REVIEW ARTICLE

MEDICAL PROGRESS

Congenital Adrenal Hyperplasia

Phyllis W. Speiser, M.D., and Perrin C. White, M.D.

From the Department of Pediatrics, Schneider Children's Hospital–North Shore–Long Island Jewish Health System, New Hyde Park, N.Y. (P.W.S.); New York University Medical Center, New York (P.W.S.); and the Department of Pediatrics, University of Texas Southwestern Medical Center at Dallas, Dallas (P.C.W.). Address reprint requests to Dr. Speiser at Schneider Children's Hospital, 269-01 76th Ave., New Hyde Park, NY 11042, or at pspeiser@LIJ.edu.

N Engl J Med 2003;349:776-88.

Congenital adrenal hyperplasia is a group of autosomal recessive disorders resulting from the deficiency of one of the five enzymes required for the synthesis of cortisol in the adrenal cortex. The most frequent is steroid 21-hydroxylase deficiency, accounting for more than 90 percent of cases. Since the last Medical Progress article on this topic was published in the *Journal* in 1987,[1] much has been learned about the genetics of the various clinical forms of 21-hydroxylase deficiency, and correlations between the genotype and the phenotype have been extensively studied. Gene-specific prenatal diagnosis is now feasible, and prenatal treatment has been more widely implemented. This discussion will be limited to the most common form of congenital adrenal hyperplasia.

BIOCHEMISTRY

Steroid 21-hydroxylase (CYP21, also termed CYP21A2 and P450c21) is a cytochrome P-450 enzyme located in the endoplasmic reticulum. It catalyzes the conversion of 17-hydroxyprogesterone to 11-deoxycortisol, a precursor of cortisol, and the conversion of progesterone to deoxycorticosterone, a precursor of aldosterone (Fig. 1).

Owing to this loss of enzyme function, patients with 21-hydroxylase deficiency cannot synthesize cortisol efficiently, and as a result, the adrenal cortex is stimulated by corticotropin and overproduces cortisol precursors. Some of these precursors are diverted to the biosynthesis of sex hormones, which may cause signs of androgen excess, including ambiguous genitalia in newborn girls and rapid postnatal growth in both sexes. Concomitant aldosterone deficiency may lead to salt wasting with consequent failure to thrive, hypovolemia, and shock.

CLINICAL MANIFESTATIONS

A spectrum of phenotypes is observed. A severe form with a concurrent defect in aldosterone biosynthesis (salt-wasting type) and a form with apparently normal aldosterone biosynthesis (simple virilizing type) are together termed classic 21-hydroxylase deficiency. There is also a mild, nonclassic form that may be asymptomatic or associated with signs of postnatal androgen excess.[2]

Classic 21-hydroxylase deficiency is detected in approximately 1 in 16,000 births in most populations.[3] The nonclassic form occurs in approximately 0.2 percent of the general white population but is more frequent (1 to 2 percent) in certain populations, such as Jews of Eastern European origin.[4] The lower general frequency is similar to that estimated on the basis of *CYP21* genotyping of newborns in New Zealand (0.3 percent).[5]

SALT WASTING

Approximately 75 percent of patients with classic 21-hydroxylase deficiency have severely impaired 21-hydroxylation of progesterone and thus cannot adequately synthe-

