSD gene findings are in agreement with the normal type I and II 3β-HSD gene sequence reported by Zerah and co-workers[60] in hirsute girls with variably mildly decreased adrenal 3β-HSD activity. Brazilian investigators have reported finding mutations in the type II gene in three of nine girls with nonsalt-wasting 3β-HSD deficiency who presented with premature pubarche and ACTH-stimulated 17-hydroxypregnenolone levels elevated six or more SD above the mean.[32a] In contrast, no mutations were identified among 13 hirsute women with mildly elevated hormone levels.[32b]

The ACTH-stimulated hormonal profiles in a few proven DNA carriers for severe 3β-HSD deficiency at the author's clinic were normal.[8, 49] These findings suggest that moderately decreased adrenal 3β-HSD activity based on the hormonal findings in premature pubarche children and in hirsute women is not caused by a mild variant of 3β-HSD deficiency CAH resulting from type II 3β-HSD gene mutation in either one or both alleles. Children with premature pubarche and hirsute females with mildly or moderately decreased adrenal 3β-HSD activity and normal type II 3β-HSD gene sequences do not have a variant of the mild, late-onset, nonclassic form of 3β-HSD deficiency CAH. It remains to be elucidated whether this mild degree of decreased adrenal 3β-HSD activity associated with modestly increased Δ5 steroid abnormality is caused by an alteration of another adrenal 3β-HSD enzyme not yet discovered from the family of 3β-HSD in humans. Alternative explanations may include a complex effect of an altered intra-adrenal hormonal milieu on the regulation of intra-adrenal 3β-HSD activity, altered fetal programming of adrenal Δ5 steroid secretion, or dysregulation of 17,20 desmolase

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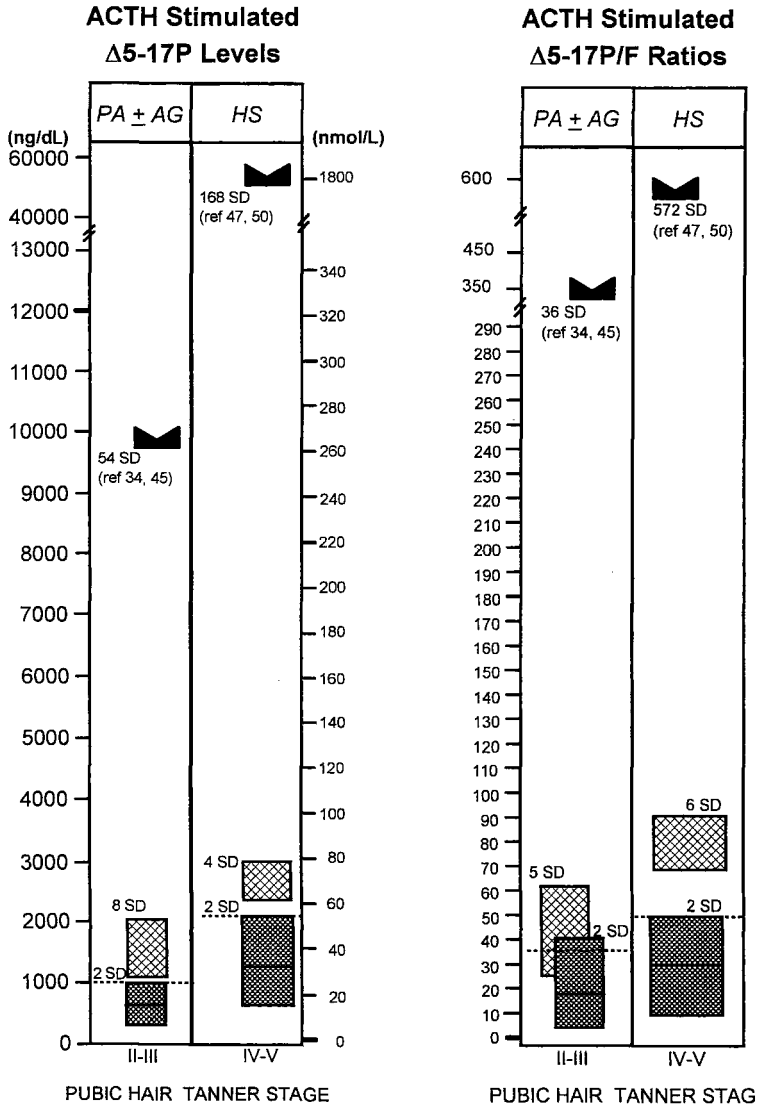

Figure 4. Adrenocorticotropic hormone (ACTH) stimulated Δ5-17P levels and Δ5-17P to F ratios (post 1-hour 0.25-mg IV bolus) in children with premature adrenarche with or without ambiguous genitalia, and in hirsute females with or without type II *3β-HSD* gene mutation. Shaded bars represent hormonal values (mean and range) of control or normal subjects. The cross-hatched bars represent the area of hormonal values in patients with mildly to moderately elevated Δ5-17P levels and Δ5-17P to F ratios without type II *3β-HSD* gene mutation. Solid bars indicate the area of hormonal values reported in patients with severe *3β-HSD* deficiency congenital adrenal hyperplasia and type II *3β-HSD* gene mutation. SD = the number of standard deviations above the normal mean level. PA = premature adrenarche; AG = ambiguous genitalia; HS = hirsutism. (*Data from* references 8, 34, 37–39, 45, 47, 48, 49, and 50.)

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activity. In addition, whether this condition is a marker for a subgroup of patients with polycystic ovarian disorder associated with either intrinsic or extrinsic metabolic abnormalities is unknown. The author's preliminary study in obese teenagers with moderately increased adrenal Δ5-17P and DHEA levels indicating decreased adrenal 3β-HSD activity in the presence of a normal sequence of type II 3β-HSD gene revealed markedly decreased insulin sensitivity.[41] Body mass index was increased in the 60% of hirsute women with hormonal evidence of mildly decreased adrenal 3β-HSD activity; however, this hormonal profile was not a consistent finding in obese women. It is unknown whether and to what extent children with premature pubarche and hirsute girls will eventually exhibit polycystic ovaries or an insulin-resistant state. The pathogenic mechanism for mild to moderately decreased adrenal 3β-HSD activity without 3β-HSD deficiency CAH is yet to be explored.

Much progress has occurred in defining the hormonal criteria for nonclassic variants of genetically defined 3β-HSD deficiency CAH. Data suggest that patients with mild homozygous or compound heterozygous mutations in the type II 3β-HSD gene express a substantially greater degree of hormonal abnormality[24] than previously reported for the nonclassic disorder. Investigation to uncover the factors related to the mildly decreased adrenal 3β-HSD activity in women and children with hyperandrogenic symptoms will clarify the nature of the mild spectrum of 3β-HSD deficiency.

GONADAL FUNCTION

Varying degrees of gonadal function have been reported in patients with severe 3β-HSD deficiency CAH.[2, 5, 31, 59] In a few salt-waster subjects, spontaneous pubertal maturation occurred[16, 52]; however, pubertal gynecomastia and testicular hypertrophy accompanied the development of male secondary sex chracteristics in male pseudohermaphrodites.[5, 40, 47, 52] In non–salt-wasting patients, spontaneous onset of puberty was complicated with primary or secondary amenorrhea and polycystic ovary–like changes on pelvic ultrasound in women.[5] The results of gonadotropin dynamic studies have been reported in a few pubertal subjects with 3β-HSD deficiency CAH.[5, 51] Although basal luteinizing hormone (LH) and follicle-stimulating hormone levels were normal, increased and normal LH-releasing hormone–stimulated LH levels have been observed.[5, 47, 59] Hypothalamic-pituitary-gonadal axis function is variable in patients with gonadal 3β-HSD deficiency. It is puzzling that pubertal LH dynamics were apparently normal in proven gonadal 3β-HSD deficiency in the presence of anatomic evidence of gonadal hyperplasia.[5] Perhaps gonadal steroidogenic activity is maintained with normal tropic hormone secretion after initial hyperplastic changes of gonads in partial gonadal 3β-HSD deficiency subjects. Polycystic ovary syndrome in women with genetically proven 3β-HSD deficiency CAH was not accompanied by elevated androstenedione and testosterone levels in patients receiving glucocorticoid replacement therapy.[5] This hormonal

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pattern differs from the presentation of polycystic ovaries that is associated with increased ovarian androstenedione or testosterone secretion.[5]

There are no follow-up data regarding infertility in adults with 3β-HSD deficiency CAH. It is probable that either genital anomaly or inadequate testicular steroidogenic activity in men is likely to cause infertility. In women, polycystic ovary changes and primary or secondary amenorrhea clearly indicate evidence of infertility owing to ovarian 3β-HSD deficiency.[5, 47]

MEDICAL MANAGEMENT

The principles of glucocorticoid therapy and mineralocorticoid therapy (if necessary) for patients with 3β-HSD deficiency are the same as for other nonhypertensive forms of CAH, except that monitoring of hormonal control requires periodic measurement of ACTH, Δ5-17P, DHEA, and dehydroepiandrosterone sulfate in these subjects.

Male pseudohermaphrodites with 3β-HSD deficiency who are raised as girls require estrogen replacement therapy with a low dose of conjugated estrogen (0.3 mg/d) at age 10 to 11 years when female puberty normally begins. These patients have usually undergone gonadectomy during early childhood. The dose of conjugated estrogen needs to be increased to 0.625 mg/d after 6 months to 1 year of treatment. Special attention to psychosexual orientation and counseling are necessary during adolescence. In male pseudohermaphrodites raised as males, spontaneous pubertal development and progression and serum testosterone and gonadotropin levels should guide the need for testosterone replacement therapy. If testicular function is inadequate for development of satisfactory secondary sex characteristics, testosterone depot treatment is necessary using initially a low dose, 50 to 100 mg every 4 weeks for 6 months to 1 year, and subsequently a greater dose, 150 to 200 mg every 4 weeks. In all patients receiving testosterone replacement therapy, liver function monitoring is recommended periodically to detect any untoward effects of testosterone treatment. The dose of testosterone can be monitored by the patient's clinical response and by serum testosterone level measurement during therapy. If the serum testosterone is above the physiologic level (1000 ng/dL), the dose should be decreased. Transdermal testosterone therapy may also be considered.

In genetic girls with severe 3β-HSD deficiency CAH, spontaneous puberty will not occur, and estrogen replacement therapy is necessary beginning at age 12 to 13 years. Initially, these girls can be treated with a conjugated estrogen, 0.3 to 0.625 mg daily, as described previously. After 1 to 1.5 years of unopposed estrogen therapy, combined estrogen and progestogen cyclic therapy is recommended to prevent endometrial hyperplasia and to initiate the menstrual cycle. The conjugated estrogen (Premarin) treatment is 0.625 mg daily for cycle days 1 to 25 and progestogen (Provera), 5 to 10 mg daily, for cycle days 15 to 25. Other low-dose estrogen- and progestogen-containing oral contraceptives, such as Triphasil, may also be used for cyclic treatment. Periodic liver function

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and lipid profile studies and gynecologic evaluation are recommended in all female patients receiving long-term oral sex hormone replacement therapy to monitor for any untoward effects of therapy. Transdermal estrogens can be equally therapeutic and carry a lower rate of hepatic complications. There seems to be relatively little experience with the latter mode of therapy in young women.

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THE GENETICS, PATHOPHYSIOLOGY, AND MANAGEMENT OF HUMAN DEFICIENCIES OF P450c17

Richard J. Auchus, MD, PhD

P450c17 commands a central role in human steroidogenesis as the qualitative regulator of steroid hormone flux (Fig. 1). Analysis of P450c17 deficiencies in humans illustrates many aspects of the physiology of steroid biosynthesis and demonstrates poignant features of the genetics and biochemistry of P450c17. 17-Hydroxylase deficiency was first described in patients with sexual infantilism and hypertension.[10] It is now recognized to occur in partial and selective forms with variable phenotypes. This article reviews the genetics and biochemistry of P450c17 as a prelude for understanding the pathophysiology of such deficiencies and approaches to their diagnosis and management.

P450c17 AND *CYP17*

Patients who carry the diagnosis of 17-hydroxylase deficiency harbor alterations in the *CYP17* gene that encodes the P450c17 enzyme. P450c17 actually performs multiple chemical transformations. Human P450c17 17α-hydroxylates Δ^5-pregnenolone and Δ^4-progesterone with roughly equal catalytic efficiency,[3, 35] whereas all other reactions show prominent differences between Δ^5 and Δ^4 substrates. The 17,20-lyase activity is roughly 50 times more efficient for the 17α-hydroxypregneno-

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Figure 1. *See legend on opposite page.*

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lone-to-dehydroepiandrosterone (DHEA) reaction than for the 17α-hydroxyprogesterone-to-androstenedione reaction.[3, 35] Although the rate of the lyase reaction can be increased more than 10-fold by the addition of cytochrome b_5,[3, 31, 35] the Δ^5 preference persists, and the lyase rate never quite achieves the rate of the hydroxylase reactions. In addition, human P450c17 16α-hydroxylates progesterone but not pregnenolone.[3, 37, 62] In the presence of cytochrome b_5, P450c17 converts approximately 10% of pregnenolone substrate to a Δ^{16} andiene product,[35] which is also formed by porcine P450c17 and acts as a pheromone precursor in pigs.[48] Although experiments to study the chemistry of P450c17 often require certain conditions, such as detergent solubilization that could be considered nonphysiologic, the remarkable consistency of substrate preferences and kinetic constants observed for the modified solubilized P450c17 expressed in *Escherichia coli*,[31, 35] the native P450c17 expressed in yeast microsomes[3] or intact COS-1 cells,[37, 38] and that obtained from human tissues and cells[3, 62] strengthens these conclusions.

One consequence of this Δ^5 preference of human P450c17 for the 17,20-lyase reaction is that the vast majority of sex steroids in humans derive from DHEA as an intermediate. This Δ^5 preference also allows the phenomenon of adrenarche to occur in humans, an event that is characterized by a dramatic rise in adrenal DHEA production that occurs at about age 8 to 10 years,[12, 60] whereas cortisol production remains relatively constant. Adrenarche is an exemplary manifestation of the biochemistry of P450c17, in which the 17α-hydroxylase and 17,20-lyase activities are differentially regulated. In fact, this dichotomy between adrenal 17α-hydroxylase activity, reflected by relatively constant cortisol production, and 17,20-lyase activity, reflected by drastically age-dependent changes in DHEA production, previously suggested that distinct enzymes performed the two transformations; however, later copurification of the 17α-hydroxylase and 17,20-lyase activities of neonatal pig testes suggests otherwise.[47] This controversy was settled when the cDNA for bovine P450c17 was expressed in COS-1 cells, conferring 17α-hydroxylase and 17,20-lyase activities to these nonsteroidogenic cells[77] and proving genetically that the 17α-hydroxylase and 17,20-lyase enzymes

Figure 1. Major steroidogenesis pathways in humans and feedback loops controlling glucocorticoid and mineralocorticoid production. Ordinarily, cortisol is the major glucocorticoid produced by the adrenal zona fasciculata/reticularis, and cortisol exerts negative feedback inhibition *(double vertical bars)* to regulate pituitary adrenocorticotropic hormone (ACTH) production. Aldosterone is the principal mineralocorticoid of the adrenal zona glomerulosa, and aldosterone synthase (P450c11AS) expression is stimulated by volume depletion, which activates the renin-angiotensin (AII) system, and to a lesser extent, by ACTH. Aldosterone acts to stimulate kaluresis and salt and water retention, which feeds back on the kidney to suppress renin production. The production of corticosterone, a weak glucocorticoid, and of 11-deoxycorticosterone (DOC), a potent mineralocorticoid, is relatively low and unimportant in healthy individuals with intact feedback systems. Note that P450c11β in the zona fasciculata also 18-hydroxylates (18OH) DOC and corticosterone as minor products.

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were, in fact, both embodied in a single enzyme, P450c17. Differential regulation of the two principal activities of P450c17 is possible because the abundance of P450-oxidoreductase[37] and the addition[31, 35, 50] or coexpression[3] of cytochrome b_5 preferentially augments the 17,20-lyase activity, and phosphorylation[7, 76] also selectively enhances 17,20-lyase activity. Recent data showing high expression of b_5 in the zona reticularis of monkeys[40] and humans[71] suggest that the developmentally regulated expression of b_5 might be a key event in the genesis of adrenarche in higher primates.

PATHOPHYSIOLOGY

P450c17 deficiencies are a form of congenital adrenal hyperplasia in which not only adrenal but also gonadal steroidogenesis is impaired. In humans, one gene for P450c17 is expressed in the adrenals and gonads[11] instead of two tissue-specific isozymes. A single 2.1-kb mRNA species yields a 57-kd protein in these tissues, and mutations in this gene produce a spectrum of deficiencies in 17-hydroxysteroids and C_{19} steroids. Loss of P450c17 in the adrenal gland impairs cortisol and DHEA production, whereas gonadal deficiency of P450c17 abrogates sex steroid production. The initial description of 17-hydroxylase deficiency was a case in which both 17α-hydroxylase and 17,20-lyase products were absent.[10] When the gene for human P450c17 was cloned,[54] patients with 17-hydroxylase deficiency were found to harbor mutations in the *CYP17* gene,[4, 67] but molecular techniques and subsequent clinical evaluations failed to implicate *CYP17* mutations as the cause of isolated 17,20-lyase deficiency.[73] Recently, three cases of isolated 17,20-lyase deficiency have been confirmed by molecular genetics,[8, 20] demonstrating that amino acid substitution mutations in P450c17 can cause an isolated loss of 17,20-lyase activity.