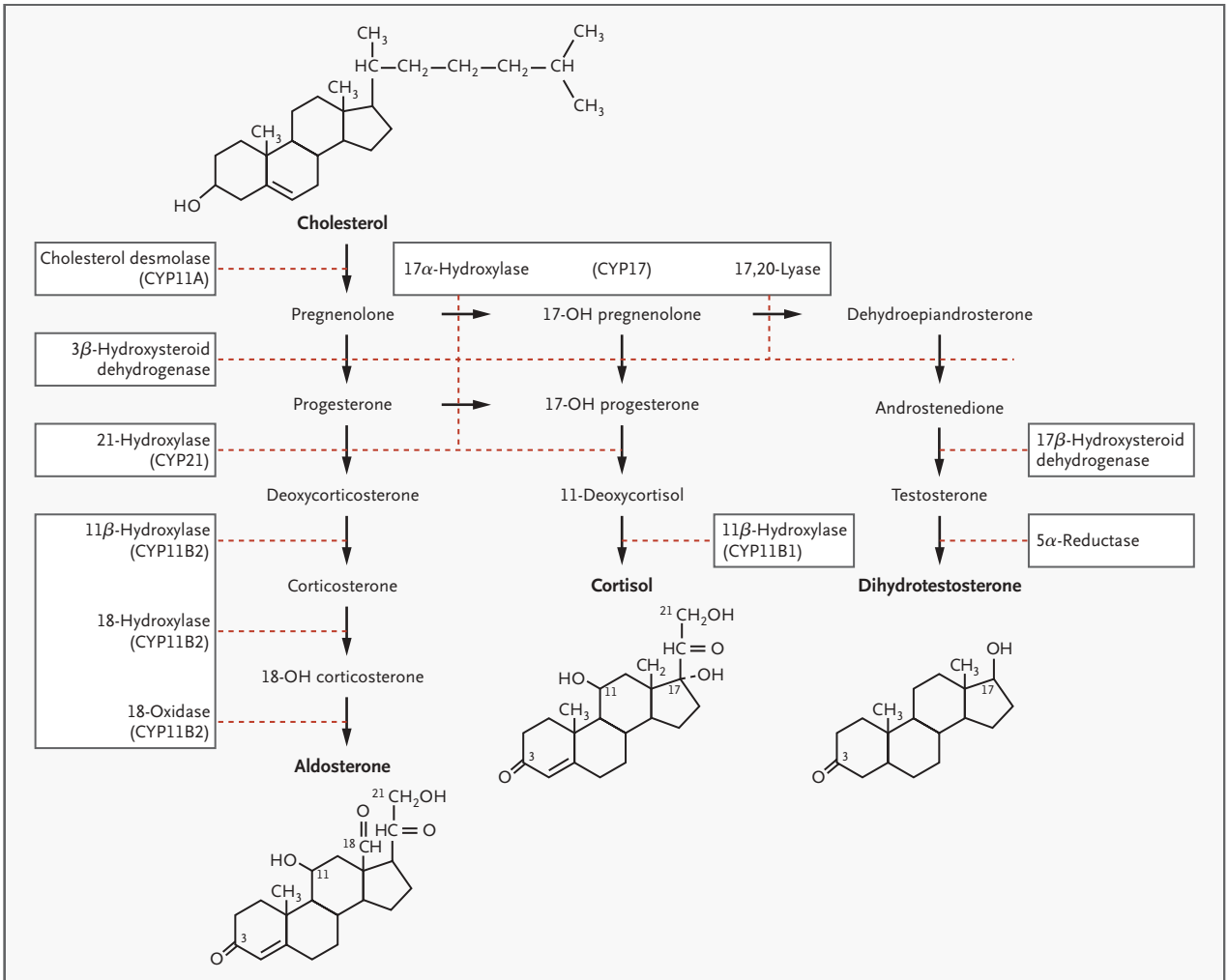

Figure 1. Pathways of Steroid Biosynthesis in the Adrenal Cortex.

The pathways for the synthesis of progesterone and mineralocorticoids (aldosterone), glucocorticoids (cortisol), and androgens (testosterone) are shown. Enzymes that are encoded by a single gene are shown in boxes. For activities mediated by specific P-450 cytochromes (CYP), the systematic name of the enzyme is given in parentheses. CYP11B2 and CYP17 have multiple activities. The planar structures of cholesterol, aldosterone, cortisol, and testosterone are shown. Deficient 21-hydroxylase activity prevents the synthesis of aldosterone and cortisol and shunts precursors such as 17-hydroxypregnenolone into the pathway for androgen biosynthesis. Androstenedione is secreted by the adrenal cortex and then converted to testosterone in the periphery. Testosterone may be aromatized to estradiol or converted by 5α-reductase to dihydrotestosterone.

size aldosterone. Elevated levels of 21-hydroxylase precursors — progesterone and 17-hydroxyprogesterone — may act as mineralocorticoid antagonists, exacerbating the effects of aldosterone deficiency.[6] Since aldosterone regulates sodium homeostasis, renal sodium excretion in untreated patients is excessive and can result in hypovolemia and hyperreninemia. Such patients cannot excrete potassium efficiently and are prone to hyperkalemia, especially in infancy. Cortisol deficiency in these patients contributes to poor cardiac function, poor vascular response to catecholamines, a decreased glomerular filtration rate, and increased secretion of antidiuretic hormone.[7] Thus, cortisol and aldosterone deficiency together cause hyponatremic dehydration and shock in inadequately treated patients. Moreover, since the development of the adrenal medulla is in part dependent on glucocorticoids, patients

with salt-wasting 21-hydroxylase deficiency may also have catecholamine deficiency, potentially further exacerbating shock.[8]

Patients with the salt-wasting form are identified through the measurement of serum electrolytes, aldosterone, and plasma renin and the finding of expected abnormalities — hyperkalemia, low levels of aldosterone, and hyperreninemia. Age-specific reference values for renin should be used, since plasma renin activity is normally higher in neonates than in older children.[9]

AMBIGUOUS GENITALIA

Girls with classic 21-hydroxylase deficiency are exposed to high systemic levels of adrenal androgens from approximately the seventh week of gestation. Thus, such girls have ambiguous genitalia: a large clitoris, rugated and partially fused labia majora, and a common urogenital sinus in place of a separate urethra and vagina. The uterus, fallopian tubes, and ovaries are normally formed, but there is no development of the wolffian duct. In contrast, affected boys have no overt signs of the disease except variable and subtle hyperpigmentation and penile enlargement.

POSTNATAL VIRILIZATION

In patients who either are not treated or are inadequately treated, long-term exposure to high levels of sex hormones promotes rapid somatic growth (predominantly an androgen effect) and advanced skeletal age, which leads to premature epiphyseal fusion (predominantly an effect of extragonadal aromatization of androgens to estrogens). Pubic and axillary hair may develop early. Clitoral growth may continue in girls. Young boys may have penile growth despite having small testes, since the androgens are adrenal in origin. Long-term exposure to androgens may activate the hypothalamic–pituitary–gonadal axis, causing centrally mediated precocious puberty.

LINEAR GROWTH

Linear growth is affected by congenital adrenal hyperplasia, even with close therapeutic monitoring. A meta-analysis of data from 18 centers showed that adult heights in patients with classic congenital adrenal hyperplasia averaged 1.4 SD below the population mean.[10] Both undertreatment and overtreatment put patients at risk for short stature, the former causing premature epiphyseal closure induced by high levels of sex steroids and the latter resulting in glucocorticoid-induced inhibition of the growth axis.[11,12]

REPRODUCTIVE FUNCTION

In girls with any form of 21-hydroxylase deficiency, signs of reproductive problems, such as oligomenorrhea or amenorrhea, may develop in adolescence.[13,14] The issue of fertility is inextricably related to psychosocial adjustment. Women with classic salt-wasting or simple virilizing disease who were born and treated in the 1940s and 1950s had a tendency to shun heterosexual relationships, especially if the introitus was inadequate or androgen levels were chronically elevated.[15] Prenatal exposure to androgens may influence subsequent sex-role behavior.[16] Indeed, most affected females have been reported to exhibit increased behavior more typical of boys during childhood in terms of toy preferences, rough play, and aggressiveness.[17] However, most women are heterosexual, and their sexual identity is almost invariably female.[17]

As surgical, medical, and psychological treatments have improved, more women with 21-hydroxylase deficiency have successfully completed pregnancies and given birth, most by cesarean section.[18,19] About 80 percent of women with simple virilizing disease and approximately 60 percent of those with the severe salt-wasting form are fertile.[18]

As compared with affected women, affected men have fewer problems with reproductive function, specifically gonadal function. Most have normal sperm counts and are able to father children.[20] One relatively common form of gonadal abnormality in affected males is the development of testicular adrenal rests, detectable by sonographic imaging before they become palpable.[21,22] Such tumors have been detected even in childhood,[23] suggesting that screening should begin no later than adolescence. In males with salt wasting, testicular rest tissue may be accompanied by deficient spermatogenesis despite treatment. Infertility can be circumvented by intracytoplasmic sperm injection.[24] These tumors, although almost invariably benign, have prompted biopsies and even partial orchiectomy.[25] Proper medical treatment consists of pituitary suppression with dexamethasone, since the tumors are usually responsive to corticotropin.

PRESENTATION IN PATIENTS WITH SIMPLE VIRILIZING 21-HYDROXYLASE DEFICIENCY

Patients with simple virilizing 21-hydroxylase deficiency do not synthesize cortisol efficiently, but adequate aldosterone secretion remains and thus sodium balance is maintained. Whereas the disease is usually diagnosed in female patients shortly after birth owing to genital ambiguity, the diagnosis is of-

ten delayed for several years in male patients. Without newborn screening, affected boys are usually identified when signs of androgen excess develop. Later diagnosis is associated with greater difficulty in achieving hormonal control, abnormal tempo of puberty, and short stature.

PRESENTATION IN PATIENTS WITH NONCLASSIC DISEASE

Patients with nonclassic 21-hydroxylase deficiency produce normal amounts of cortisol and aldosterone at the expense of mild-to-moderate overproduction of sex hormone precursors. A few nonclassic cases are detected by newborn-screening programs, but most are missed because of the relatively low base-line levels of 17-hydroxyprogesterone.[26-28] It is not known what proportion of cases ascertained in this manner eventually become symptomatic. Family studies indicate that overt androgen excess never develops in many patients with nonclassic disease. Some children grow rapidly or have advanced bone age, and pubic or axillary hair prematurely develops in some.

Hirsutism is the single most common symptom at presentation in approximately 60 percent of symptomatic women, followed by oligomenorrhea (54 percent) and acne (33 percent).[29] Thus, nonclassic 21-hydroxylase deficiency and polycystic ovarian syndrome may present in similar ways. Decreased fertility is an indication for glucocorticoid treatment in both men and women, but the prevalence of nonclassic 21-hydroxylase deficiency is not greater among patients in infertility clinics than in the general population, and infertility appears to be a presenting symptom in only 13 percent of women with nonclassic 21-hydroxylase deficiency.[29]

PRESENTATION IN HETEROZYGOTES

Patients who are heterozygous for *CYP21* mutations often have slightly higher 17-hydroxyprogesterone levels after adrenal stimulation than do unaffected subjects. Although it has been suggested that heterozygotes might be more likely to have signs of androgen excess than would genetically unaffected subjects, case–control studies do not support this concept.[30] Thus, it would seem likely that ascertainment bias caused the apparent association.