Combined 17α-Hydroxylase/17, 20-Lyase Deficiency

Loss of P450c17 in the human adrenal gland prohibits the biosynthesis of cortisol and C_{19} steroids. Curiously, the adrenal glands of patients with 17-hydroxylase deficiency are similar to those of rodents, which do not express P450c17,[63] such that rodents rely on corticosterone as their principal glucocorticoid, and their adrenal glands cannot make C_{19} steroids. Patients with 17-hydroxylase deficiency rarely[26] manifest symptoms of adrenal insufficiency owing to sustained corticosterone production. Because corticosterone is a weaker glucocorticoid than cortisol, abnormally high corticosterone production is necessary before feedback inhibition on pituitary corticotropin (ACTH) secretion occurs,[45] establishing a new steady state (Fig. 2). To produce sufficient corticosterone to make up for the absence of cortisol, dramatically elevated quantities of intermediate steroids, such as progesterone and 11-deoxycorticosterone

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(DOC), must accumulate, as well as unusual metabolites, such as 18-hydroxycorticosterone[33] and 19-nor-deoxycorticosterone.[23] This ACTH-driven overproduction of mineralocorticoids leads to hypertension, a characteristic presenting feature of this disease. The hypertension usually develops in early adulthood[9] but can present in infancy[15] and can be severe.[46] As is true in other hypertensive disorders caused by mineralocorticoid excess,[39] the hypertension can become fixed if the disease is not treated for many years.[52]

Although the general description given herein is true for most patients with this disorder, considerable variation in phenotype and laboratory findings has been described. These variables include the degree of genital virilization in 46,XY subjects and the capacity for menstruation in 46,XX subjects; the severity of the hypertension and hypokalemia; the aldosterone secretion rate; the type and amount of adrenocortical hyperplasia; the gonadal morphology and histology; and the coexistence of additional disorders, such as 21-hydroxylase deficiency[53] or maternal androgen excess.[14] This heterogeneity has not been completely explained, but many factors, including the severity of the P450c17 deficiency, variations in genes regulating hormone responsiveness, diet (sodium consumption), and environment, undoubtedly contribute. The reader is referred to a detailed discussion of case reports,[72] which is beyond the scope of this article.

Isolated 17, 20-Lyase Deficiency

This disoder is extremely rare because mutations that cause this phenotype must not only destroy most 17,20-lyase activity but preserve most 17α-hydroxylase activity. Patients who are 46,XY present with ambiguous genitalia at birth or with inguinal hernias with or without pubertal delay as adolescents[72] (Table 1). Patients do not show the consequences of mineralocorticoid excess because preserved cortisol production prevents excessive DOC and corticosterone accumulation. Clinical laboratory findings vary considerably owing to the age of diagnosis, the severity of the disease, and the discrepancy between the 17α-hydroxylase and 17,20-lyase activities in a given individual. Nonetheless, C_{19} steroid production is severely, although not completely, impaired, whereas 17-hydroxylated steroid production is nearly or completely normal.

DIAGNOSIS

Unlike forms of congenital adrenal hyperplasia, such as the lipoid type and 21-hydroxylase deficiency, in which glucocorticoid and mineralocorticoid production are impaired, patients with 17-hydroxylase deficiency do not have an adrenal crisis in the postnatal period. Consequently, the diagnosis is often not entertained until hypertension, hypokalemia, or pubertal delay is evaluated during adolescence or early

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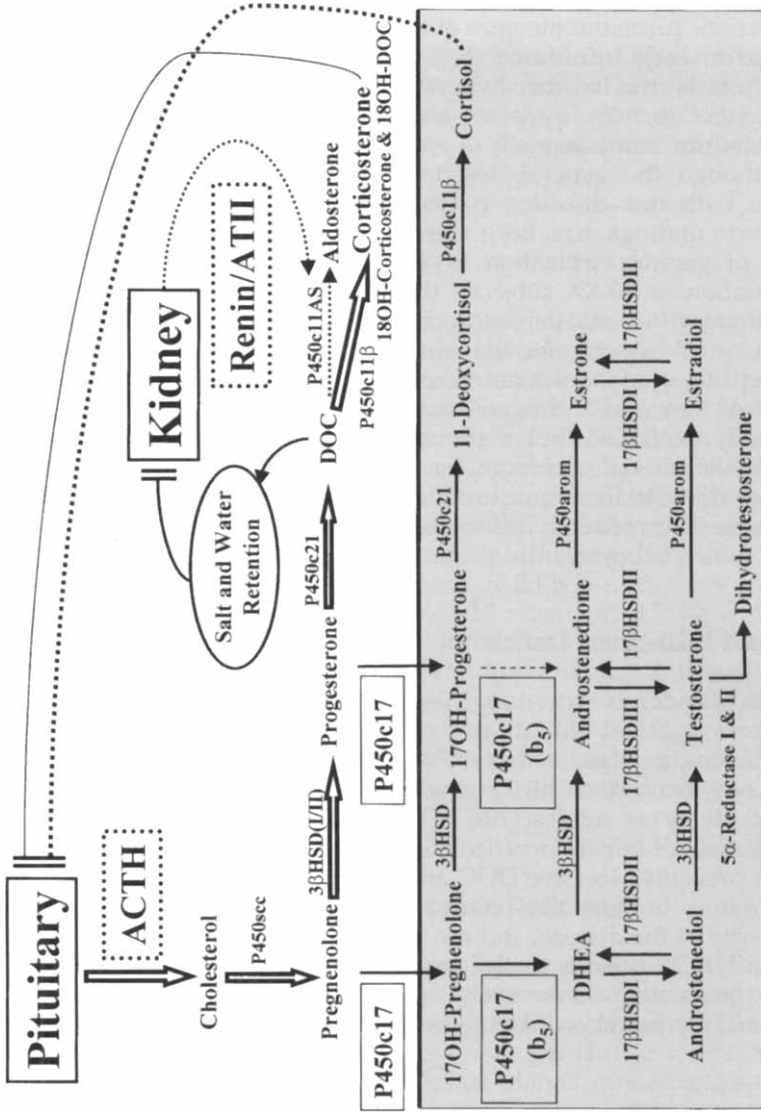

Figure 2. *See legend on opposite page.*

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adulthood. Patients with a 46,XY karyotype and incomplete deficiency may be misdiagnosed with androgen insensitivity or defects in later steps of dihydrotestosterone biosynthesis. As is true for all steroidogenic enzyme deficiencies, the diagnosis is most convincingly established by measuring precursor-to-product ratios during ACTH stimulation testing. In particular, circulating concentrations of the 17-deoxysteroids progesterone, corticosterone, and DOC rise to 5 to 10 times normal after ACTH administration.[15] In addition, 17-hydroxylase deficiency, in distinct contrast to 11-hydroxylase and 21-hydroxylase deficiencies, is characterized by elevated production of 18-hydroxycorticosterone and 18-hydroxy-DOC (Table 2).[33] The ratio of corticosterone to DOC (or of their 18-hydroxy-derivatives) distinguishes 17- from 11-hydroxylase deficiency. Table 3 compares the clinical, laboratory, and genetic characteristics of the various mineralocorticoid excess states that may arise in children and young adults.

Although production of the precursors corticosterone and DOC is markedly elevated in 17-hydroxylase deficiency, DOC production can be much greater in 11-hydroxylase deficiency, whereas plasma 18-hydroxy-DOC concentrations are not elevated.[33] The reason for this apparent discrepancy is that P450c11β (the product of the *CYP11B1* gene) is not exclusively an 11β-hydroxylase but exhibits weak 18-hydroxylase activity[49, 64] (Fig. 2) and trace amounts of aldosterone synthase activity.[75] The low 18-hydroxy-DOC production in 11-hydroxylase deficiency, despite enormous DOC concentrations, is compelling genetic evidence that P450c11β is responsible for elevated 18-hydroxy-DOC and 18-hydroxy-corticosterone production in 17-hydroxylase deficiency. Analogously, in glucocorticoid-remediable aldosteronism, abundant 18-oxygenase activities in the zona fasciculata owing to the presence of a chimeric *CYP11B2/11B1* gene[36] lead to excessive 18-oxygenated steroid production.[16] Patients with 17-hydroxylase deficiency with paradoxically measurable, if

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Figure 2. Physiologic disturbances in glucocorticoid, mineralocorticoid, and sex steroid homeostasis in complete 17-hydroxylase deficiency. The inability to 17α-hydroxylate C_{21} steroids in the adrenal gland eliminates all steroids within shaded region and shunts pregnenolone flux to progesterone, 11-deoxycorticosterone (DOC), corticosterone, and possibly aldosterone *(large open arrows)*. Absence of negative feedback by cortisol *(dashed line)* causes overproduction of adrenocorticotropic hormone (ACTH) *(top-most large open arrow)*, and the resultant abundance of the weak glucocorticoid corticosterone provides adequate systemic glucocorticoid action and feedback on ACTH secretion *(solid line)*. The hypothalamic-pituitary-adrenal axis then reaches a steady state at a higher set-point; however, the drive to overproduce corticosterone allows the accumulation of intermediates such as the potent mineralocorticoid DOC, and high DOC production stimulates salt and water retention, which suppresses renin secretion *(dashed arrow)*. Thus, aldosterone production is low *(dashed arrow)*, but hypertension and hypokalemia develop because of DOC excess. In addition, the unusually high concentrations of DOC and corticosterone in the presence of robust P450c11β expression leads to excessive production of ordinarily minor metabolites 18-hydroxy (18OH)-DOC and 18-OH-corticosterone. Because 17-hydroxy (17OH)-pregnenolone and dehydroepiandrosterone (DHEA) synthesis is nil *(shaded region)* in the fetus and at puberty, no androgen or estrogen synthesis is possible, and sexual infantilism results.

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Table 1. COMPARISON OF COMBINED 17α-HYDROXYLASE/17,20-LYASE DEFICIENCY AND ISOLATED 17,20-LYASE DEFICIENCY

Deficiency	Plasma Steroids	Urinary Steroids	Clinical Presentation
Combined 17α-hydroxylase and 17,20-lyase	↓ 17-OH-steroids, DHEA, androgens, estrogens ↑ Progesterone, DOC, DOC metabolites, corticosterone	↓ 17-OHCS, 17-KS pregnanetriolone ↑ Tetrahydro-DOC	Hypertension/hypokalemia, sexual infantilism
Isolated 17,20-lyase	Normal 17-OH-steroids ↓ DHEA, androgens ↑ 17-OHP/AD (>10 after hCG)	↓ 17-KS	Ambiguous genitalia in 46,XY

17-OHP = 17-hydroxyprogesterone; AD = androstenedione; hCG = human chorionic gonadotropin; DHEA = dehydroepiandrosterone; 17-OHCS = 17-hydroxycorticosteroids; 17-KS = 17-ketosteroids; DOC = 11-deoxycorticosterone.

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Table 2. COMPARISON OF STEROID PROFILES IN ADULTS WITH 17-, 11-, AND 21-HYDROXYLASE DEFICIENCES

Type of Deficiency	DOC ng/dL	18-OH-DOC ng/dL	Corticosterone ng/dL	18-OH-Corticosterone ng/dL	Aldosterone ng/dL
17-OH	25–500	100–600	4000–40,000	60–1000	<10
11-OH	50 to >1000	<10	<200	<10	<3
21-OH	10–100	3–20	100–500	10–200	10–60
Normal	2–20	1–20	100–500	10–40	10–30

DOC = 11-deoxycorticosterone; 18-OH-DOC = 18-hydroxy-DOC; OH = hydroxylase.
Data adapted from Kater CE, Biglieri EG: Disorders of steroid 17 alpha-hydroxylase deficiency. Endocrinol Metab Clin North Am 23:341–357, 1994.

not elevated, aldosterone production have been described. It is possible that, in these instances, artifacts owing to laboratory methods or intercurrent glucocorticoid therapy confound the data.[32] It is equally likely that other genetic and environmental modifiers contribute to these variations in disease manifestations, such as polymorphisms that alter the aldosterone synthase activity of P450c11β. The latter hypothesis is consistent with the finding that most 17-hydroxylase deficiency cases with measurable aldosterone production are from Japan.[32, 72] Until a large series of patients with 17-hydroxylase deficiency is compiled with uniform evaluation, these conundrums will persist.

Although heterozygous family members of patients with 17-hydroxylase deficiency without other endocrine abnormalities usually have clinically normal adrenal and gonadal physiology, it is sometimes possible to detect heterozygosity using biochemical testing. Elevated corticosterone and 18-hydroxycorticosterone concentrations, as well as the 18-hydroxycorticosterone-to-aldosterone ratio, after ACTH stimulation are perhaps the most readily available means to detect heterozygotes if an index case has been identified.[65] More precisely, the ratio of total urinary metabolites of corticosterone to those of cortisol is elevated (reflecting low 17α-hydroxylation), and the ratio of total urinary metabolites of C_{19} steroids to those of C_{21} steroids is low (reflecting low 17,20-lyase activity).[13] If a compelling reason for ascertainment of an individual's zygosity exists, molecular genetics provides a highly sensitive, although tedious, method that must be performed in a research laboratory.[20]

MOLECULAR GENETICS

Deletions, Premature Truncations, Frameshifts, and Splicing Errors

Among the genetic abnormalities described in the *CYP17* gene, the largest deletion reported involves the substitution of 518 bp (most of exon 2 and part of exon 3) with 469 bp of unknown DNA, disrupting the protein near its beginning and causing complete 17α-hydroxylase

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Table 3. COMPARISON OF MINERALOCORTICOID EXCESS STATES WITH SUPPRESSED PLASMA RENIN ACTIVITY

Disease	Laboratory Findings	Key Features	Molecular Basis
*17-Hydroxylase deficiency	↑ DOC, corticosterone, and 18-OH derivatives ↓ 17-OH-steroids, C_{19} steroids ↓ Aldosterone	Sexual infantilism/ambiguity	*CYP17* mutations
Primary aldosteronism	↑ Aldosterone, often ↑ 18-OH-corticosterone	Normal cortisol axis	Unknown
DOC-oma	↑ DOC, normal aldosterone, variable other steroids	Normal cortisol axis	Unknown
*Syndrome of apparent mineralocorticoid excess	↓ Cortisone metabolites ↓ Aldosterone	Dexamethasone suppression of hypertension, kaluresis	*HSD11B2* mutations
*Glucocorticoid-remediable aldosteronism	↑ 18-OH- and 18-oxocortisol, variable aldosterone	Dexamethasone suppression of hypertension, kaluresis	*CYP11B2/11B1* chimeric gene
*Cushing syndrome	↑ Cortisol production, variable other steroids	Symptoms of cortisol excess, variable mineralocorticoid excess	Unknown
*Glucocorticoid resistance	↑ Cortisol production ↑ C_{19} steroids	Symptoms of cortisol insufficiency, androgen excess	GR mutations, other

*ACTH-dependent mineralocorticoid excess.
DOC = 11-deoxycorticosterone; OH = hydroxy; GR = glucocorticoid receptor.

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deficiency.[6] A 4-bp duplication of the sequence CATC following Ile479[30] was originally observed in Canadian Mennonites[29] and has been subsequently found in at least six Dutch Frieslander families.[28] This duplication leaves 95% of the protein unaffected and creates a mutant P450c17 that has an altered sequence in its last 25 residues, that is truncated three residues prematurely, and that is wholly devoid of enzymatic activity. The crucial nature of the carboxy terminus of P450c17 is also shown by the complete absence of activity in the 9-bp, in-frame deletion of residues Asp487, Ser488, and Phe489[19] and in a Gln461→stop mutation.[73] Although these mutants retain the heme-binding region, these ostensibly minor alterations in the extreme carboxy terminus are catastrophic for enzymatic activity.

A computer model of human P450c17 suggests why the enzyme is so sensitive to alterations in its carboxy terminus.[5] The last 48 residues of P450c17 are involved in an extended β-sheet structure that folds down from the protein surface to form the "roof" of the active site, which is critical for proper substrate binding and subsequent catalysis. The CATC duplication after Ile479,[30] the deletion of residues 487 to 489,[19] and the mutant Gin461→stop,[73] which all retain the heme-binding site, disrupt or lack this critical stretch of residues required for activity.

The mutation delTG300,301 shifts the reading frame and alters the codon use beginning within exon 5.[43] Mutation 7bp dup 120 changes the reading frame from exon 2 onward.[70] The premature truncation Trp 17→stop has been found in a homozygous[68] and a compound heterozygous[61] patient, and mutations Glu194→stop and Arg239→stop each comprise separate alleles in a single patient with complete 17α-hydroxylase deficiency.[56] These three early truncations are not informative for structure/function studies because they delete the heme-binding region as well as residues important for substrate and redox partner binding.

Two deleterious intronic mutations have been described, a G to T substitution at nucleotide +5 in intron 2[61] and an analogous G to A substitution at position +5 of intron 7.[66] These splice junction mutations delete exons 2 or 7, respectively, during RNA processing ("exon skipping"). The excision of these exons introduces early premature stop codons well before the heme-binding region. The deletion of a G within codon 438 has been found in a homozygous patient.[51] This mutant gene encodes a protein in which the Gly-Pro-Arg-Ser-Cys-Ile motif at residues 438–443 (the underlined Cys ordinarily donates the axial sulfhydryl to the heme iron) is converted to Asp-Leu-Ala-Pro-Val-Stop, which destroys all enzymatic activity. An ATG→ATC substitution in the intiating methionine codon has been described in a patient with complete 17α-hydroxylase deficiency and hypokalemic myopathy.[58]

Amino Acid Substitutions—Combined 17α-Hydroxylase/17,20-Lyase Deficiency

Careful biochemical and computational analyses of mutant enzymes from patients with unusual phenotypes can provide insight into the

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functional roles of specific amino acids in P450c17. For example, the mutation His373Leu, when expressed in *E. coli,* lacks the classical P450 difference spectrum,[44] strong evidence that this protein does not bind heme properly. Modeling studies[5] predict that His373 lies distant from the heme moiety, suggesting that structural changes elsewhere in the His373Leu mutant secondarily abolish heme binding. In contrast, the mutation Arg440His[18] lies two residues away from the heme-liganding Cys442, and the reason for loss of activity in this mutant is more straightforward. In most P450 enzymes, an analogous arginine residue in this position is critical for neutralization of a negative charge on a heme proprionate and stabilization of heme incorporation[25]; hence, this mutation also interferes with heme binding.