DIAGNOSIS

Classic 21-hydroxylase deficiency is characterized by markedly elevated serum levels of 17-hydroxyprogesterone, the main substrate for the enzyme. Basal 17-hydroxyprogesterone values measured by radioimmunoassay usually exceed 10,000 ng per deciliter (300 nmol per liter) in affected infants, whereas the levels in normal newborns are below 100 ng per deciliter (3 nmol per liter). This difference makes it possible to screen newborns (Fig. 2) for the disorder with the use of dried blood spots on filter paper. Screening minimizes delays in diagnosis, especially in male patients, and reduces morbidity and mortality from adrenal crises.[3]

Approximately 10 percent of severely affected term newborns have low initial base-line 17-hydroxyprogesterone levels.[26] False negative results occur when infants are discharged early from the hospital and thus have been screened before they are two to three days old, a time for which there are no established normative data. Conversely, most sick or premature infants have elevated 17-hydroxyprogesterone levels without having inborn errors in steroid biosynthesis, especially those with gestational ages of less than 31 weeks.[5,31] To ensure that no infant with classic 21-hydroxylase deficiency is missed, normal limits must be set so low that the positive predictive value of an abnormal 17-hydroxyprogesterone screening test is only 2 percent.[32] The high rate of false positive values not only increases the real cost of screening but also causes psychological distress to the parents while testing is being repeated. Delays in accurate diagnosis can lead either to unnecessary steroid therapy or to failure to institute therapy in a timely manner.

To improve accuracy, some screening programs have set reference levels for serum 17-hydroxyprogesterone in infants that are based on weight and gestational ages,[33,34] whereas others have emphasized concurrent measurement of serum cortisol.[35] Measurement of 17-hydroxyprogesterone by fluoroimmunoassay[36] or tandem mass spectrometry[37] may improve both the sensitivity and the specificity of screening.

The gold standard for differentiating 21-hydroxylase deficiency from other steroidogenic enzyme defects is the corticotropin (cosyntropin) stimulation test, performed by injecting a 0.125-mg or 0.25-mg bolus of cosyntropin and measuring base-line and stimulated levels of 17-hydroxyprogesterone. Blood samples are obtained at base line and 60 minutes after the administration of cosyntropin. This test should be distinguished from the low-dose (0.5-μg) stimulation test that is becoming popular for evaluating the integrity of the hypothalamic–pituitary–adrenal axis.[38] Except for premature infants, there are no age-related differences in the criteria for the