The mutation Ser106Pro, found in two apparently unrelated Guamanian patients,[38] introduces a helix-breaking proline into what is predicted to be the B′-helix, near residues that form a lateral boundary of the substrate-binding pocket. P450c17 is sensitive to perturbations in this region, such that even the conservative replacement of Ser106 with Thr (the corresponding residue found in rainbow trout P450c17[57]) abolishes most enzymatic activity.[37] Specifically, Ile112 is predicted to interact directly with substrate, suggesting why mutation insIle112 is devoid of measurable activity.[27] Nearby, mutations Gly90Asp[67] and Arg96Trp[34] are predicted to reposition the second strand of β-sheet 1, containing the key residue Gly95. Computer simulations predict that 3β-hydroxyl and 3-keto groups of Δ^5 and Δ^4 substrates, respectively, form hydrogen bonds to the carbonyl group or the amide hydrogen of Gly95.[5, 41] The four mutants insIle112, Ser106Pro, Arg96Trp, and Gly90Asp may all primarily impair substrate binding.

Three mutations that retain partial enzymatic activity have also been described. Mutations Tyr64Ser[27] and Pro342Thr[1] retain approximately 15% and 20% of wild-type activity, respectively. The loss of one of two contiguous Phe residues in the ΔPhe53/54 mutation[69] destroys all but a trace of enzymatic activity,[72] and this mutation has been found in other cases of 17-hydroxylase deficiency in Japan,[42] suggesting a founder effect. The structural alterations responsible for the loss of activity in these mutants are not entirely clear, but these regions of the protein must be somewhat more tolerant of such structural changes than, for example, the active site and the heme-binding region.

Mutations Causing Isolated 17,20-Lyase Deficiency

The first patient with isolated 17,20-lyase deficiency in whom the *CYP17* gene was sequenced proved to be a compound heterozygote for the Gln461→stop and Arg496Cys mutations.[73] When studied in transfected cells, the Gln461→stop mutant was inactive, but the Arg496Cys mutant retained a small amount of 17α-hydroxylase and 17,20-lyase activities.[73] When restudied as an adult,[74] the patient's steroid hormone profile reflected nearly complete deficiencies of 17α-hydroxylase and 17,20-lyase activities, consistent with the molecular genetics and bio-

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chemistry of the mutant proteins. This case illustrates many of the pitfalls in the diagnosis of isolated 17,20-lyase deficiency and emphasizes that the clinical features, the molecular genetics, and the biochemistry of the mutant P450c17 protein(s) must all be congruent to ensure an accurate diagnosis.

Recently, two 46,XY Brazilian patients presented with convincing clinical evidence of isolated 17,20-lyase deficiency, that is, genital ambiguity and diminished C_{19} steroid production yet normal 17-hydroxycorticosteroid production. One patient was homozygous for mutation Arg347His and the other for Arg358Gln, whereas each parent was heterozygous for the respective mutant allele.[20] When expressed in COS-1 cells, the mutants hydroxylated progesterone and pregnenolone,[20] but only a trace of 17,20-lyase activity could be reconstituted by coexpressing an excess of oxidoreductase and b_5.[21] Although 17α-hydroxypregnenolone is a poor substrate for the mutant enzymes, competition experiments unequivocally show that the affinity of the mutant proteins for 17α-hydroxypregnenolone is equivalent to that of the wild-type enzyme,[20, 21] suggesting that arginines 347 and 358 do not lie in or near the active site.

Computer modeling studies demonstrate that R347H and R358Q neutralize positive charges in the redox partner binding site.[5, 20] Biochemical studies confirm that mutations R347H and R358Q impair interactions of P450c17 with its electron donor P450-oxidoreductase and with cytochrome b_5[21]; therefore, isolated 17,20-lyase deficiency is not caused by an inability of the mutant enzymes to bind the intermediate 17α-hydroxypregnenolone but rather by subtle disturbances in interactions with redox partners.[5, 20, 21] Another patient subsequently shown to have isolated 17,20-lyase deficiency was found to harbor mutation F417C,[8] which is predicted to lie on the edge of this redox partner binding surface.[5] The biochemistry of the F417C mutant has not been studied in detail, so it is not known if the same mechanisms as for the R347H and R358Q mutants apply to F417C.

A male pseudohermaphrodite with congenital methemoglobinemia owing to a mutation in the gene for cytochrome b_5 has been described.[22] It is possible that this patient was incompletely virilized because of low (but not absent) testicular 17,20-lyase activity and testosterone deficiency in utero owing not to a P450c17 mutation but rather to the loss of b_5, the cofactor protein that stimulates 17,20-lyase activity. Neither circulating steroid hormone concentrations nor a genetic analysis of the *CYP17* gene were reported for this subject. If this patient has isolated 17,20-lyase deficiency owing to the loss of b_5, the physiologic importance of b_5 in P450c17 chemistry would be proved.

MANAGEMENT

The child with 17-hydroxylase deficiency is chronically exposed to elevated circulating mineralocorticoid (DOC) concentrations but roughly normal amounts of glucocorticoids (as corticosterone). Mineralocorticoid

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excess in the neonatal period is of no consequence because mineralocorticoid (aldosterone) production is normally high in infants[17]; however, as the child ages and begins to consume solid foods, sodium intake rises, and mineralocorticoid excess can lead to sodium retention, hypertension, and hypokalemia. The hypertension can become fixed if not treated for many years[39]; hence, some control of DOC production is desirable. Moderation of dietary sodium content is prudent as an adjunct to pharmacologic therapy, which consists of glucocorticoid supplementation to reduce aberrant DOC production. Special considerations in the child with 17-hydroxylase deficiency include the avoidance of highly potent fluorinated glucocorticoids, such as dexamethasone, that have disproportionately large detrimental effects on linear growth and bone mineral accrual. Hydrocortisone administered in two or three divided doses will generally suffice, although direct comparison of steroid regimens in this uncommon disease are lacking. The glucocorticoid dose should be titrated to normalization of blood pressure and plasma potassium concentrations, as well as restoring plasma renin activity to the measurable range as endpoints. The frank normalization of plasma DOC and corticosterone concentrations may require overtreatment with glucocorticoids.[52] It is preferable to err on the side of undertreatment because the dire consequences of glucocorticoid excess throughout childhood are less desirable than modest mineralocorticoid excess.

As is true for patients with Turner's syndrome, gonodal dysgenesis, androgen insensitivity, or some other steroid biosynthetic defects, patients with 17-hydroxylase deficiency fail to exhibit pubertal development, and fetal testosterone deficiency causes all but the most mildly affected patients to present phenotypically as prepubertal females. In addition, the testosterone surge that occurs during the first year of life in 46,XY children is absent in 17-hydroxylase deficiency, which could theoretically impair responsiveness to testosterone later in life for mildly affected individuals. In most cases, estrogen replacement therapy is initiated at the time of expected puberty or on diagnosis if that time has already passed. Estrogen replacement not only allows the development of female secondary sexual characteristics but stimulates the increase in bone mass that normally occurs during puberty.[24] In a few cases, testosterone supplementation has been given to mildly affected 46,XY patients to stimulate penile development[15]; however, as is true for patients with partial androgen insensitivity, the rearing of these individuals as males and the choice of appropriate therapy are complex decisions that unfortunately may yield less than satisfactory results.

The treatment of 17-hydroxylase deficiency in the adult patient strives to achieve four goals: (1) reduction of the production or action of mineralocorticoids; (2) avoidance of the untoward effects of glucocorticoid excess; (3) replacement of sex steroids; and (4) prevention of the long-term consequences of the abnormal physiology. Although the caveats and special considerations are somewhat different in the two age groups, the cornerstone of the therapeutic plan remains sodium restriction plus glucocorticoid supplementation, traditionally consisting of a

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daily dose of dexamethasone. Patients with 17-hydroxylase deficiency given dexamethasone demonstrate a prompt reduction in DOC and corticosterone production, with naturesis and resolution of kaluresis.[33] The hypertension usually resolves with glucocorticoid therapy,[52] but if the diagnosis has been delayed for many years, the hypertension can become fixed.[39] The goal of glucocorticoid therapy is to restore the blood pressure and plasma potassium concentration to normal using the minimal amount of drug possible, usually 0.25 to 1 mg/d of dexamethasone or 2 mg/d to 5 mg/d of prednisone. Circulating concentrations of DOC and corticosterone may not completely normalize on this regimen,[52] but a rise in renin and aldosterone during glucocorticoid administration indicates that the therapeutic goal of eliminating ACTH-dependent mineralocorticoid excess has been achieved.[33] As is true in other states of ACTH-driven mineralocorticoid excess, such as glucocorticoid-remediable aldosteronism and apparent mineralocorticoid excess, care must be taken not to suppress the hypothalamic-pituitary-adrenal axis overzealously, which can lead to complications of glucocorticoid excess.[16] Instead, small doses of mineralocorticoid antagonists, such as spironolactone or potassium canrenoate, can be added to the regimen, allowing modest glucocorticoid doses during long-term therapy in the adult as well.[39] Particularly with the development of newer mineralocorticoid antagonists lacking the side effects of spironolactone, such as eplerenone, blockade of mineralocorticoid action is likely to assume a larger role in the management of ACTH-dependent mineralocorticoid excess states. If hypertension persists despite adequate blockade of mineralocorticoid production or action, the addition of a calcium channel blocker to the regimen is usually sufficient.[32, 39]

Patients who have 17-hydroxylase deficiency also fail to produce DHEA, and beneficial effects of DHEA supplementation in women (but not in men) with adrenal insufficiency have been demonstrated.[2] Because it is not clear whether the benefits of DHEA in these women are caused by the direct action of DHEA or the conversion of DHEA to active androgens, definitive recommendations cannot be made, and any regimen would require a preparation and dosing that accommodates a narrow therapeutic window to avoid the undesirable consequences of androgen excess. Nonetheless, some form of C_{19} steroid supplementation could be beneficial in 17-hydroxylase deficiency. Some 46,XX females with partial 17-hydroxylase deficiency have been reported to have spontaneous menses,[59] but, as a general rule, 46,XX patients require cyclical or combined estrogen-progestin replacement therapy to prevent endometrial hyperplasia from unopposed estrogen stimulation. In contrast, 46,XY females lack Müllerian structures and can be treated with an estrogen replacement regimen without the progestin. One astonishing report describes the successful in vitro fertilization of a 46,XX patient with 17-hydroxylase deficiency after stimulating ovarian development, despite low intrafollicular estradiol concentrations.[55]

Affected 46,XY individuals require gonadectomy to prevent malignant degeneration in their intra-abdominal testes. The need for genetic

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and psychologic counseling of these individuals, particularly if some intersex features exist, should not be neglected. Most of these patients are diagnosed at an age at which their gender identity and role have been firmly established but before an age when they can fully grasp the complexity of their condition.

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NEW IDEAS FOR MEDICAL TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

Deborah P. Merke, MD, and Gordon B. Cutler, Jr, MD

Dramatic improvement has occurred in the lives of patients with congenital adrenal hyperplasia (CAH) during the past 50 years through the introduction of glucocorticoid and mineralocorticoid replacement therapy[18] and luteinizing hormone–releasing hormone (LHRH) agonist treatment to arrest secondary LHRH-dependent precocious puberty.[43] Owing to the discovery of cortisone therapy, patients with classic CAH can have a long lifespan. Despite these advances, existing treatment has failed to normalize the growth and development of many children with CAH, and the clinical management of adults with CAH is often complicated by iatrogenic Cushing's syndrome, inadequately treated hyperandrogenism, or infertility.[8] Many of these unresolved clinical problems exist even when compliance with treatment has been excellent. Recently, adrenalectomy has been proposed as an alternative therapy for CAH that is difficult to manage with medical therapy.[32, 54] Nevertheless, existing therapy has been less effective than hoped, and it is unknown whether new medical approaches can improve on earlier results. This article addresses the clinical problems patients with CAH continue to have and new medical strategies that offer the prospect of an improved outcome of treatment. The goal of new treatment approaches is to normalize the growth and development of children with CAH and to

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optimize the quality of life of adults with CAH. Some of these strategies are being tested, whereas others await medical and technical advances.

UNRESOLVED PROBLEMS IN THE TREATMENT OF 21-HYDROXYLASE DEFICIENCY

In their pioneering studies of glucocorticoid therapy for CAH, Bartter[2] and Wilkins[57] and their colleagues reasoned that a defect in the pathway of cortisol biosynthesis led to a compensatory increase in pituitary corticotropin (ACTH) secretion. The resulting increase in ACTH caused the hypersecretion of adrenal androgens. Because ACTH, like other anterior pituitary hormones, is regulated by negative feedback, they hypothesized that the administration of glucocorticoid at physiologic doses would restore the levels of ACTH and adrenal androgen to normal.

Experience during the past 50 years requires certain revisions to this hypothesis. The administration of physiologic hydrocortisone doses does not normalize plasma ACTH levels in patients who have CAH.[8, 33] Exogenous hydrocortisone (divided two or three times daily) fails to replicate the close temporal relationship between ACTH pulses and the subsequent cortisol pulses that would normally restrain ACTH by negative feedback. Moreover, a decreased sensitivity of feedback inhibition is often observed in patients with CAH. Recent studies in mice with 21-hydroxylase deficiency suggest that intrauterine glucocorticoid deficiency results in hyperactivity of the hypothalamic-pituitary-adrenal axis and insensitivity to glucocorticoid feedback.[48] Decreased glucocorticoid sensitivity may further blunt the central effects of glucocorticoid therapy, whereas peripheral glucocorticoid sensitivity is preserved, leading to growth inhibition among other undesirable side effects.

Even the restoration of normal ACTH secretion in CAH cannot normalize androgen production because, with any adrenal activity, the block at 21-hydroxylase shunts a greater than normal proportion of steroid intermediates into the androgen pathway. To overcome the intrinsic tendency of the adrenal gland to overproduce androgens in CAH, the rate of cholesterol side-chain cleavage must be decreased to below normal levels to prevent the excessive accumulation of 17-hydroxyprogesterone and shunting into the androgen pathway. To suppress the rate of cholesterol side-chain cleavage to below normal levels by negative feedback requires supraphysiologic doses of glucocorticoid. Patients with treated CAH may have normal cortisol levels and excessive androgen secretion, excessive cortisol levels and normal androgen secretion, or an intermediate state consisting of mild hypercortisolism and mild hyperandrogenism (Fig. 1).

Conventional medical treatment is often a difficult balancing act between the undesirable states of hypercortisolism and hyperandrogenism. Signs of glucocorticoid excess, such as obesity, poor growth velocity, or other features of Cushing's syndrome, are frequent among treated

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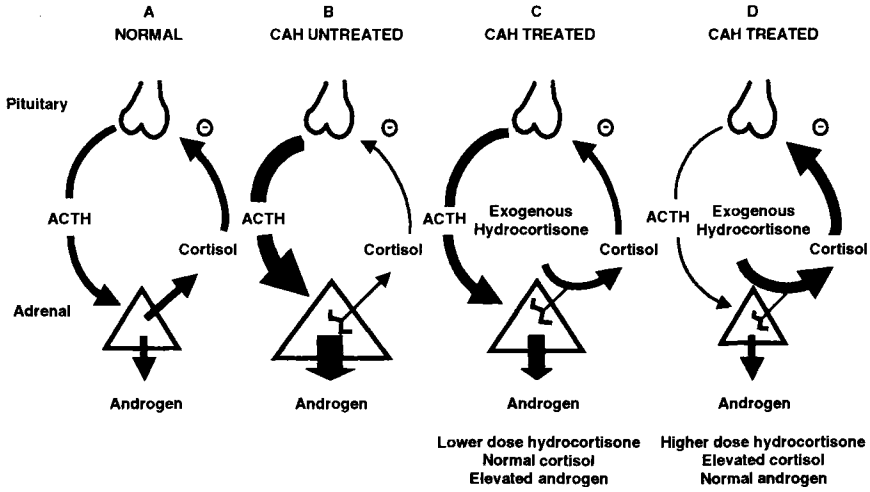

Figure 1. Relation between androgen production and cortisol production in untreated and treated patients with congenital adrenal hyperplasia (CAH). *A*, Normally, the adrenal gland produces cortisol and androgen, which is regulated by negative feedback. *B*, In the untreated patient with CAH, low levels of cortisol secretion are achieved in most patients through the compensatory hypersecretion of adrenocorticotropic hormone (ACTH) and adrenal hyperplasia. Overproduction of androgen is often massive. *C* Physiologic doses of hydrocortisone reduce androgen production, but not to normal levels. *D*, Supraphysiologic doses of hydrocortisone can normalize androgen levels, but at the expense of exposing the patient to elevated cortisol levels. Mineralocorticoid treatment has been omitted for clarity. (*Adapted from* Merke DP, Cutler GB: New Approaches to the treatment of congenital adrenal hyperplasia. JAMA 277:1073–1076, 1997; with permission.)

patients.[5, 26, 50] The symptoms and signs of hyperandrogenism include virilization of women, precocious virilization of men, and adult short stature in men and women. An additional complication in children is central precocious puberty, which is most likely to develop when the diagnosis of CAH is delayed or when control of adrenal androgen secretion is poor.[43] The premature elevation of gonadal steroid secretion compounds the problem of excess secretion of adrenal androgens.

The adult short stature frequently observed in patients who have CAH may be caused by hypercortisolism, by hyperandrogenism owing to indirect effects on the growth axis caused by hyperestrogenism, or by a combination of both. Retrospective studies indicate that the final height of treated patients is relatively independent of the degree of control of adrenal androgen levels.[11] Adrenal androgen secretion is best controlled in patients in whom the hydrocortisone dose, on average, is highest relative to their individual requirement and thus contributes the most to the ultimate adult short stature. Theoretically, patients with the most nearly physiologic hydrocortisone dose would tend to have the poorest control of adrenal androgen levels and bone maturation rates and, consequently, decreased final height because of early epiphyseal fusion; how-

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ever, glucocorticoid excess suppresses growth. A randomized prospective crossover trial showed that patients treated with 15 mg/m^2/d of hydrocortisone were less likely to show growth suppression when compared with patients taking doses of 25 mg/m^2/d.[45]

Once growth and development are complete, women with CAH often continue to have problems with hirsutism, amenorrhea, and infertility. Delay of the average age of menarche in girls with classic CAH is common,[25, 40] and a polycystic ovarian syndrome (PCOS) type of ovarian dysfunction has been described.[16] Androgens may directly prevent the maturation of follicles or affect the hypothalamic-pituitary-gonadal axis; however, menstrual irregularities, anovulation, and infertility in girls with CAH are not always caused by the undertreated state of hyperandrogenism. Girls with CAH have increased adrenal progesterone secretion[23] and elevated estrogen of adrenal origin.[40] Ovarian dysfunction in girls with CAH may be caused by abnormalities at the hypothalamic, pituitary, or ovarian level, and inadequate control of excessive adrenal sex steroids (androgens, progestins, and estrogens alone or in combination) may contribute to menstrual and reproductive disorders.