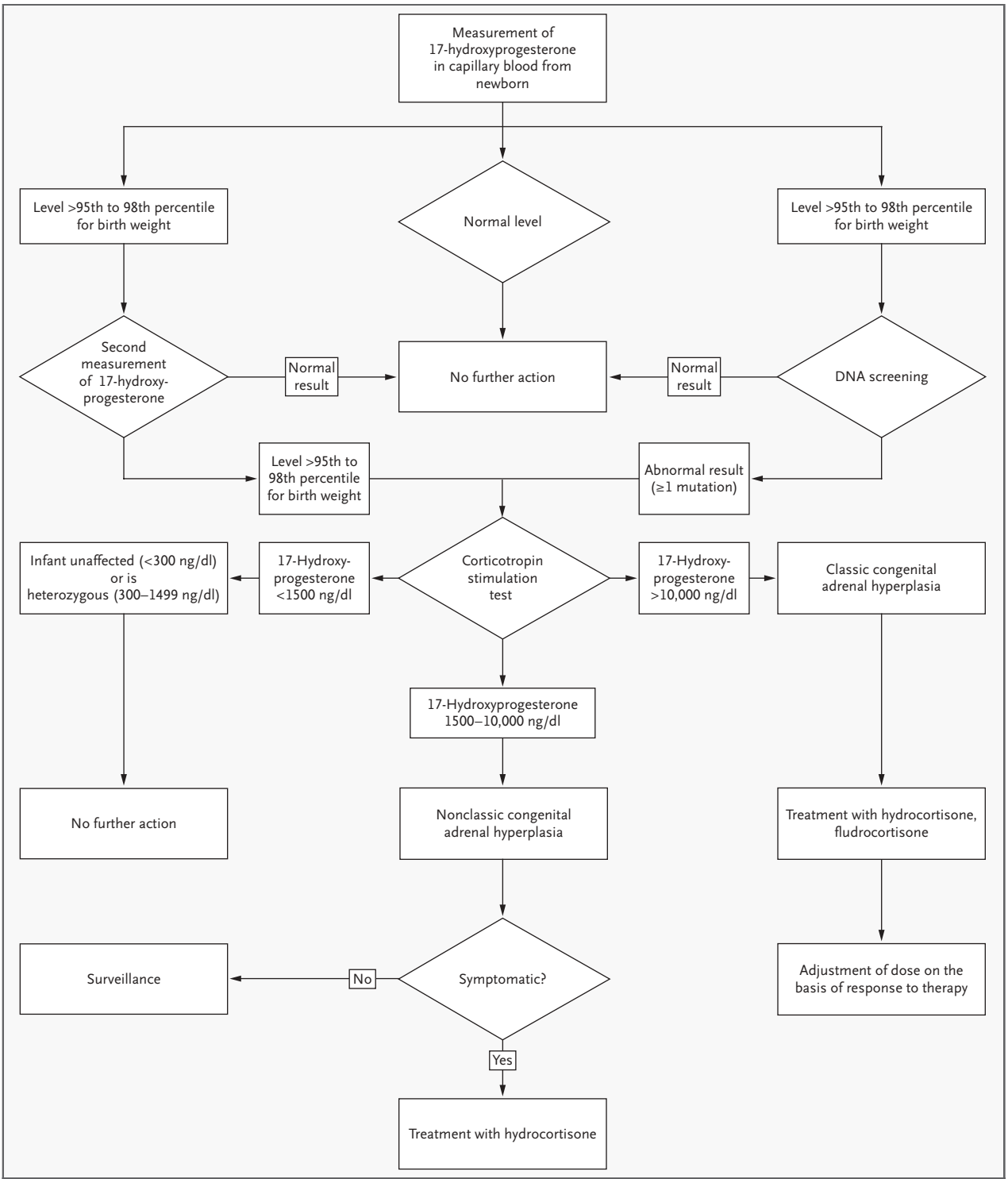

Figure 2. Algorithm for Screening of Newborns for 21-Hydroxylase Deficiency.

Protocols vary from center to center. Screening is first performed on dried capillary blood. A 17-hydroxyprogesterone level in the 95th to 98th percentile for birth weight prompts a second screening test, which may be either a repeated hormonal assay or a molecular test to determine whether the infant is a carrier of at least one common mutation in the gene for 21-hydroxylase (*CYP21*). If the results of either of these tests are abnormal, the infant undergoes a corticotropin (cosyntropin) stimulation test. Depending on results, the infant is classified as unaffected, a probable heterozygote, having nonclassic disease, or having classic disease. The need for further management is dictated by these results.

diagnosis of 21-hydroxylase deficiency on the basis of 17-hydroxyprogesterone levels.

The severity of hormonal abnormalities depends on the type of 21-hydroxylase deficiency. Patients with salt-wasting disease have the highest 17-hydroxyprogesterone levels (up to 100,000 ng per deciliter [3000 nmol per liter] after corticotropin stimulation), followed by patients with simple virilizing disease, who usually have somewhat lower levels (10,000 to 30,000 ng per deciliter [300 to 1000 nmol per liter]). Patients with nonclassic disease have smaller elevations (1500 to 10,000 ng per deciliter [50 to 300 nmol per liter]),[39] especially in the newborn period.[27,28] Random measurements of basal serum 17-hydroxyprogesterone levels are often normal in patients with nonclassic disease unless the values are obtained in the early morning. Thus, the diagnosis is most reliably made by measuring the patient's response to corticotropin stimulation.

Other hormones whose levels are usually elevated in patients with 21-hydroxylase deficiency include progesterone, androstenedione, and to a lesser extent, testosterone. An atypical steroid, 21-deoxycortisol, is also elevated but is not routinely assayed.[40] Mutation analysis can confirm the diagnosis and is used in some newborn-screening programs.[5,41,42] It is currently available to a limited extent in the United States, but automated methods are likely to increase its availability.[43]

GENETICS

MUTATIONS

Mutations in the *CYP21* (*CYP21A2*) gene, which is located in the highly polymorphic HLA histocompatibility complex on chromosome 6p21.3 along with a pseudogene, *CYP21P* (*CYP21A1P*), are responsible for causing 21-hydroxylase deficiency. Although *CYP21* and *CYP21P* have 98 percent nucleotide-sequence identity, the latter has accumulated several mutations that totally inactivate its gene product. These include an 8-bp deletion in exon 3, a frame shift in exon 7, and a nonsense mutation in exon 8 (Fig. 3).[44,45] Additional mutations in *CYP21P* affect messenger RNA (mRNA) splicing or amino acid sequence. Most mutations causing 21-hydroxylase deficiency arise from two types of recombination between *CYP21* and *CYP21P*. Approximately 75 percent represent deleterious mutations found in the pseudogene that are transferred to *CYP21* during mitosis by a process termed "gene conversion." About 20 percent are meiotic recombinations that delete a 30-kb gene segment[46] that encompasses the 3′ end of the *CYP21P* pseudogene, all of the adjacent *C4B* complement gene, and the 5′ end of *CYP21*, producing a nonfunctional chimeric pseudogene. More than 60 additional mutations account for the remaining 5 percent.[47]

CYP21 is one of the most polymorphic human genes.[48] In sperm, spontaneous recombinations between *CYP21* and *CYP21P* (i.e., gene conversions or deletions) are detected in 1 in 1000 to 1 in 100,000 cells.[49] In patients, 1 to 2 percent of affected alleles are spontaneous mutations not carried by either parent.[50] Therefore, it is important to ascertain parental genotypes for prenatal counseling.

CORRELATIONS BETWEEN PHENOTYPE AND GENOTYPE

Correlations between the *CYP21* genotype and phenotype have been studied in various ethnic and racial groups.[50-55] *CYP21* mutations can be grouped into three categories according to the level of enzymatic activity predicted from in vitro mutagenesis and expression studies.[51,56-58] The first group consists of mutations such as deletions or nonsense mutations that totally ablate enzyme activity; these are most often associated with salt-wasting disease. The second group of mutations, consisting mainly of the missense mutation Ile172Asn (I172N),[59] yields enzymes with 1 to 2 percent of normal activity. These mutations permit adequate aldosterone synthesis and thus are characteristically found in patients with simple virilizing disease. The final group includes mutations such as Val281Leu (V281L)[60] and Pro30Leu (P30L)[58] that produce enzymes retaining 20 to 60 percent of normal activity; these mutations are associated with the nonclassic disorder.

When the 21-hydroxylase deficiency phenotype is quantitated with the use of 17-hydroxyprogesterone levels or scores for signs of androgen excess or salt wasting, allelic variation in the *CYP21* genotype accounts for 80 to 90 percent of phenotypic variation. Compound heterozygotes for two different *CYP21* mutations usually have a phenotype compatible with the presence of the milder of the gene defects.[50]

Although 21-hydroxylase deficiency is a monogenic disorder, the patient's genetic background will inevitably influence its expression, as has been observed in other human diseases.[61,62] Modifying genes presumably include those regulating the pro-

duction of — and tissue-specific sensitivity to — androgens and estrogens and those involved in sodium conservation.

Another source of phenotype–genotype variability is the so-called leakiness of splice mutations. A mutation in the second intron (at nucleotide 656 in which guanine is substituted for adenine, transferred from *CYP21P* by gene conversion) comprises 25 percent of all classic 21-hydroxylase deficiency alleles and usually results in abnormally spliced mRNA transcripts.[51,56] Experimental and clinical observations suggest, however, that a small amount of the mRNA is normally spliced. A mere 1 or 2 percent of normal functional enzyme activity can

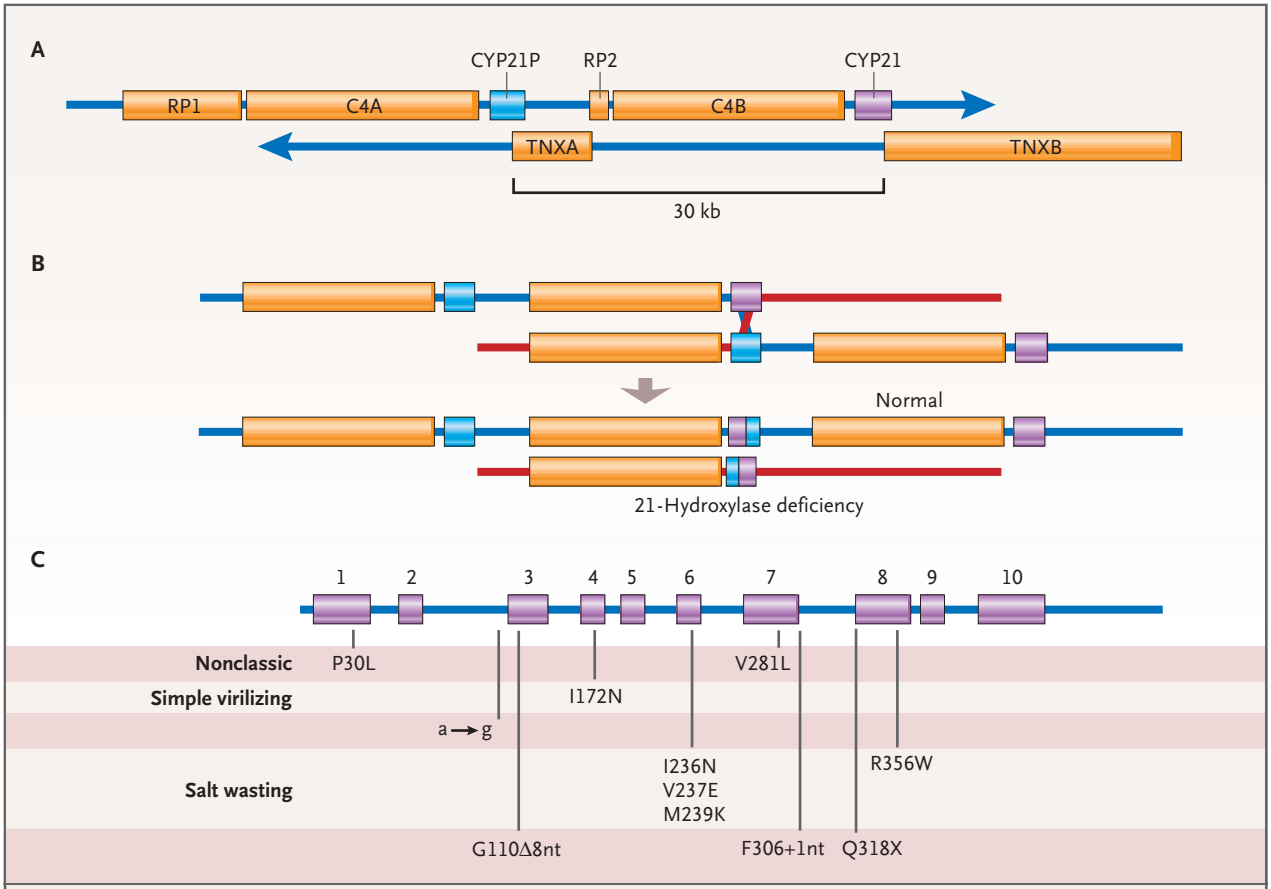

Figure 3. The Chromosomal Region of 6p21.3 Containing the 21-Hydroxylase Genes (Panel A), 21-Hydroxylase Genes Undergoing an Unequal Crossover during Meiosis (Panel B), and Mutations in Steroid 21-Hydroxylase Causing Congenital Adrenal Hyperplasia (Panel C).