Once growth and development are complete, men with CAH may have infertility owing to the development of testicular adrenal rest tumors.[4] Inhibition of gonadotropins owing to excessive adrenal androgens usually does not occur, even in untreated adult patients with classic CAH.[52] The majority of adult men with CAH have normal fertility.[52] As many as 30% of males with classic CAH have evidence of adrenal rest tissue on a screening testicular ultrasound.[1] The clinical significance of small (<5 mm) testicular adrenal rest masses, which are detected only by screening ultrasound, is unknown.

Unresolved clinical problems in the management of classic 21-hydroxylase deficiency include an inadequate response to glucocorticoid and mineralocorticoid replacement therapy, iatrogenic Cushing's syndrome,[5, 26, 50] adult short stature,[9, 38] the activation of ectopic adrenal tissue resulting in adrenal rest tumors,[1] and infertility.[25, 37] Current understanding of the molecular defect in CAH does not permit prevention of the many clinical consequences of this disorder. The following sections describe new ideas for the medical treatment of CAH, which represent potential solutions to these unresolved issues (Fig. 2).

PERIPHERAL BLOCKADE OF ANDROGEN ACTION AND ESTROGEN PRODUCTION

Most of the adverse outcomes in treated children with CAH are attributable to supraphysiologic levels of glucocorticoid, androgen, progestin, or estrogen. Higher doses of glucocorticoid are often needed to achieve satisfactory androgen suppression, which exposes children with CAH to excessive levels of glucocorticoid. Glucocorticoid excess can be avoided by restricting the dose of glucocorticoid to the physiologic range[31]; however, the reduced glucocorticoid dose leads to elevated sex

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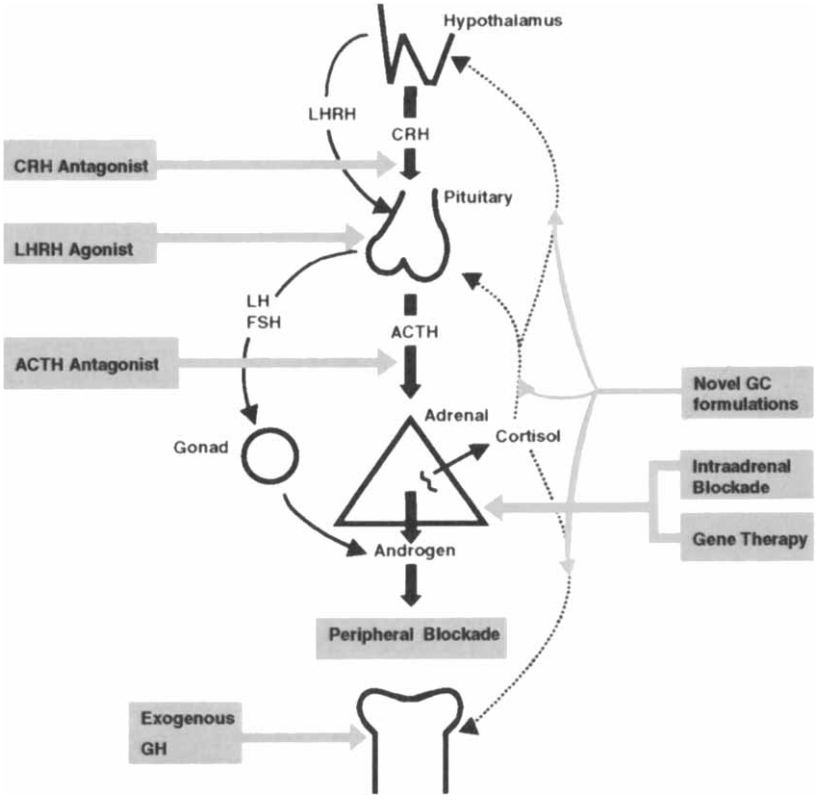

Figure 2. New treatment approaches for congenital adrenal hyperplasia (CAH). The hypothalamic-pituitary-adrenal axis is disturbed in CAH. In the undertreated state, there is increased production of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone(ACTH), and adrenal androgens caused by impaired cortisol production. Exogenous glucocorticoid treatment reduces androgen production by negative feedback on the hypothalamus and pituitary, but higher doses have a direct effect on bone and suppress growth. Promising new treatment approaches include optimization of negative feedback with the use of novel glucocorticoid formulations; intra-adrenal blockade of adrenal androgen production through the use of drugs that inhibit the enzymes of steroid synthesis; correcting the genetic defect through gene therapy; peripheral blockade of androgen action and estrogen production; enhancing growth with exogenous growth hormone; delaying puberty with luteinizing hormone-releasing hormone (LH-RH) agonist therapy; and blocking ACTH or CRH production through the use of antagonists. Stimulatory effects are indicated by solid lines; inhibitory effects are indicated by dashed lines. GC = glucocorticoid; GH = growth hormone.

hormone levels in most children. As an alternative approach to the treatment of CAH, the pathophysiologic effects of elevated androgen could be prevented through the use of an antiandrogen to block androgen action and an aromatase inhibitor to block the conversion of androgen to estrogen[8, 29, 33, 34] (Fig. 3). This treatment approach is based on the earlier successful use of spironolactone, an antiandrogen, and testolac-

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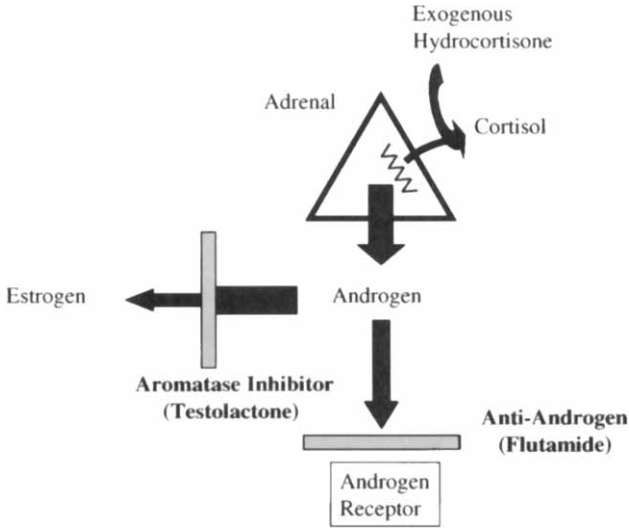

Figure 3. An investigational approach to the treatment of classic congenital adrenal hyperplasia. Fludrocortisone is given in the usual manner. The hydrocortisone dose is reduced to physiologic levels, resulting in elevated androgen production. An antiandrogen agent is administered to block the effect of the elevated androgen levels, and an inhibitor of androgen-to-estrogen conversion is given to block conversion of the increased amount of androgen to estrogen.

tone, an aromatase inhibitor, for the treatment of familial male-limited precocious puberty.[28, 30]

An investigational regimen of flutamide, testolactone, and reduced hydrocortisone dose (approximately 8 $mg/m^2/d$) is being studied at the authors' center in an ongoing clinical trial.[34] Instead of using high-dose hydrocortisone to suppress ACTH and androgens, lower doses of hydrocortisone are used, and androgens are allowed to remain elevated. The effects of androgens are then antagonized with an androgen receptor antagonist, flutamide, and the conversion of androgen to estrogen blocked with an aromatase inhibitor, testolactone. Estrogen blockade is important because estrogens advance bone age and cause children to enter central puberty precociously.

Preliminary 2-year results of this trial are promising.[34] Despite the reduced hydrocortisone dose and the expected increase in the levels of androgens, both of which would be expected to increase the rate of growth and bone maturation, growth and bone maturation rates declined significantly during flutamide and testolactone treatment. This investigational regimen fully normalized growth rate and bone maturation during the 2-year duration of the observations.

Flutamide and testolactone were chosen because of prior experience with these drugs. Although no liver toxicity has been observed in the

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subjects studied thus far, vigilance is required to use flutamide safely in children. Ongoing pharmaceutical development of improved antiandrogens and aromatase inhibitors offers the prospect of further improvement in the ability to achieve a peripheral blockade of excessive adrenal androgen. The ultimate test of this treatment is whether it will maintain normal growth and development throughout childhood and adolescence.

Peripheral blockade of androgens may also be useful in women with CAH. Women with CAH have an increase in PCOS,[16] and flutamide, or a similar antiandrogen, may antagonize intraovarian levels of androgens and reduce the incidence of PCOS. The use of an antiandrogen would also permit a lower glucocorticoid dose. Women of reproductive age taking an antiandrogen should be counseled regarding contraceptive use. Taking an antiandrogen during pregnancy may result in ambiguous genitalia in a male fetus. Other potential teratogenic effects are unknown.

INTRA-ADRENAL BLOCKADE OF ADRENAL ANDROGEN PRODUCTION

Intra-adrenal blockade of hormone production represents an additional approach to the medical treatment of patients with CAH. This approach has been successfully used in the treatment of familial male-limited precocious puberty[22] and Cushing's syndrome[47] with the use of ketoconazole. Ketoconazole blocks adrenal steroid production at several enzymatic steps and is effective in achieving a reversible medical adrenalectomy. Although the risk of hepatic toxicity caused by ketoconazole necessitates periodic monitoring of liver function, this risk has not prevented the successful long-term use of this drug in familial male-limited precocious puberty. A regimen of ketoconazole and physiologic hydrocortisone (and fludrocortisone), by reducing adrenal androgen production to normal levels and by avoiding glucocorticoid excess, would be predicted to improve growth and development in children with CAH. To the authors' knowledge, such an approach has not been attempted in patients with CAH.

Drugs have been developed that specifically inhibit many of the enzymes of steroid synthesis,[13] including cholesterol side-chain cleavage, 3β-hydroxysteroid dehydrogenase, 11-hydroxylase, and aromatase. Nevertheless, there is no specific inhibitor of 17,20-lyase activity. With the cloning of the *CYP17* gene that encodes this enzyme and the discovery that serine dephosphorylation of the enzyme greatly reduces 17,20-lyase activity,[59] the development of such inhibitors should eventually be achieved. By blocking the conversion of 17-hydroxyprogesterone to androstenedione, a 17,20-lyase inhibitor could provide a further medical strategy for normalizing adrenal androgen production without resorting to a supraphysiologic glucocorticoid dose.

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LUTEINIZING HORMONE–RELEASING HORMONE AGONIST AND GROWTH HORMONE TREATMENT

A common complication of CAH is central precocious puberty, which is most likely to develop when the diagnosis of CAH is delayed or when control of adrenal androgen secretion is poor. Excess secretion of adrenal androgens and estrogens results in advanced skeletal maturity, which is associated with premature activation of the hypothalamic-pituitary-gonadal axis. The premature elevation of gonadal steroid secretion compounds the problem of excess secretion of adrenal androgens. The result of excessive adrenal androgen production and precocious puberty is adult short stature. Children with CAH and central precocious puberty can be treated with an LHRH agonist to arrest secondary LHRH-dependent precocious puberty.[43] The beneficial effects of this combination of glucocorticoid and mineralocorticoid therapy to suppress adrenal hormone production and LHRH analogue to arrest gonadal activation include regression of the physical signs of puberty, improvement of behavioral changes (personal experience by DPM), and restraint of rapid growth rate and skeletal maturation.[44]

Treatment with an LHRH analogue has been shown to improve adult height in children with LHRH-dependent precocious puberty when compared with the height of untreated historical controls,[42] pretreatment height predictions,[20, 39] or midparental potential height.[20, 39] Nevertheless, adult height is not always fully restored to the patient's genetic potential. Patients who are treated at a younger bone age tend to achieve taller adult height.[42]

Numerous studies support the hypothesis that extended pubertal delay would improve the final adult height of children who have CAH. The adult height of patients with isolated hypogonadotropic hypogonadism and delayed puberty is greater than the normal height of the American population, and final height correlates significantly with the duration of pubertal delay.[53] Rare cases of estrogen insensitivity,[46] and aromatase deficiency[7] show remarkable tall stature with delayed epiphyseal fusion. A randomized double-blind, placebo-controlled clinical trial to determine the efficacy of LHRH analogue treatment on growth in children with non–growth hormone–deficient short stature and normally timed puberty revealed a significant increase in the adult height (average, 6 cm) in the LHRH analogue–induced pubertal delay group.[58] Similarly, a moderate pubertal delay (i.e., to age 13 years in girls and 14 years in boys) would be expected to improve adult height in children with CAH.

Growth hormone therapy in combination with LHRH analogue treatment has also been used to improve the adult height of children with central precocious puberty.[27, 41, 55] Moreover, growth hormone administration can increase adult height in children with idiopathic short stature.[21] Growth hormone may improve the adult height of children with short stature owing to CAH.

A LHRH agonist–induced pubertal delay, with or without the addi-

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tion of growth hormone therapy, may improve the final adult height of children who have CAH. Further studies are needed to determine the efficacy of these treatments in CAH.

ALTERNATIVE GLUCOCORTICOID FORMULATIONS OR DOSE SCHEDULES

Normally, the hypothalamic-pituitary-adrenal axis is controlled by negative feedback. In normal individuals, each ACTH pulse is followed within minutes by a pulse of cortisol that acts to restrain the magnitude and duration of the ACTH pulse by negative feedback. In patients with CAH, exogenous hydrocortisone administration fails to normalize plasma ACTH levels because it lacks the normal close temporal relationship to the ACTH pulses. Alternative methods of administering glucocorticoid, which would provide a greater feedback during the early morning surge of ACTH, hold promise as a strategy to improve treatment outcome. More than a decade ago, a dose schedule in which one third of the glucocorticoid dose was given at 3 AM was found to be much more effective in suppressing 17-hydroxyprogesterone than the daytime administration of the same total dose.[35]

The question remains how to best administer glucocorticoid to suppress the early morning ACTH surge and the tendency toward increased ACTH production in patients with CAH. Randomized clinical trials comparing treatment regimens are lacking. Some clinicians ask their patients to administer a midnight dose. Other physicians administer long-acting glucocorticoids, such as prednisone, at the bedtime dose. Dexamethasone has been used but is avoided in children because of the risk that glucocorticoid excess will suppress growth. A carefully designed clinical research protocol to individualize a nighttime dexamethasone dose, with monitoring of dexamethasone levels, might allow improved control of ACTH without growth suppression.

The development of a delayed-release hydrocortisone formulation may improve ACTH suppression. The development of formulations similar to the delayed-release synthetic glucocorticoids that have been used in inflammatory bowel disease is possible. Hydrocortisone is the preferred steroid rather than the synthetic glucocorticoids because its production rate in children and adults is known,[14, 31] because its metabolic clearance is relatively invariant (except after certain drugs), and because a great deal is known about the total daily dose that can be given without producing the complications of glucocorticoid excess.

Two approaches in the development of delayed-release glucocorticoids have been used for the purpose of delivering glucocorticoids to the colon in patients with inflammatory bowel disease: (1) a pH-dependent methacrylic acid copolymer (Eudragit L or S) coated formulation that dissolves at pH 6 or 7 and that is stable in an acid environment,[15] and (2) the synthesis of steroid esters that are resistant to degradation in the upper gastrointestinal tract.[19] There is an expected 4-hour delay from

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Eudragit S,[15] whereas the use of steroid esters is expected to have an approximate 6-hour delay.

Key pharmacokinetic properties of the formulations, such as the time of initial appearance of cortisol in plasma, the time to peak cortisol level, the time course of the decline in cortisol levels, and the bioavailability relative to the standard hydrocortisone formulation, need to be studied. If greater ACTH suppression by the delayed-release formulation is proven, a new treatment regimen can be tested in children with CAH in a randomized crossover design against a regimen using the standard hydrocortisone formulation. Strategies for developing a new hydrocortisone formulation would be expected to improve the suppression of early morning ACTH surge without increasing the total glucocorticoid dose, resulting in improved clinical outcome.

Preliminary studies in adult patients with CAH indicate that carbenoxolone, an inhibitor of the 11β-hydroxysteroid dehydrogenase enzyme that inactivates cortisol, can boost endogenous cortisol levels without increasing hydrocortisone dose, leading to improved hormonal control.[24]

CORTICOTROPIN-RELEASING HORMONE ANTAGONIST

Efforts in the treatment of CAH to date have focused on negative feedback by glucocorticoid to control ACTH secretion. This situation is analogous to the use of negative feedback by medroxyprogesterone to control gonadotropin secretion in precocious puberty. In precocious puberty, the effectiveness of treatment was improved dramatically when it became possible to block the hypothalamic LHRH signal by long-acting LHRH agonists.[6] Likewise, an effective antagonist of corticotropin-releasing hormone (CRH) or ACTH would dramatically improve the treatment of CAH by eliminating the need to rely solely on glucocorticoid negative feedback to prevent excessive adrenal androgen production.

What is the current feasibility of a new approach to CAH treatment using ACTH or CRH antagonist? With the cloning of the ACTH family of receptors,[36] new approaches to the development of ACTH antagonists are possible. Preclinical results with a CRH antagonist are promising.[3, 56] A prototype CRH receptor antagonist, antalarmin, binds to the CRH receptor type I and blocks the effects of CRH on that receptor. It acutely and chronically decreases ACTH and cortisol secretion without causing adrenal insufficiency.[3] In rats, chronic treatment with antalarmin is well tolerated. ACTH and corticosterone levels are decreased and adrenal size reduced owing to increased apoptosis in the outer zona fasciculata.

Corticotropin-releasing hormone has an important role in mediating behavioral, autonomic, and inflammatory stress-related response.[10, 12] Extensive preclinical research is exploring the effects of the CRH antagonist class of drugs because of their potential use in a variety of human conditions, including common disorders such as depression and eating

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disorders. The use of a CRH antagonist represents a novel therapeutic approach to CAH. Once toxicology studies are complete and human studies are possible, the therapeutic usefulness of a CRH anatagonist in the treatment of CAH should be tested.

GENE THERAPY

Although 21-hydroxylase deficiency is not an appropriate candidate for gene therapy at this time, methodologic advances will eventually make gene therapy safer, more effective, and possibly less expensive than alternative approaches. Pharmacologic approaches are not completely safe or effective, and they are costly in terms of drugs, monitoring, and the quality of life (e.g., the inconvenience of lifelong drug administration). Gene therapy represents a potential solution to the inherent problems of pharmacologic therapy and may be the ideal treatment in the future.

21-Hydroxylase deficiency has several features that may facilitate gene therapy. First, the 21-hydroxylase gene is expressed almost exclusively in the adrenal cortex. Second, because the cortisol pathway is regulated by negative feedback, precise regulation of 21-hydroxylase gene expression is not essential (e.g., heterozygotes for 21-hydroxylase deficiency have no clinical phenotype, and modestly increased expression of the gene would probably not be harmful). Moreover, the ability to remove adrenal glands by laparoscopy, combined with established techniques for adrenal autotransplantation, would make it feasible to perform the gene transfer ex vivo.

The 21-hydroxylase–deficient mouse is an ideal model in which to evaluate possible approaches to gene delivery and has proved useful in testing novel therapeutic strategies. As a model system for the treatment of human disease by genetic therapy, a recombinant DNA fragment containing the murine genomic gene for 21-hydroxylase has been successfully introduced into mutant mice.[17] Only 15% of newborns are typically rescued by synthetic steroid therapy, whereas the efficiency of rescue was increased to 80% through the use of gene therapy.[17]

The defective enzyme was recently replaced in the adrenals of the mouse model using an adenoviral vector encoding the genomic sequence of the human *CYP21* gene.[49] In homozygous 21-hydroxylase–deficient mice, intra-adrenal injections of this adenoviral vector allowed expression of human CYP21 mRNA and 21-hydroxylase activity in the adrenal, normalized plasma hormone levels, and corrected adrenal structural abnormalities. At the same time, the adenoviral vectors induced almost no inflammatory response in the adrenals, suggesting that high local glucocorticoid concentration suppresses the immune response caused by these vectors in other tissues. The adrenal may be a privileged site for gene therapy, an observation made earlier.[51]

In contrast to exogenous hormone replacement therapy, correcting the gene defect in 21-hydroxylase deficiency by adrenal gene transfer

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corrects the endocrine abnormalities characteristic of 21-hydroxylase deficiency.[49] The development of novel viral vectors with adrenal-specific promoters will be required to improve the efficiency and duration of gene transfer in the adrenal. With these advances, gene therapy may become a feasible option for the treatment of CAH.

SUMMARY

During the past 50 years since the discovery of cortisone therapy as an effective treatment for CAH, many advances have been made in the management of 21-hydroxylase deficiency. Despite these advances, the clinical management of patients with CAH is often complicated by abnormal growth and development, iatrogenic Cushing's syndrome, inadequately treated hyperandrogenism, and infertility.

New treatment approaches to classic CAH represent potential solutions to these unresolved issues. At the National Institutes of Health, a long-term randomized clinical trial is investigating a new treatment regimen: a reduced hydrocortisone dose, an antiandrogen, and an aromatase inhibitor.[34] Peripheral blockade of androgens may also be helpful in the adult woman with CAH and PCOS. Other promising new treatment approaches include LHRH agonist–induced pubertal delay with or without growth hormone therapy, alternative glucocorticoid preparations or dose schedules, CRH antagonist treatment, and gene therapy. The applicability and success of these new approaches await the results of current research.

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SURGICAL TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA

Jay J. Schnitzer, MD, PhD, and Patricia K. Donahoe, MD

The surgical treatment of patients with congenital adrenal hyperplasia (CAH) has evolved and improved over the past 3 decades since the authors have been caring for these children. Such improvements have led to earlier performance of surgery and particularly to the wider use of early single-stage surgery rather than relying on earlier external and delayed vaginal pull-through procedures. Evaluation of the outcome of surgery on these patients should be reserved for patients operated on during the last 15 years because techniques have improved, and because surgeons are better trained to perform these procedures and have a greater understanding of the molecular, genetic, embryologic, and endocrinologic issues involved in the pathophysiology of this disorder and the subtleties of the associated genotypes and phenotypes. A well-disciplined team including an endocrinologist, geneticist, pediatrician/neonatologist, surgeon, and anesthesiologist must understand and appreciate each other's role in caring for these patients. Although the birth of a child with ambiguous genitalia is a social emergency, care must be taken in making decisions of gender assignment. The treatment team must take the responsibility to educate the parents to take an active role in the process. Although gender assignment is easier for the patient with CAH, the medical crises associated with this disorder and their potential lethality demand accuracy in the differential diagnosis. Genetic counseling combined with endocrine treatment can lessen the phenotype for

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subsequent siblings and the trauma associated with their care and gender assignment.

This article discusses CAH in the context of normal sex determination and differentiation. Topics include how CAH can be differentiated from other causes of ambiguous genitalia that can produce similar phenotypes, how a rapid diagnosis can be made and early treatment used to avoid adrenal crisis, the timing of surgery after the child stabilizes, the optimization and use of early surgical correction, and follow-up for anticipated problems that may manifest at adolescence. With adequate diagnosis and treatment, a good outcome can be expected. The future holds promise for even better results for this cohort of patients and their families who have been the focus of research for generations of clinicians and investigators.

SEX DETERMINATION

One must understand how normal sex differentiation occurs to appreciate the variations that result when abnormalities occur. After decades of study, it was discovered that the sex-determining region of the Y chromosome *(SRY)* provides a genetic switch that initiates normal sex differentiation. *SRY*[18] was delimited to the distal pseudoautosomal 1A1 region of the short arm of the Y chromosome where small deletions were found to be associated with human sex reversal.[46] A single copy gene found on the Y chromosome of all mammals[21] and homologous to the high-mobility group (HMG) family of DNA-binding proteins was cloned[53] and found to be expressed in the urogenital ridge just before gonadal differentiation in a tissue-specific and male-specific pattern.[32] Definitive proof that SRY is the molecular switch for male differentiation was the finding that a 14kb fragment of the *sry* gene, transgenically expressed in chromosomal female mice, resulted in male development.[31] A decade of search has failed to reveal a gene or genes targeted by SRY, although the authors originally speculated[24] but eventually disproved[25] that Müllerian-inhibiting substance (MIS) would be such a target.

The steps between SRY expression and MIS and testosterone production are not fully clarified but include early gonadal ridge expression of SOX 9,[17, 30, 56] another HMG protein with homology to *sry* in the DNA-binding domain that is later male specific in its testicular expression pattern, and DAX-1[4], WT-1[23, 29, 33], SF-1[49], and GATA 4.[58] All of these transcription factors must be expressed in appropriate combinatorial patterns for the male phenotype to manifest. DAX-1 is an orphan nuclear hormone receptor mapped in the region of Xp21 where duplication causes male-to-female sex reversal. Expression of the *DAX-1* gene suppresses male gonadal ridge differentiation, permitting female gonad and reproductive duct development.[34] Mutations in WT-1 and SF-1, which are early determinants of differentiation of mesonephric mesoderm into a bipotential gonad, also lead to complete failure of gonadal development. GATA 4, which is male specific in its testicular expression pattern,

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is required along with SF-1 and SOX 9 for MIS expression.[5, 58, 61] Although there is a regulatory gene hierarchy in sex differentiation, that is, SRY, SOX 9 for testis, and DAX-1 inhibition of SF-1 for ovarian function, it is still not clear how SRY initiates the necessary downstream events. Architectural changes in chromatin may be as important as sequence-specific DNA binding. Although an SRY consensus sequence exists that is known to bind and to bend DNA,[19] it has not yet been shown to be essential for activation of SOX 9, MIS, or GATA 4, for expression of DAX-1, or for activation or repression of any other gene with sex-specific expression.

SEX DIFFERENTIATION

Male-specific morphologic changes characterized after germ cell migration[55] by seminiferous tubular formation and Leydig cell development—as well as biochemical changes characterized by the production of MIS and testosterone–ensue and perdure for variable periods of time. These biochemical products then direct reproductive duct development. MIS causes regression of the Müllerian duct, preventing development of the uterus, fallopian tubes, and vagina,[35] whereas testosterone stimulates the Wolffian ducts to form the vas, seminal vesicles, and epididymis.

DIAGNOSIS OF CONGENITAL ADRENAL HYPERPLASIA AND DIFFERENTIATION FROM OTHER CAUSES OF INTERSEX ABNORMALITIES

Congenital adrenal hyperplasia can result in intersex abnormalities at birth. One must differentiate accurately between CAH and other abnormalities that can cause sexual ambiguity at birth because CAH can be lethal if untreated. Abnormalities that result in gender ambiguity at birth (among them CAH) fall into three major categories: chromosomal abnormalities, excessive androgen syndromes, and deficient androgen syndromes.[13]

Chromosome Abnormalities

Chromosomal abnormalities result in syndromes of pure gonadal dysgenesis,[28] mixed gonadal dysgenesis,[16] and true hermaphroditism.[9, 57] Patients with pure gonadal dysgenesis are phenotypically female but have a 46,XY karyotype and streak gonads that fail to differentiate. In these cases, the 1A1 region of the Y chromosome can be abnormal owing to deletions.[46] In rare cases, mutations have been detected in the *sry* gene, mostly in the DNA-binding domain.[38, 39] Mutations in SOX 9[30] associated with campomelic dysplasia[17, 59] or WT-1 associated with Fra-

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sier syndrome[3] also result in pure gonadal dysgenesis. Fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) technologies can be used to confirm abnormalities in these candidate genes. The undifferentiated streak gonads fail to produce either testosterone or MIS, resulting in retained Müllerian duct derivatives and deficient Wolffian derivatives. These patients, who are more commonly discovered in utero because of ultrasonographic findings of a female perineum in the presence of a 46,XY karyotype, are gender assigned to the female sex at birth. Gonadotropin levels are high, whereas MIS and testosterone levels are undetectable. The authors recommend early removal of the streak gonads to avoid neoplastic changes.[52] Progressive incremental endocrine replacement therapy should be started before the onset of adolescence at a time dictated by the patient's growth characteristics, that is, earlier if the patient is exceeding the growth curve and delayed if short stature is observed.

Persons with mixed gonadal dysgenesis comprise the largest group of patients with chromosomal abnormalities leading to ambiguous genitalia.[16] Although the karyotype of 45,X/46,XY permits an early diagnosis, in the authors' experience, 40% of mixed gonadal dysgenesis patients can have a 46,XY karyotype.[10, 51] In these cases, asymmetry of the gonads and retained Müllerian ducts can help to establish the diagnosis. The characteristic phenotype is an intra-abdominal streak gonad and a partially descended small testis. Retained Müllerian ducts are characteristic of the disorder, but the vagina can enter the urethra at variable levels to create a urogenital sinus defect because of failure of the vagina to migrate normally to the perineum.

Frequently, enough testosterone is produced by the small testis to cause clitoral hypertrophy. MIS levels are usually low to normal, as are serum testosterone levels, which can be better detected after human chorionic gonadotropin (hCG) stimulation. Because the gonads are dysgenetic, this group of patients has the highest risk for neoplastic transformation.[52] For this reason, early gonadectomy is recommended. The decision toward male or female gender assignment is dependent on the time of presentation and the size of the phallus. If the phallus is small, indicating lesser degrees of androgenic stimulation, and if the infant presents early, a female gender assignment is logical. If the patient presents later in life and has a larger phallus, the male role is supported. In the case of female gender assignment, clitoral recession, vaginal exteriorization, labioscrotal reduction, and gonadectomy are performed at an early age. When the male role is to be supported, staged hypospadias repair with construction of a neourethra must be done, often coupled with presurgical testosterone treatment to enhance phallic size. The Müllerian structures need to be removed with preservation of the vas, if possible. Many of these children also require prepenile scrotal repair[13, 14] and orchidopexy with careful follow-up by palpation and serial sonography for neoplastic changes in the preserved testis.

Although chromosomal abnormalities can presumably cause true hermaphroditism, which is rare in North America but common in Af-

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rica[57] and Europe, its etiology is not clear. Deletions and mutations in SRY have not been found, although noncoding regions of the *SRY* gene have not yet been studied. Over 90% of these patients have a 46,XX karyotype, with the remainder more complex. The most common phenotypes are asymmetric with a descended testis and an intra-abdominal ovary or a more normal gonad on one side and an ovotestis on the other. Also possible but more rare are bilateral ovotestes, which may be symmetrical.[9] The presence of a testis can be confirmed by the detection of serum MIS and testosterone. Often there is sufficient testosterone to stimulate phallic growth. Müllerian ducts are often retained, but a urogenital sinus defect accompanies the clitoral hypertrophy. Longitudinal biopsies of ovotestes often contain ovarian tissue at the poles with central testicular tissue.[45] With careful dissection, it may be possible to preserve the gonadal tissue concordant with the sex of rearing. If these children are to be raised as females, clitoral recession, vaginal exteriorization, and labioscrotal reduction should be done early in infancy with preservation of ovarian tissue if possible. If the patient presents already committed to the male role later in life, staged hypospadias repair, removal of Müllerian structures with preservation of the vas,[7] prepenile scrotal repair,[12] and orchidopexy with preservation of testicular tissue should be performed.

Androgen-Deficient Syndromes

Individuals with a 46,XY karyotype can be insufficiently androgenized, leading to male pseudohermaphroditism, which can occur because of deficient testosterone production,[42] androgen receptor deficiency,[64] or an inability to metabolize testosterone to dihydrotestosterone.[27, 60] The enzymes of steroid metabolism necessary for testosterone production may be defective. These enzymes include steroid acute regulatory protein (Star), resulting in lipoid adrenal hyperplasia (formerly thought to represent defects in cholesterol desmolase, P450scc), 17-hydroxylase (P450c17), 3β-hydroxysteroid dehydrogenase, and 17-ketosteroid reductase. Androgen receptor abnormalities can cause either a mild or severe phenotype. In the former, it may be possible to raise the patient as a male; in the latter, the testicular feminization syndrome results, and the appropriate gender assignment is female. Failure of conversion of testosterone to dihydrotestosterone results from abnormalities in the 5α-reductase genes.[2] Patients with insufficient testosterone production may have a sufficient male phenotype to be raised in the male gender. In other situations, the phenotype may be predominantly female, and that gender should be supported. The karyotype is 46,XY; testosterone levels are low, even after hCG stimulation; but MIS levels are normal or even elevated. With hCG stimulation and measurement of precursors, the specific enzyme defect may be pinpointed and confirmed by genetic analysis of the specific gene.

The larger number of patients with male pseudohermaphroditism

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have deficiencies of the androgen receptor. The androgen receptor gene[6, 36, 37, 54] can be analyzed with PCR or complete DNA sequencing. The phenotype may be severe, in which case the female role is supported, or mild, in which case the male role is supported. Serum testosterone levels are elevated, as are MIS levels in these patients with a 46,XY karyotype. The testes may be fully descended or undescended but are symmetrical. Because of the high MIS levels, Müllerian structures are absent. An abnormal 5α-reductase enzyme[1, 2] can also result in insufficient androgenization, but most patients can be supported in the male role. Such patients may have severe penoscrotal hypospadias, but the phallus size is often sufficient for reconstruction,[8, 11, 22] and virilization takes place at puberty.

Androgen Overexpression: Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia with virilization is a syndrome that occurs when a female fetus is exposed to excessive endogenous androgens. Other designations are the adrenogenital syndrome or female pseudohermaphroditism.[62, 65] Morphologic changes occur in the adrenal gland where excessive adrenocorticotropic hormone (ACTH) results in hyperplasia of the fasciculata, which produces the glucocorticoid cortisol, and of the glomerulosa, which produces the mineralocorticoid aldosterone. The absence of these products and failure of their negative feedback on the pituitary results in excessive ACTH, which is thought to stimulate local intra-adrenal growth factors, such as basic fibroblast growth factor (FGF) and insulin-like growth factor (IGF) II,[40, 41] which are speculated to cause the hyperplasia. Similar phenotypic changes can be recapitulated by exposure to exogenous androgenic agents, as observed when they previously were used to prevent spontaneous abortions. Over 90% of CAH cases result from a deficiency of the enzyme CYP21, otherwise known as P450c21 or 21-hydroxylase. The genetics of CAH are complex. The *CYP21* gene, mutations of which are most commonly the cause of the disorder, resides on chromosome 6p in the HLA complex between *HLA B* and *HLA DR* as a tandem repeat of a functional (*CYP21B*) and a nonfunctional region or pseudogene (*CYP21A*). The 98% homology between these two isoforms leads to recombination, resulting in incorporation of deletions from the pseudogene that disable the functional gene.[20, 43, 44, 63] Five percent of CAH cases occur because of deficiencies of the CYP11B1 (P450c11 or 11β-hydroxylase) or 3β-hydroxysteroid dehydrogenase (Δ4,5-isomerase) enzymes.[15, 47]

The adrenal differentiates in the human fetus after 11 weeks' gestation, which is after the internal reproductive ducts have differentiated and the gonads have formed. The external genitalia, which differentiate after 11 weeks, are the most severely affected by excessive androgens. Under the influence of androgens, the genital tubercle becomes hypertrophied and has a more penile appearance. The labia majora become fused posteriorly and take on a scrotal appearance. The ovaries are normal

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and maintain a normal intra-abdominal position, never descending into the labioscrotal folds. The Müllerian structures (fallopian tubes, uterus, and upper vagina) are normal, but the lower vagina is foreshortened, conjoining with the urethra to form a high urogenital sinus defect rather than migrating normally to the perineum. Severely masculinized infants are often found to have firm prostate-like tissue surrounding the urogenital sinus where the vagina enters the urethra.