In Panel A, *CYP21P* and *CYP21* are the steroid 21-hydroxylase pseudogene and active gene, respectively. *C4A* and *C4B* encode the fourth component of serum complement. *RP1* encodes a nuclear protein of unknown function, and *RP2* is the corresponding truncated pseudogene. On the opposite chromosomal strand, *TNXB* encodes tenascin-X and *TNXA* is the corresponding truncated pseudogene. A bar delineates the 30-kb region that is deleted in approximately 20 percent of 21-hydroxylase deficiency chromosomes. In Panel B, 21-hydroxylase genes undergo an unequal crossover during meiosis, and the resulting daughter chromosomes possess either three *CYP21* alleles or one nonfunctional *CYP21* gene as a result of a large deletion. Panel C shows the positions of mutations normally found in *CYP21P;* any of these can be transferred to *CYP21* in gene-conversion events. Recombinant enzymes carrying each missense mutation have been expressed in cultured cells. Numbered boxes represent exons. The percentage of normal activity seen in each mutant enzyme is denoted by the position of each mutation on a vertical scale; the mutations that most severely affect activity, causing salt-wasting disease, are at the bottom of the figure. The mutations are as follows: P30L, Pro30Leu; a→g, mutation in intron 2 activating a cryptic splice site; G110Δ8nt, deletion of eight nucleotides ; I172N, Ile172Asn; I236N, V237E, and M239K are a cluster of three mutations almost invariably inherited together (Ile236Asn, Val237Glu, and Met239Lys, respectively); V281L, Val281Leu; F306+1nt, the insertion of a single nucleotide; Q318X, a nonsense mutation of Gln318; R356W, Arg356Trp; and P453S, Pro453Ser.

change the patient's phenotype from salt-wasting to simple virilizing disease. However, reports of asymptomatic subjects who are homozygous for this mutation most likely represent examples of artifacts derived from polymerase-chain-reaction–based genotyping.[63]

TREATMENT

GLUCOCORTICOIDS

Patients with classic 21-hydroxylase deficiency require long-term glucocorticoid treatment to inhibit excessive secretion of corticotropin-releasing hormone and corticotropin by the hypothalamus and pituitary, respectively, and to reduce elevated levels of adrenal sex steroids. In children, the preferred drug is hydrocortisone (i.e., cortisol itself) in maintenance doses of 10 to 20 mg per square meter of body-surface area per day in three divided doses. Doses of up to 100 mg per square meter per day are given during adrenal crises and life-threatening situations. Even these maintenance doses exceed physiologic cortisol secretion (7 to 9 mg per square meter per day in neonates[64] and 6 to 8 mg per square meter per day in children and adolescents[65]). The efficacy of treatment is best monitored by measuring 17-hydroxyprogesterone and androstenedione levels at a consistent time in relation to the administration of medication. Children should also undergo radiography annually to determine bone age, and their linear growth should be carefully monitored.

The therapeutic goal is to use the lowest dose of glucocorticoid that adequately suppresses adrenal androgens and maintains normal growth and weight gain.[66] One should not attempt to achieve normal levels of 17-hydroxyprogesterone, since this requires supraphysiologic doses of glucocorticoid that may cause Cushing's syndrome. Rather, 17-hydroxyprogesterone levels should be partially suppressed to the range of 100 to 1000 ng per deciliter (3 to 30 nmol per liter). Androstenedione and testosterone levels (measurement of the latter is useful only in prepubertal children and women) should be maintained at values that are appropriate for the patient's age and sex.

The short half-life of hydrocortisone minimizes growth suppression and other adverse effects of longer-acting, more potent glucocorticoids such as prednisone and dexamethasone. Because of the drug's short half-life, a single daily dose of hydrocortisone is ineffective in regulating adrenocortical secretion.

An oral suspension of hydrocortisone has been withdrawn from the market in the United States owing to problems with mixing the suspension, which resulted in erratic hormonal control. Crushed tablets may be substituted. Older adolescents and adults may be treated with prednisone (e.g., 5 to 7.5 mg daily in two divided doses) or dexamethasone (total, 0.25 to 0.5 mg given in one or two doses per day). Patients should be monitored carefully for signs of iatrogenic Cushing's syndrome, such as rapid weight gain, hypertension, pigmented striae, and osteopenia. Males with testicular adrenal rests detected by palpation or sonography may require higher doses of dexamethasone to suppress corticotropin.

Treatment is not indicated in asymptomatic children with nonclassic 21-hydroxylase deficiency, since the potential adverse effects of glucocorticoids probably outweigh any benefits. Glucocorticoid treatment should be reserved for children with early onset of disease and rapid progression of pubic and body hair, growth, or skeletal age. Treatment should also be considered in adolescent girls and young women with signs of virilization.