Because of the normal position of the ovaries, any patient with bilateral undescended testes should be suspected of having CAH. A 46,XX karyotype will confirm this diagnosis. Excess ACTH is often associated with excess melanocyte-stimulating hormone (MSH), which can result in darkened genitalia or breast areola, which should raise the suspicion for this diagnosis, particularly in the male. Testing for serum 17-hydroxyprogesterone,[48] which is performed routinely on all infants in many states in the United States, is definitive for the diagnosis of CYP21 deficiency and can usually be performed with a rapid turnaround time. PCR can be used to make the diagnosis in utero.[15] Although patients are protected by maternal steroids immediately after birth, within 1 to 3 weeks, patients with severe 21-hydroxylase deficiency go into an aldosterone deficiency salt-losing crisis characterized by hyponatremia, hyperkalemia, and acidosis, which leads to dehydration and vascular collapse and is potentially lethal if untreated. In males without a previously affected sibling, these early subtle changes are often undiagnosed and lethal because they are confused with pyloric stenosis or vomiting associated with gastroenteritis.[20, 44]

SURGICAL RECONSTRUCTION

Before performing one-stage surgical repair, which can take 3 to 5 hours depending on the severity of masculinization, steroid replacement is increased. Surgery is undertaken only after the infant has been stabilized medically as determined by suppression of 17-hydroxyprogesterone. In a stress regimen of steroid replacement (Table 1), a double oral

Table 1. PERIOPERATIVE STEROID MANAGEMENT OF NEONATE WITH CONGENITAL ADRENAL HYPERPLASIA

Time	Drug, Dose, and Route of Administration
Preoperative (on call to surgery)	Cortisone acetate microcrystals, 25 mg SQ
Surgery—initial (just as anesthesia and intravenous fluids are begun)	Hydrocortisone, 50 mg IV bolus
First 24 hours during and after surgery	Hydrocortisone, 50 mg IV or IM q6h
Beyond 24 hours postoperative, resume daily oral medications	Florinef, 0.1 mg PO q AM Hydrocortisone, 5 mg PO (AM), 2.5 mg PO (noon), 2.5 mg PO (PM)

SQ = subcutaneous; IV = intravenous; IM = intramuscular; PO = by mouth.

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dose is given 4 hours before surgery (for same-day admissions) or cortisone acetate, 25 mg subcutaneously, on call to surgery (for patients admitted the day before surgery). The exact dose and schedule may vary based on the patient's age, size, and the nature of the surgery. During anesthesia and after intravenous access has been established, a bolus of 50 mg of hydrocortisone is given. Thereafter, 50 mg of hydrocortisone is delivered every 6 hours for the first 24 hours. After oral feeding resumes, maintenance to maintenance doses are provided twice over the subsequent 48 hours. For simple cystoscopic procedures with a short anesthesia time, the authors give double doses 4 hours before surgery and for the first oral dose after surgery, after which maintenance doses of hydrocortisone and fluorocortisone (Florinef) are resumed.

Surgical reconstruction, which is performed to make the phenotype of the perineum concordant with the sex of rearing, is preceded by cystoscopy using either the Storz or Wolff infant cystoscopes. Full water flow allows one to better appreciate the external sphincter and to find and enter the vaginal orifice from the urogenital sinus. The vagina in most patients enters the urethra distal to the external sphincter. In a small but substantial subgroup of patients with female pseudohermaphroditism who are usually severely masculinized, the vagina enters at or above the external sphincter through a circumference of stimulated, firm, prostate-like tissue at the urogenital sinus opening. A small cystoscope can enter the vagina from the urethra, and a normal cervix can be visualized at the top of the vagina. A Fogarty catheter is secured in the vagina, and a Foley catheter is placed in the bladder. To prevent dislodgment, both are fixed together. The patient is then placed in the extreme lithotomy position for perineal reconstruction.

Virilized females with CAH and a 46,XX karyotype should undergo a feminine perineal reconstruction. The mainstays of the repair are clitoral recession, which preserves sensation and erection while reducing the enlarged clitoris, correction of the urogenital sinus defect that results from failure of the vagina to complete migration to the perineum, and exteriozation of the vagina. With increasing masculinization of the infant, the entry of the vagina into the urethra becomes more proximal and the repair more technically demanding. The labioscrotal folds need to be trimmed, thinned, and elongated to create a more normal-appearing labia majora; thus the repair consists of clitoral recession, labioscrotal reduction, and exteriorization of the vagina.

Because the techniques used to repair the most severely masculinized defects can be applied to repair less masculinized defects, the repair of the more complex anomaly is described herein, which can be varied to suit individual needs. To begin the clitoral recession (Fig. 1), the authors circumscribe 2 mm below the base of the glans (Fig. 1*A*), deglove the shaft, and divide the dorsal shaft skin to create Byars' flaps in a manner originally used for hypospadias repair (Fig. 1*B*). This incision is carried far enough cephalad to allow the glans to sit comfortably at the mons pubis in the normal female clitoral position.

Longitudinal parallel incisions are then made through Buck's fascia

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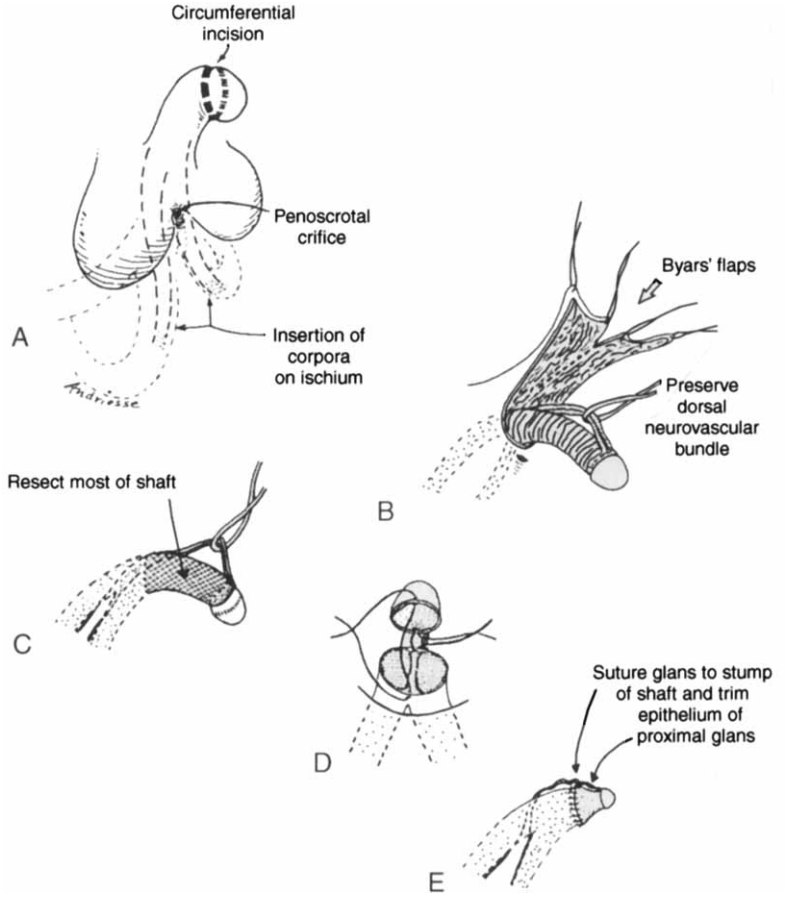

Figure 1. Clitoral recession. *A,* The enlarged clitoris is circumscribed below the glans. *B,* The shaft skin, which is predominantly dorsal, is mobilized, unfolded, and divided in the midline, while the skin of the shaft is removed down to the base of the corpus spongiosa; microdissection is used to preserve the dorsal neurovascular bundle. *C,* The shaft is resected between the base of the glans and a point just distal to the bifurcation of the corpora cavernosa. *D* and *E,* The glans is reapproximated to the base of the shaft with fine interrupted nonabsorbable sutures. (*From* Donahoe PK, Schnitzer JJ: Ambiguous genitalia in the newborn. *In* O'Neil JAJ, Rowe MI, Grosfeld JIL, et al (eds): Pediatric Surgery. St. Louis, Mosby, 1998, pp 1797–1818; with permission.)

on either side of the dorsal neurovascular bundle, which includes the left and right dorsal phallic arteries. Each artery branches from its respective internal pudendal artery as it egresses from Alcock's canal near the ischial tuberosity (Fig. 2) and then courses ventrally to the medial aspect of each corpora cavernosa and then to the dorsal aspect of the phallus, where it joins in the midline to complete its parallel course distally to the glans alongside its mate from the opposite side.

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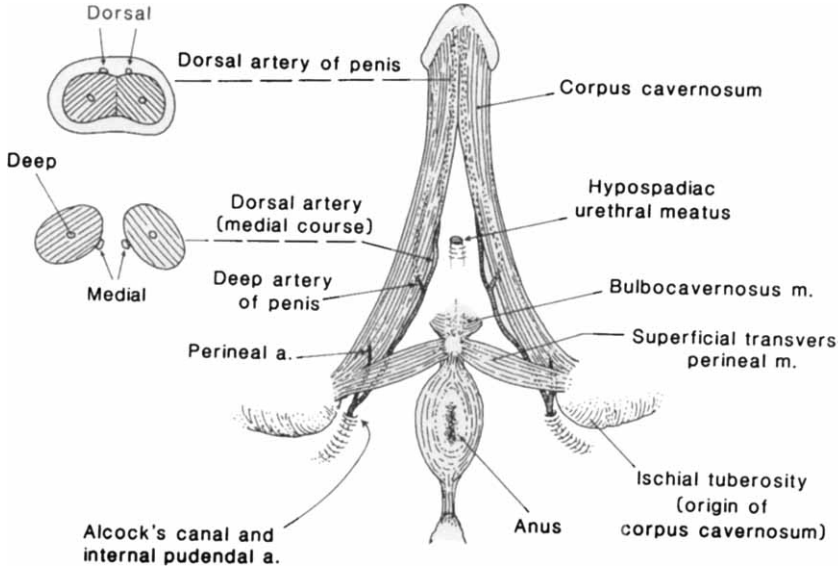

Figure 2. Neurovascular anatomy of the phallus. The internal pudendal artery exits from Alcock's canal at the ischial tuberosity and bifurcates. The perineal artery courses ventrally toward the scrotum. The dorsal phallic artery travels medially with one branch penetrating to become the deep artery of the phallus. Each medial branch curves around the corresponding bifurcated corpus cavernosum medially and dorsally to become the dorsal phallic artery at the point where the corpora conjoin. a = artery; m = muscle. (*From* Donahoe PK, Schnitzer JJ: Ambiguous genitalia in the newborn. *In* O'Neill JAJ, Rowe MI, Grosfeld JIL, et al (eds): Pediatric Surgery. St. Louis, Mosby, 1998, pp 1797–1818; with permission.)

Using a microtechnique and loupes for magnification, dissection is carried down to the fascia covering the corpora (Fig. 1*C*) where the neurovascular bundle is lifted off Buck's fascia covering the corpora and dissected back to a point where the conjoined corpora begin to divide and course laterally. The midbody of the clitoral corpora is then resected between the base of the glans and the bifurcation of the corpora after retracting the preserved dorsal neurovascular bundle out of the way. Selected hemostasis is secured in the corpora of the clitoris, taking care to avoid fibrosis to preserve future erectile function. The now redundant dorsal neurovascular bundle is carefully displaced out of the way while the corpora is sutured to the base of the glans with interrupted nonabsorbable 4-0, 5-0, or 6-0 sutures, depending on the age of the child and the size of the clitoris (Fig. 1*D*). If the glans is enlarged, it is trimmed circumferentially to appropriate size, except where the neurovascular bundle enters the dorsum of the glans, with preservation of only that epithelium that is to be exposed as the new clitoris (Fig. 1*E*). The preserved glans epithelium is then sutured around its edge to the mons cephalad and to the proximal separated Byars' flaps as they wrap around ventrally.

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The incisions and flaps needed to reduce the labioscrotal folds and to exteriorize the vagina are shown in Figure 3*A*. The caudal U-flap based on the rectum is advanced inward to join the posterior wall of the mobilized vagina. This maneuver creates the new introitus on the perineum. An anterior island flap to augment the introitus originates from the base of the glans and extends posteriorly to the top of the caudal U-flap. This square island flap becomes the anterior wall of the new introitus after it is rotated inward to meet the distal anterior wall of the mobilized vagina.

The anatomy as viewed from the side is shown in Figure 3*B*. The vagina, which enters the urethra as a urogenital sinus, proximal to the external sphincter in the most severely masculinized patients with adrenogenital syndrome, is by far the most difficult tissue to repair in an attempt to prevent subsequent narrowing. The anterior island flap from beneath the new clitoris is swung in (*arrows*) to meet the anterior wall of the mobilized vagina (*see below*), and the posterior flap is turned in to meet the posterior wall of the vagina, which diminishes the distance to the perineum that the newly mobilized vagina separated from the urethra must traverse. A finger in the rectum (Fig. 3*C*) allows the surgeon to appreciate the thinness of the plane separating the vagina and the rectum and to stretch out the plane to avoid angulation during the dissection and inadvertent entry into the rectum. A Fogarty balloon placed at the time of cystoscopy into the vagina can be palpated for orientation throughout the dissection, which must be carried well up to the peritoneal reflection, again with meticulous care to avoid entering the rectum. If the rectum is entered, it may be necessary to perform a diverting colostomy. Posterior crossing fibrous bands near the introitus must be divided to avoid stenosis of the introitus, but care must be taken to preserve the perineal body. The dissection is carried upward to identify the point where the vagina conjoins the urethra. A nerve stimulator is helpful in identifying and preserving the external sphincter.

U-flaps are drawn out on each labioscrotal fold (Fig. 3*D*). The most distal vagina is opened caudally first at the point where it conjoins the urethra (Fig. 3*E*). It is risky to attempt to circumscribe the vagina blindly at this point rather than to open it caudally because the proximal urethra can be injured or narrowed at this site. The previously placed Fogarty balloon is deflated, brought out this opening, and retracted upward, which exposes the Foley balloon in the urethra, which is then closed over the Foley catheter with interrupted absorbable sutures in at least two, and preferentially three, layers. Subsequently, the dissection is carried more proximally to separate the anterior wall of the vagina from the posterior bladder neck. To achieve sufficient mobilization to advance the anterior wall of the vagina beyond the closed urethral suture line, a move calculated to avoid fistula formation after surgery, it is necessary in most cases to continue the dissection up to the peritoneal reflection (Fig. 3*F*). The anterior island flap on the perineum is then swung downward and rotated inward to meet the mobilized anterior wall of the vagina where it is sutured with interrupted absorbable sutures. The

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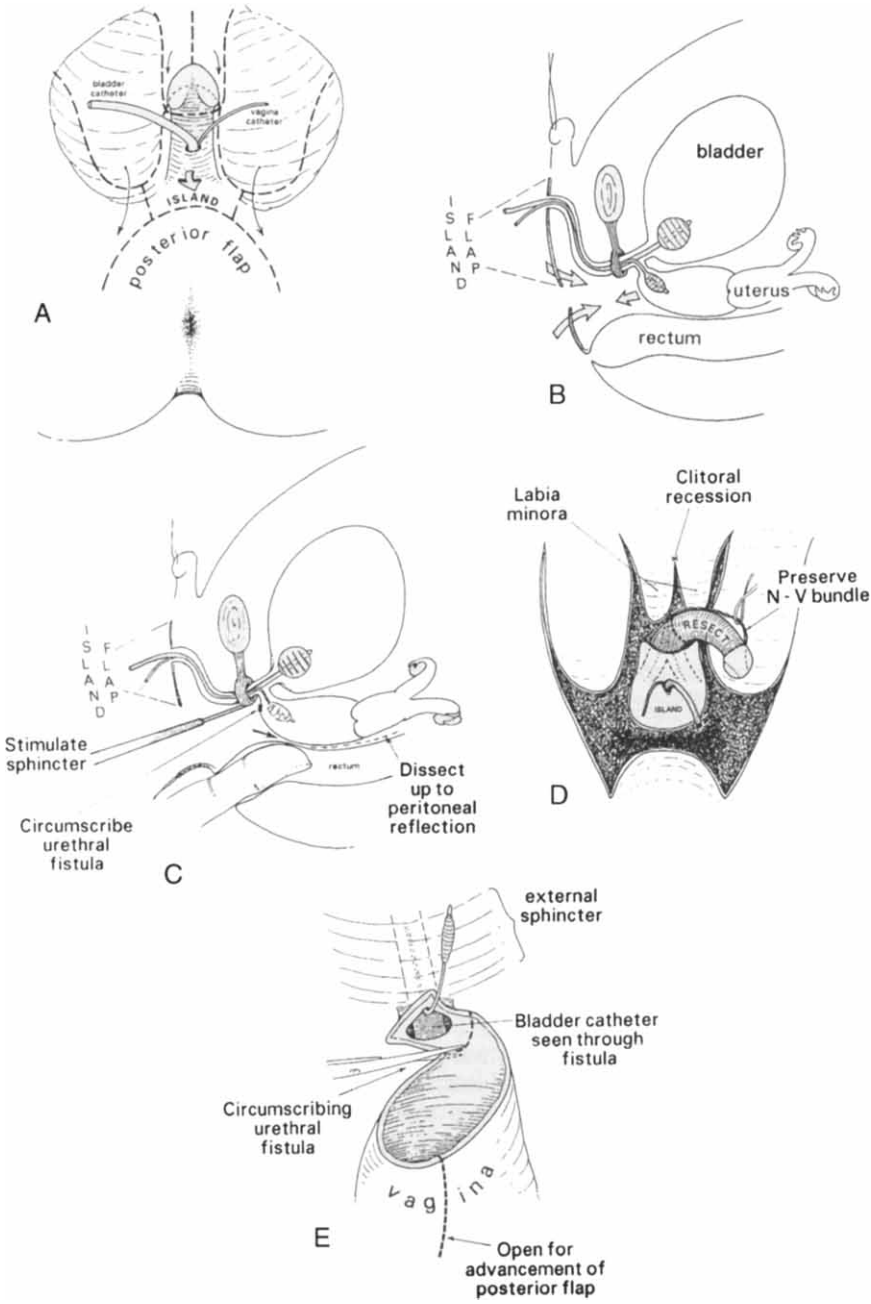

Figure 3. Surgical reconstruction for female gender assignment. *A,* Locations of incisions as viewed from the surgeon's perspective from the perineum with the patient in lithotomy position. *B,* Sagittal view, showing the downward and inward direction of movement of the anterior island flap *(right-pointing arrows)* and inward position of the posterior flap. The *smaller arrow* shows the distance that must be traversed by the vagina once it has been separated from the urethra. *C,* Dissection between the vagina and the rectum posteriorly, and beginning of the anterior separation of the vagina from the urethra. *D,* The completely dissected flaps. *E,* Separation of the vagina from the urethra.

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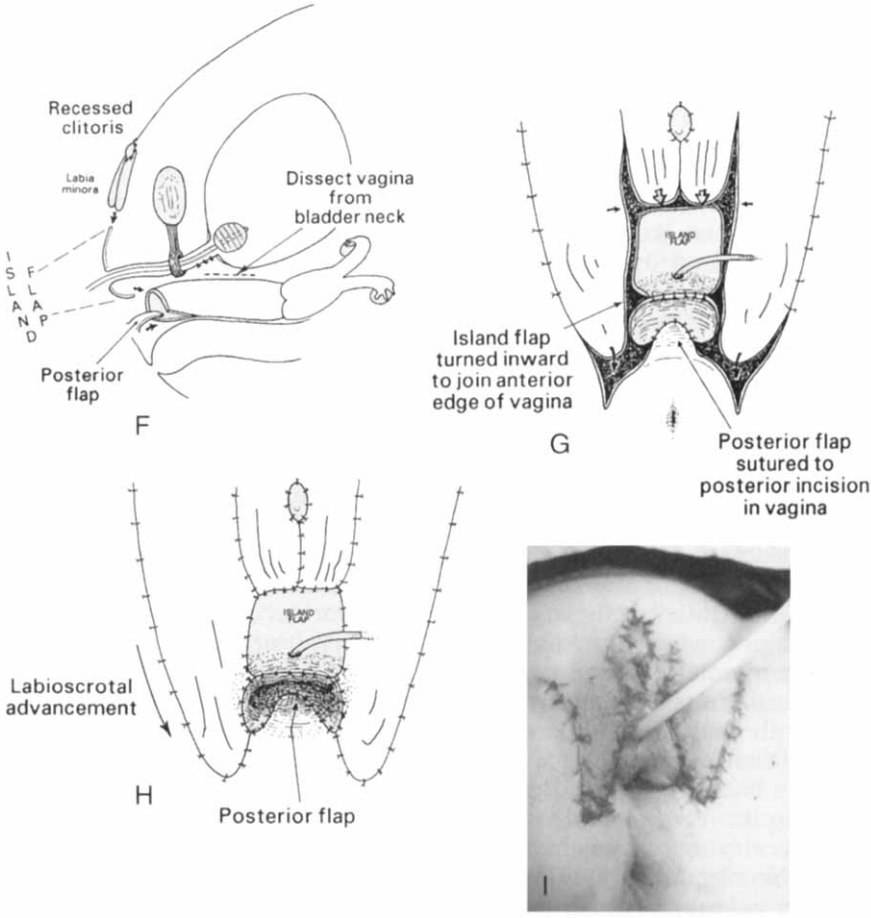

Figure 3 *(Continued). F,* The anterior plane of dissection behind the urethra and bladder. *G,* Advancement of the labioscrotal flaps to cover defects, and inward rotation of the anterior island flap and the posterior U-flap to augment the introitus. *H* and *I,* The completed reconstruction. (*From* Donahoe PK, Schnitzer JJ: Ambiguous genitalia in the newborn. *In* O'Neill JAJ, Rowe MI, Grosfeld JIL, et al (eds): Pediatric Surgery. St. Louis, Mosby, 1998, pp 1797–1818; with permission.)

posterior U-flap is turned in to meet the back wall of the mobilized vagina that has been divided in the midline to widen the introitus. The anterior and posterior introital suture lines are shown in Figure 3*G*. Byars' flaps are suitably trimmed, taking care to retain a broad base, advanced posteriorly, and wrapped around the clitoris, then joined together in the midline, using as much tissue as necessary to fill in the gap left when the anterior island flap is rotated inward. The labia minora created from Byars' flaps are closed in the midline below the clitoris until they meet the superior aspect of the already rotated anterior island

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flap. The U-flaps on each labioscrotal fold are trimmed and defatted and much of the scrotal fat is removed to become the new labia majora flaps, which are advanced posteriorly into the lateral arms of the inverted posterior U-flap to complete the reconstruction (Fig. 3*H* and *I*).

In the authors' experience, this one-stage approach is advantageous when compared with previous methods that first repaired the clitoris and created labia minora and majora but delayed the vaginal pull through. When these procedures are performed together, all available tissue unscarred by previous surgery can be used to provide a more ample introitus. Meticulous dissection is required to perform the surgery in infants, but because one does not have to stretch the thin anterior wall of the vagina, introital stenosis is avoided, which has plagued this operation in the past. The infant's pelvis is also shorter, making mobilization of the vagina less difficult than in the adult. Because the urogenital sinus worsens or lengthens with age, the pull-through procedure becomes much more difficult if delayed until adolescence. The authors have appreciated improved outcomes using the early one-stage procedure over the past decade; however, the true test of its efficacy will be satisfactory intercourse in adulthood. Although the anterior island flap refinement of the procedure has not been used long enough to assess fully this functional outcome, the short-term outcome has been encouraging when compared with the results from procedures that do not incorporate the square anterior island flap.

The authors examine the repair endoscopically 6 weeks to 2 months later with the patient under general anesthesia to ensure that a urethrovaginal fistula has not occurred and to assess the introitus, confirming that the back wall has not adhered to the anterior wall of the vagina. The introitus is measured with a sound, and the parents are taught how to dilate during diaper changes for the next 2 months in an attempt to make the introital suture line more supple during this phase of healing.

REPAIR AT ADOLESCENCE

Narrowing of the introitus may occur but seems to be less severe than observed before the anterior island flap (C. Nihoul-Fekete, MD, personal communication) was incorporated into the procedure. The authors do not perform dilatations in children beyond the early infant years because of the potential for emotional trauma and more practically because the children simply will not permit it. Once the child is well into adolescence, she can be motivated to participate in a program of dilatations. A local introitoplasty will often suffice if dilatations fail. If needed, a larger flap swung down from the buttock can be used. Use of the one-stage repair at an early age generally ensures that surgery, if necessary at adolescence, will be simpler and less onerous for the patient.

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SUMMARY

Unraveling of the genetics of CAH offers the possibility of earlier detection and prenatal treatment or, alternatively, blastocyst embryo selection and eventually in utero gene therapy. Endocrine, surgical, and anesthesia management after birth have improved, leading to a better outcome for these patients. In the authors' experience, early one-stage reconstructive surgery, although demanding, allows one to use all available tissue. Once mastered, the repair is actually technically easier than vaginal pull-through surgery in the adolescent. Patients go through childhood with a body image that is more concordant with normal. Neither the child nor the parents must suffer the anticipation of a major operative intervention at puberty that can cause great emotional stress and that may be more difficult. The authors have encountered situations in late adolescence in which it has been impossible to separate the urogenital sinus from below. Under these circumstances, one can consider a posterior sagittal approach in which the rectum is bivalved to allow one to approach the vagina from below[50] in an attempt to separate it safely from the urethra and to mobilize it to the perineum. It is also feasible to consider fashioning a segment of sigmoid colon[26] as a neovagina, realizing that mucosal drainage needs to be managed daily. The authors have also encountered the rare 46,XX patient raised as a male and committed to the male role. In these cases, the patient can be offered gonadectomy, followed by staged complex hypospadias repair, and surgery to remove Müllerian structures and, if possible, to preserve the vas, followed by prepenile scrotal repair and insertion of testicular prostheses.[14]

Children with CAH require a lifetime of care with surgical approaches that are age appropriate. These patients can lead a full and productive life. It is the physician's responsibility to make certain that these children reach their full potential with the least number of interventions, which should be designed and optimized to produce the best possible outcome.

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GENDER AND SEXUALITY IN CLASSIC CONGENITAL ADRENAL HYPERPLASIA

Heino F. L. Meyer-Bahlburg, Dr.rer.nat.

Until the early 1990s, the psychosocial management of children born with ambiguous genitalia was widely guided by the policy developed in the mid-1950s by Money and the Hampsons[50] in collaboration with the pediatric endocrinologists at Johns Hopkins University in Baltimore, Maryland. Herein, this management is referred to as the "optimal-gender policy."[36] As applied to 46,XX newborns with classic congenital adrenal hyperplasia (CAH), the policy recommends the establishment of the definitive endocrine diagnosis and treatment as early as possible to minimize the period of gender uncertainty (in addition to meeting the urgent endocrine needs of the patient), assignment of the infant to the female gender, and feminizing surgery of the masculinized external genitalia as necessary to facilitate consistent gender-typical rearing by the family, to prevent stigmatization by family members and others, and to make later penovaginal intercourse possible. The female gender is usually considered optimal for 46,XX patients with CAH because, with adequate glucocorticoid replacement therapy, ovarian sex hormone production can be preserved, sex hormone replacement therapy is unnecessary, and fertility can be retained. Genital surgery, when needed, permits later penovaginal intercourse.

In recent years, significant aspects of the optimal-gender policy have

This work was supported in part by grant HD38409-01 from the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.

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been questioned. Based on scattered data on poor long-term functional outcomes, patient activists have emphasized the risk genital surgery constitutes for sexual functioning. This critique forms the basis for the "Recommendations for Treatment" of the Intersex Society of North America,[25] which strongly objects to genital surgery performed for other than purely medical indications when the patient is too young to give informed consent. Another critique comes from biologic determinists who have extrapolated from the neuroendocrine and behavioral endocrine research that shows the decisive role of prenatal androgens in the masculinization of brain and behavior in rodents to gender identity in humans. Diamond and Sigmundson[11] have on that basis proposed new guidelines for the gender assignment of newborns with intersexuality, which include the recommendation of assigning patients who have 46,XX CAH with severe degrees of genital masculinization (Prader stage 5[62]) to the male gender. These critical points have received increased attention more than 40 years after the optimal-gender policy was formulated. One reason is the fact that problems with sexual functioning do not usually develop, or come to professional attention, before late adolescence or adulthood. Significant problems of gender identity and gender change sometimes come to full expression only in adulthood.[43] Both problem areas may be accentuated at an age when many patients are no longer under the care of pediatric endocrinologists. Even if they still see these specialists, the physician-patient relationship may be such that quality-of-life issues specific to sexuality and gender are not openly discussed. Thus, the professionals who make decisions regarding early gender assignment and surgery often do not receive the feedback on the patient's functioning 20 to 40 years later that is crucial for evaluation of the long-term outcome of the psychosocial management policy implemented in infancy and childhood. Moreover, the increased number of female physicians working in pediatric subspecialties is likely to heighten the understanding of problems female patients may have with sexual functioning.

This article briefly reviews the evidence on the development of gender and sexuality in 46,XX persons with classical CAH and the implications for clinical practice. Because the effects of prenatal androgens on the development of genitals and brain differ from postnatal effects, the discussion herein is not directly relevant to patients with nonclassic CAH.

GENDER DEVELOPMENT

In the development of gender, one differentiates between (core) gender identity and gender-role behavior. Gender identity refers to the basic sense of belonging to or fitting into the male or female gender or something else, for instance, a *hermaphroditic identity.* Gender-role behavior denotes the behavior typical of one gender versus the other in a given historical time and place.

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Gender-Role Behavior

Shortly after animal research discovered the crucial role of the prenatal and perinatal sex hormonal milieu in the sexual differentiation of brain and behavior,[8] 46,XX persons with classic CAH became the initial model (and remain a key model today) for testing the applicability of animal findings to analogous human behavior. The path-breaking initial findings were published by Ehrhardt and Money in the late 1960s.[17, 18, 49] These findings have been replicated, with few exceptions, by numerous studies in the United States and elsewhere.[5, 6, 13, 16, 33, 77] Overall, most published studies agree that 46,XX persons with classic CAH as a group show marked degrees of masculinization of sex-dimorphic behavior when compared with girls and women without CAH. These behavioral shifts have been observed not only in the cohort of women growing up before cortisone treatment became available who experienced a combination of prenatal and postnatal effects of hyperandrogenemia[18] but also in girls and women treated with glucocorticoids from infancy on.[5, 6, 13, 17] The effects on gender-role behavior have been best documented for children (from preschool age on) but can also be shown in adolescents and adults, that is, age groups for which satisfactory global assessment instruments of gender-role behavior are more difficult to develop. The masculinization effects have been demonstrated with diverse modes of assessment, including systematic behavior observation in laboratory situations, written self-report questionnaires, parent-administered questionnaires, and semistructured systematic interviews conducted with patients or parents. All categories of sex-dimorphic behaviors seem to be affected in CAH, including toy preferences, rough-and-tumble play, aggressiveness, interest in sports, maternal behavior, and vocational preferences.

The specific causes of these behavioral shifts are more difficult to demonstrate. Given the variability in gender-role behavior among non-CAH girls and boys, it seems likely that such variability is accounted for, in part, by gender-related genes (other than the gene defects characteristic of the various forms of CAH).[39] Better established is the role of social factors, particularly the family and the peer group, in the development of gender role behavior, involving various mechanisms of social learning.[66] In addition, a few investigators have pointed out that the illness status may have an impact on the development of gender-atypical behavior. Most researchers working in this area agree that the most significant factor accounting for the difference in sex-dimorphic behavior between CAH girls and women and controls is the effect of the prenatal hyperandrogenemia on the sexually differentiating brain. This working hypothesis is based on the vast amount of evidence for this neuroendocrine mechanism in nonhuman mammals, including primates. In the disorder of CAH, one would ideally conduct studies that correlate fetal androgen levels with later sex-dimorphic behavior in childhood, adolescence, or adulthood. In view of the logistic difficulties inherent in such an approach, such data do not exist. Several researchers have interpreted

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the degree of genital masculinization in terms of Prader stages or clitoral size as an indicator of the degree of brain androgenization. Correlational findings have been inconsistent, with some researchers finding the expected correlations, and others failing to confirm them. In this context, the hormonal mechanisms of genital differentiation are not identical to those of brain differentiation.[40] Genital differentiation proceeds through the action of at least three hormones, testosterone, dihydrotestosterone, and müllerian-inhibiting factor, whereas brain differentiation is affected by testosterone and by estrogen derived from testosterone by aromatization within brain target cells. A new theory[19] has added postnatal sensitive periods during which low-dose estrogens are especially important. Currently, the most convincing human evidence supports an androgen-based masculinization shift in CAH. That evidence is based on observations that the effects are particularly strong in the salt-wasting variant of classic CAH and are much weaker in the simple virilizing variant.[6, 14] The salt-wasting variant, characterized by a more complete block of the enzyme 21-hydroxylase, is likely to generate higher androgen levels and is associated, on average, with increased genital masculinization.[63, 71, 75]

Gender-role behavior varies widely within the major subtypes of classic CAH, however. This observation is not surprising given the well-known variability in endocrine phenotype, even for a specific molecular genotype.[28, 60] (For a more detailed discussion of this topic, the reader is referred to the article by Speiser elsewhere in this issue.) Whether the molecular genotype contributes to the explanation of the variability in gender-role behavior, beyond its association with the endocrine phenotype, is currently under investigation.

If, as expected, much of the shift toward increased masculinization of behavior in 46,XX CAH can be attributed to the atypical prenatal androgen milieu, a reduction of prenatal androgen levels by prenatal dexamethasone treatment should reduce the degree of masculinization. This effect has been shown for the external genitalia,[9, 20] and preliminary analyses of an analogous behavioral study by the author show weak shifts in the same direction for sex-dimorphic behavior (H.F.L. Meyer-Bahlburg, Dr.rer.nat., unpublished data). Concerns about the potential behavioral side effects of this treatment[44, 72] are also being addressed in ongoing studies.

Gender Assignment, Reassignment, and Gender Identity

Almost all 46,XX patients, if appropriately diagnosed, are raised as girls to preserve endogenous sex hormone production and fertility. Some patients who are born with markedly masculinized genitalia (Prader stages 3, 4, and 5) are inadvertently assigned to the male gender. A few with particularly strong degrees of genital masculinization are deliberately assigned to the male gender. When, in such cases, the correct

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diagnosis is made later on but still in infancy or early childhood, many physicians recommend sex reassignment to the female gender, with appropriate parental consent. The optimal-gender policy stipulates that any sex reassignment in intersex children after the age of 18 months should take into account the behavioral development of the child and, at older ages, the explicit wishes of the child. Most patients who have been assigned or reassigned at an early age to the female gender will remain females lifelong. A small group of patients self-initiate gender reassignment around puberty or later,[49] sometimes as late as in mid-adulthood.[43] Most 46,XX patients with CAH who have been reared in the male gender until late childhood or early adolescence elect to remain boys once they are correctly diagnosed and informed in late childhood or early adolescence, although occasional patient-initiated reassignments to the female gender have been reported.[45] Thus, an apparently stable gender identity in childhood does not necessarily preclude gender dysphoria and gender change at a later age, nor does a successful gender reassignment in childhood, especially when physician initiated, rule out later gender problems. Predictors of patient-initiated gender change to male are not well researched. The few studies addressing this issue for intersex patients suggest an important role of prolonged genital ambiguity and of virilization by endogenous androgens in childhood and adolescence rather than just the prenatal hormonal milieu.[43, 48] The model of human gender identity and gender-role determinism based on prenatal hormone exposure does not fully account for the behavioral outcome.

Assigning all 46,XX newborns with Prader 5 genital stage to the male gender[11] would have a clear advantage. The fully formed penile structure, which involves a penile urethra, may not require any surgery placing the neuronal and vascular supply to the clitorophallus at risk. On the other hand, later ovarectomy and the abolishment of fertility potential, and the need for sex hormone replacement therapy (unless the patient decides to forego glucocorticoid replacement therapy and thereby the suppression of adrenal androgen production, which carries other risks), are almost obligatory sequelae of this decision. The advantage of assigning these infants to the female gender is that endogenous sex hormone production and fertility can be maintained; however, surgical procedures are required to permit penovaginal intercourse. Even if these surgeries are limited to opening, widening, or deepening the vagina, they place the blood supply and innervation at risk. That risk increases if gender-confirming surgery of the external genitalia is performed. Leaving the external genitalia masculinized may constitute an increased risk of social rejection or ambiguous gender rearing. The extent of the latter risk is also not well established; the evidence is mostly limited to case reports and undocumented clinical experience.

The recommendation by Diamond and Sigmundson[11] is largely based on extrapolation from findings of prenatal and perinatal hormonal effects on the sexual differentiation of brain and behavior in lower mammals, from data collected on a small number of 46,XY individuals

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with intersex and related conditions who were raised as females and later changed to males, and from the follow-up of male-raised 46,XX CAH cases. Although it is assumed that the extreme masculinization of the genitalia also indicates complete masculinization of brain and behavior, the fact that 46,XX individuals raised throughout childhood as boys decide to stay boys in adolescence and adulthood, once they have been informed of the correct diagnosis, does not necessarily indicate a complete determination of the behavioral outcome by prenatal hormones but, rather, a combination of the effects of prenatal hormones, postnatal hormones, and postnatal rearing. Moreover, many, if not the majority, of the published cases raised as boys or changed to boys seem to have been staged Prader 3 and 4 rather than Prader 5. The few attempts at correlating genital Prader stage with the degree of behavioral masculinization have at best been moderately successful, which implies considerable variability of gender-role behavior of individuals with a given Prader stage. The only fully satisfactory scientific test of this recommendation would be a clinical trial randomly assigning 46,XX children with extreme degrees of genital masculinization to either the female or male gender with the associated prevalent medical and psychosocial management, with patients in both groups compared for the long-term outcome in terms of gender and sexual functioning by mid-adulthood. Given the political and ethical quandaries involved in such a trial, it is unlikely to be performed. A less compelling but nevertheless important alternative to a full clinical trial would be a comparison of 46,XX individuals with CAH and extreme degrees of genital masculinization who have inadvertently been raised as boys or girls. To date, the long-term outcomes in a few male-raised cases have been documented in detail.[47, 74] A study involving samples of sufficient size is needed to provide a satisfactory basis for a general policy decision about the gender assignment in cases of 46,XX CAH with marked genital masculinization. As neonatal screening for CAH becomes more prevalent, it is unlikely that such an analysis will be feasible.

The marked changes in physician-patient and physician-parent relationships that have occurred over the last 2 decades imply increased participation of the parents in the gender assignment decision, which, in turn, requires that more detailed information be provided to the parents during the informed consent process. The demands by intersex patient organizations for more openness instead of the secrecy rules used for earlier cohorts imply that more complete education about the medical history and condition be provided for the patients in line with their cognitive development. Given the dearth of empirical data on counseling outcomes in this area, clinical judgment will be required on the part of well-trained mental health personnel concerning the maturity and coping ability of patients and their families and at what age and in what form the medical education should be provided. It is impossible to guarantee that such counseling will in all cases have the desired positive outcome.

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Clinical Implications

Gender Assignment at Birth

Unless a solid empiric basis emerges for changing the current policy, the evidence seems insufficient for recommending a systematic policy change. In the absence of such a database, clinicians must continue to make decisions on gender assignment based on their clinical judgment because such children (and their parents) cannot be left in a gender limbo. In all likelihood, Prader stage 5 at birth indicates an earlier onset of a sufficient degree of hyperandrogenemia to bring about a more complete masculine development of the external genitalia but not necessarily a higher degree of hyperandrogenemia during the (somewhat later) time of sexual differentiation of the brain. This notion should be empirically substantiated. It seems conceivable that Prader 4 (and perhaps even Prader 3) genital status and Prader 5 differ in the time of onset of marked hyperandrogenemia but little, or not at all, in the androgen levels at later stages of differentiation. Even if data are collected to show that Prader 5 patients have, at least on average, higher androgen levels at the time of brain differentiation than Prader 4 patients, one would have to take into consideration the likely variability in terms of gender-role behavior outcome for patients born with Prader 5 genitalia and raised either as girls or boys.

The societal changes in terms of physician-patient relationships and the increased (although still insufficient) knowledge of the long-term outcome of such patients demand that the patients' families and, later in development, the patients themselves participate in decision making. Patients and their families must be adequately informed by clinicians about the diversity of long-range outcomes and not given overoptimistic assurances. This counseling requires a careful balance between providing gross oversimplification on the one hand and too much detail on the other, with a resulting paralysis of decision making. The individual physician would be overwhelmed if the status of the empiric evidence needed updating for each new patient. Respective professional societies should develop consensus guidelines that are revised at regular intervals or whenever new major evidence comes to light regarding the long-term outcome and the resulting conclusions for gender assignment policies.

Gender Reassignment in Childhood

Any gender reassignment must carefully take into consideration the child's development, the family's beliefs, and the cultural environment. Uncertainty exists regarding how long into postnatal development gender reassignment can be done without an increased risk of conflict with the child's emerging sense of gender identity and, if old enough, gender wishes. The early data collected by Money suggested a cut-off age of 18 months. Newer data on normative development suggest an earlier cut-off date, perhaps around 9 months of age.[38] After the cut-off age, the

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child's emerging habitual gender-role behavior and identity must be carefully evaluated for compatibility with the recommended gender and, from later preschool age with the child's (persistent) gender wishes. Such an evaluation can be an arduous task because of the child's cognitive limitations at this stage of development and because of the potential emotional sensitivity of the topic. Moreover, children tend to be very dependent on their parents and other authority figures. Even if a child seems to go along with a physician-initiated gender reassignment based solely on anatomic or other biologic considerations, the gender reassignment may not necessarily be successful. The real test comes in adolescence or even later.

In addition to considering the child's gender development, the clinician must evaluate the role expectations the family has for the child, the family's flexibility in this regard, and the societal context. The child and the family must be able to cope with the gender reassignment. In some Muslim countries, there is great resistance to reassign a male-assigned child to the female gender for reasons of social ostracism and economics.[26, 59, 69] The clinician's role is not to superimpose her or his cultural values on those of others but to come to a decision that minimizes potential harm to the patient in her or his cultural environment.

Gender dysphoria can be difficult to diagnose, especially in middle childhood and early adolescence when the child has learned to hide cross-gender wishes because of pressures from the social environment. If the physician suspects a problem in this area, he or she should refer the child for evaluation by an appropriate mental health specialist.

Gender Reassignment in Older Adolescence and Adulthood

Although intersex patients are not the same as (nonintersex) transsexuals, the clinician is well advised to consider the recently revised guidelines[22] of the Harry Benjamin International Gender Dysphoria Association for orientation on what to consider in making decisions on patient-initiated gender assignment at older ages. The guidelines suggest the type of personnel who should perform the evaluation. They recommend a real-life test in the desired gender, especially for adults, good documentation of the behavioral history and current status, and corroboration of the history by others when available.

Gender-Atypical Behavior

Many parents become anxious and uncomfortable when their daughter with CAH develops markedly gender-atypical behavior. Children with CAH should be monitored regularly and assessed unobtrusively for the degree of gender atypicality of their behavior, as should the family milieu for the degree of acceptance or rejection of such behavior, so that appropriate psychologic/psychiatric counseling promoting the acceptance of the behavior can be undertaken. Girls with

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CAH with markedly gender-atypical behavior persisting into late childhood and early to mid-adolescence may become alienated from their gender-typical peer group. The alienation can lead to isolation, self-doubts, and depressive features,[15] and such girls may profit from individual counseling and from contacts with other girls in support groups. Money's counseling guide[46] is the outstanding text in the intersex area.

SEXUAL ORIENTATION

Sexual orientation refers to the overall erotic responsiveness to male versus female sex partners, which is not necessarily identical with the gender of the actual sex partner(s) of a person, nor with her or his sexual-orientation identity as gay, bisexual, or lesbian. Women with classic CAH have an increased rate of bisexual or homosexual orientation, as demonstrated in sexual imagery such as erotic/romantic fantasies and dreams, sexual attractions, and to a lesser degree, overt homosexual involvement,[12, 15, 18, 24, 42, 53, 54, 77] but few of these women consider themselves lesbians. The negative findings in a few studies[29, 32, 55, 68] are probably explained by methodologic factors, such as inadequate assessments of sexual orientation, high proportions of (less prenatally androgenized) simple virilizing cases in a given sample, and small sample sizes. The increased rate of bisexuality and homosexuality is usually interpreted from the perspective of *organizing* effects of prenatal and perinatal sex hormones on the developing brain and subsequent sex-dimorphic behavior,[34, 35] as demonstrated in numerous mammalian species. This interpretation is also compatible with the fact that 46,XX women with salt-wasting CAH, usually associated with the more severe enzyme abnormality[76] and more marked genital masculinization,[63, 71, 75] show the strongest increase in bisexual or homosexual orientation.[12, 24, 54, 77] Similarly, 46,XX individuals raised as males, combining prenatal and perinatal elevated androgens with postnatal and pubertal elevations of activating androgens, usually appear gynecophilic,[47, 74] as are patients who change to the male gender in adulthood.[43] Because the majority of women with CAH are heterosexual, the prenatal hormonal milieu does not dictate a bisexual or lesbian outcome. It is likely that the developmental process leading to a specific sexual orientation is influenced also by psychosocial factors.[34]

Clinical Implications

Because of conceptual misunderstandings in the medical literature, the findings on sexual orientation in 46,XX women are often misrepresented as suggesting high rates of lesbian women. Given the widespread homophobia in US society, such overstatements should be avoided in prognostic counseling of parents. On the other hand, some adolescent and adult patients need help with an emerging bisexual or homosexual

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orientation. Such help can come from individual counseling and support groups. One cannot predict the long-term development of sexual orientation, and counseling that is biased toward either heterosexuality or homosexuality is inappropriate unless the patient requests it, an important point when considering referral. For adolescent patients, disclosure of their sexual orientation to the parents can cause harm. Such disclosure should be done only when the patient requests it and by the patient herself, possibly in the presence of the counselor, and even then only with careful preparation.

SEXUAL ACTIVITY

As a group, women with classic CAH report less sexual activity than controls. This observation has been documented in a number of studies, particularly for heterosexual activity. During adolescence, these women tend to have delayed (or absent) sexual milestones, such as dating, sexual initiation, and falling in love.[12, 24, 27, 42, 52, 68, 77] In adulthood, fewer are sexually active, in steady relationships, or married.[12, 24, 27, 42, 54, 68, 77] For all of these behaviors, it is predominantly women with the salt-wasting variant who differ from controls, much more so than women with the simple virilizing variant.[12, 30, 77]

In addition to delayed or reduced overt sexual activity with a partner, the frequency of sexual imagery, such as romantic/erotic fantasies and nightdreams, the intensity of experienced sexual motivation (drive, libido), and the capacity to fall in love seem to be markedly lower in women with CAH, especially those with the salt-wasting condition when compared with controls,[12, 24, 42, 68] although a few women with CAH complain of excessive libido, especially when untreated or when treatment lapses.

The reduction of sexual activity most likely stems from several causes. One is the genital status in later adolescence and adulthood. Two aspects are particularly relevant to sexual behavior: (1) the width of the vaginal introitus and the width and depth of the vagina, and (2) the erotic function of the clitoris and the labia minora. Moderate and severe degrees of prenatal androgenization usually affect the external genitalia markedly. The outcome of corrective surgery or dilatation of the vagina varies with the technique chosen, the age at which it is done, and the adherence to postsurgical maintenance. There are many reports of women with CAH who find intercourse painful or even impossible or who stay away from heterosexual involvement altogether because of their awareness of having an inadequate vagina,[2, 10, 21, 54, 64, 68] whereas some sexually inexperienced women overrate the functionality of their vagina.[73]

The second aspect of genital status with marked implications for sexual functioning is the clitoris. Early-childhood surgery of the hyperplastic clitoris has been commonly performed in an attempt to facilitate gender-typical rearing and the development of a consistent gender iden-

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tity,[50] although the potential costs in terms of loss of erotic sensitivity have been debated since this policy was developed.[23] The diminution or loss of erotic sensitivity and orgasmic capacity and of sexual satisfaction is probably frequent when clitorectomy is applied, although some women seem to retain both to varying degrees.[23, 42, 52, 55] Clitorectomy has been replaced in Western industrialized countries by various techniques of clitoral reduction or recession, which may improve further when the results of new studies of clitoral anatomy[4, 58] are taken into consideration. Although the published functional outcomes of these techniques seem promising,[10, 57, 65] albeit not universally,[1] the performance of genital surgery for other than a life-threatening condition without the child's fully informed consent is being severely criticized by the Intersex Society of North America.[25] Erotic sensitivity may be reduced in women with CAH who lack labia minora because of more severe genital masculinization. To what extent surgically created labia minora support erotic sensitivity is unknown.

The timing of genital surgery in the course of a child's development is likely to have important psychologic implications. It is plausible that successful comprehensive genital surgery in infancy without any need for later procedures would be beneficial in comparison with other surgical regimens, but no systematic data are available on the differences in psychologic consequences of early (one-stage) genital surgery in infancy as compared with early plus late (two-stage) genital surgery that combines clitoroplasty in infancy with vaginal reconstruction in adolescence. For most adolescent girls, sexual maturation, that is, breast development, menarche, and the menstrual cycle, the sexual attention of boys, infatuation, dating, and coital initiation, provides a significant developmental challenge. The need to cope, in addition, with the awareness of genital anomalies and possible genital surgery complicates this process. It has not been studied whether it is easier for an adolescent woman to undergo vaginal reconstruction when she is ready to become coitally active versus undergoing surgery earlier, with the subsequent use of dilators until she starts engaging in intercourse regularly. Prescribing vaginal dilatation during middle childhood can be problematic. In the author's experience, cessation of the procedure was advised in several cases because of the emotional effect on the child; however, no systematic data on psychosocial acceptibility and outcome are available. Adolescents in general vary widely in the time course and pattern of their sexual socialization. The author's clinical impression is that the variability is further increased by the medical and psychologic aspects of classic CAH. Clearly, there is a need for systematic and sophisticated follow-up studies to permit a definitive comparison of the functional outcomes of the various surgical techniques and the ages at which they are performed, as experienced by the women themselves.

It seems likely that the awareness of having a functionally inadequate vagina and of experiencing reduced erotic sensitivity, orgasmic capacity, and sexual satisfaction will inhibit courtship and perhaps reduce interest in sexuality altogether. Body image problems have been

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noted or documented by several researchers with regard to general physical characteristics related to sexual attractivity, such as short stature, lack of breast development, or hirsutism, and to genital status and function.[21, 29, 31] Inadequate genital status may contribute to the strikingly low sexual motivation seen in a considerable number of women with classic CAH. The fact that women with the salt-wasting condition as a group have more severe genital masculinization and reduced sexual activity and motivation when compared with simple virilizing women is compatible with this interpretation; however, it is not known whether genital status provides a sufficient explanation of the low sexual motivation. The potential influence of concurrent atypical hormone levels needs to be ruled out. Factors to be considered are the increased progesterone and 17-hydroxyprogesterone,[3] given that progestogens are used to reduce sexual libido in sex offenders[7]; the medication-induced hypoandrogenemia,[56] given that androgens are known to contribute to male and female libido[67]; and the excess glucocorticoid levels, given their role in major depressive disorder[61] and the fact that depression is often associated with reduced sexual motivation.[70] Many patients with intersex conditions are chronically oversensitized by frequent and insensitive genital examinations during childhood and adolescence,[51] common in teaching hospitals, and some patients may develop an aversion to being exposed nude or to touch by potential sexual partners, which adds to difficulties with courtship and sexual functioning.

As the author has argued elsewhere,[37] the gender- and sexuality-related behavioral characteristics of women with classic CAH may account to a major extent for the reduced fertility rates of women with CAH,[54] in addition to the well-documented endocrine problems of dysregulation of ovulation and suppression of conception.

Clinical Implications

Genital Surgery

The earlier comments regarding the need for more detailed informed consent procedures also apply to genital surgery. Esthetic and gender-typical appearance continue to be useful as criteria for surgical outcome, but more attention must be paid to long-term sexual functioning.[41] The available database for rational decision making on the degree of genital masculinization that requires surgery, the age at which it should be performed, and the technique that should be used is woefully insufficient. Quality-of-life considerations, including the sexual life in adolescence and adulthood, must have a greater role. There is a major need for pediatric societies to develop consensus guidelines that the individual practitioner can use. Given the patient's developmental variability, genital surgery, dilatation, and their timing must be tailored to the developmental stage and personal sensitivities of the patient, with

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active participation of the patient in the decision process and embedded in preprocedure and postprocedure counseling as necessary.

Genital Examinations

Genital examinations are crucial for evaluating the need for surgery, the outcome of surgery, and the impact of lapses in hormonal treatment. The physician must be aware of the potentially adverse psychologic consequences of such examinations, even if the child seems overtly compliant. Genital examinations have more significant psychologic implications than examinations of most other body parts. Genital examinations must be performed with psychologic sensitivity, and their repetition by multiple trainees, or even in the presence of trainees, must be avoided. Alternative training strategies must be developed that are not harmful to the patient.

Sexuality Counseling

Although, as a group, women with classic CAH have more sexual problems than do controls, the physician should avoid stereotyping and acknowledge that many women with CAH function within normal limits. For the prevention and early detection of sexual problems and for early intervention, the author recommends that, at least during the adolescent years, young women with CAH have an annual visit with a female mental health professional who is familiar with the psychosocial and sexual problems of intersex patients. Having the same person involved in this capacity over the years can be beneficial, provided that the rapport with the patient is generally good. In addition, meeting one or more patients with the same or similar condition can be extremely helpful for patients with an uncommon disorder, especially if the disorder affects an organ system that cannot be easily discussed in public. The development of clinic-affiliated support groups is recommended. Patient-support groups based on the Internet can also be useful. When recommending support-group affiliation, one should always make the patient aware of the risk of biases. The patient should be encouraged to discuss novel information acquired from support groups with her physician or mental health specialist. Patients with specific sexual dysfunctions may need to be evaluated by a specialist who can review all endocrine, anatomic, surgical, and psychologic factors that may be involved and who can plan an intervention in consultation with the respective specialists.

Given the scarcity of mental health personnel familiar with intersex problems, physicians and patient organizations should press for appropriate training of mental health liaison personnel and for third-party coverage of the respective services.

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