MINERALOCORTICOIDS

Infants with the salt-wasting form of 21-hydroxylase deficiency require supplemental mineralocorticoid (usually 0.1 to 0.2 mg of fludrocortisone daily) and sodium chloride (1 to 2 g or 17 to 34 mmol of sodium chloride daily in addition to glucocorticoid treatment). The sodium content of either breast milk or infant formulas (8 mmol per liter) is insufficient to compensate for sodium losses in these infants. Older infants and children usually do not require sodium chloride supplements, and they often have reduced requirements for fludrocortisone.

Patients with the simple virilizing form of the disease by definition secrete adequate amounts of aldosterone, but nevertheless many are treated with fludrocortisone. This can aid in adrenocortical suppression, reducing the dose of glucocorticoid required to maintain acceptable 17-hydroxyprogesterone levels.

Plasma renin activity levels or direct renin immunoassays may be used to monitor the adequacy of mineralocorticoid and sodium replacement, taking into account the age-specific reference ranges for each laboratory. Hypotension, hyperkalemia, and elevated renin levels suggest the need for an increase in the dose, whereas hypertension, edema, tachycardia, and suppressed plasma renin activity sig-

nify overtreatment with mineralocorticoids. Adjustments in the dose should be made in increments or decrements of 0.05 to 0.1 mg. Excessive fludrocortisone may also retard growth.

MANAGEMENT OF AMBIGUOUS GENITALIA

Improvements in the surgical correction of genital anomalies over the past two decades have led to earlier use of single-stage surgery — between two and six months of life in girls with 21-hydroxylase deficiency, a time when the tissues are maximally pliable and psychological trauma to the child is minimized.[67] The long-term outcomes of the newer surgical procedures have yet to be evaluated. Retrospective reviews suggest that both the cosmetic and functional outcomes of genital surgery procedures as formerly practiced were often unsatisfactory.[68] Surgery during adolescence is often fraught with psychological and technical difficulties. Such technically demanding surgery must only be done by experienced surgeons. Patient-advocacy groups have appealed to physicians to inform families about all the potential surgical pitfalls so that they can carefully consider whether and when surgery should be done. In addition, there is now heightened awareness of the need for psychological support for families with an affected child.[17] Respect for patients' privacy has led to fewer genital examinations during childhood and adolescence. The transition from childhood to adulthood may require an interdisciplinary team of specialists to manage medical, gynecologic, and psychosexual concerns of patients.[69]

PRENATAL DIAGNOSIS AND TREATMENT

Prenatal genetic counseling is advised for all affected families. This raises the somewhat controversial question of prenatal treatment (Fig. 4). Maternally administered dexamethasone ameliorates genital ambiguity in affected female fetuses.[70] Dexamethasone is used because it is not inactivated by placental 11β-hydroxysteroid dehydrogenase. Dexamethasone presumably suppresses the secretion of sex steroids by the fetal adrenal glands, but the precise mechanism of action is uncertain. The recommended dose is 20 μg per kilogram of body weight per day (based on prepregnancy weight), given in three divided doses. Treatment failures have been attributed to the cessation of therapy in midgestation, noncompliance, or suboptimal dosing, though some reports provide no ready explanation.[71] Conversely, the extent of genital virilization can vary among affected females in the same family even without treatment.[72]

It is important to remember that, in this autosomal recessive condition, only one of eight fetuses will be an affected female when both parents are known carriers. To prevent genital virilization, prenatal therapy must be administered early in the first trimester to all women whose fetuses are at risk. There is an ethical concern, because seven of eight pregnancies will need to be treated unnecessarily, albeit briefly, to prevent one case of ambiguous genitalia. To minimize unnecessary dexamethasone treatment in male or unaffected female fetuses, prompt and accurate genetic diagnosis is crucial. Genotyping at closely linked, informative marker loci (microsatellites) in addition to genotyping at the *CYP21* locus reduces the likelihood of error.[50,63]

The long-term safety of prenatal treatment remains uncertain. No congenital malformations have been attributable to such therapy, and the incidence of fetal deaths in treated pregnancies does not exceed that predicted for the general population. However, subtle effects of glucocorticoids might go unnoticed during early life.[73,74]

There is a variable incidence of maternal complications. Overt Cushing's syndrome, excessive weight gain, and hypertension have been reported in approximately 1 percent of all women with treated pregnancies, usually those who were treated throughout pregnancy. A proposed decrease in the dose of dexamethasone later in pregnancy has not been tested for efficacy.[75] Thus, women must be fully informed of the potential risks to themselves and their fetus and the possible lack of benefit to an affected female. Treatment should be carried out in specialized centers with the use of approved protocols.[76,77]

NOVEL TREATMENTS

Blockade of the synthesis or action of sex steroids might permit the doses of glucocorticoid to be decreased. Preliminary results of an ongoing trial showed that a four-drug regimen consisting of low-dose hydrocortisone, fludrocortisone, testolactone (an aromatase inhibitor that will prevent estrogen-induced epiphyseal fusion), and flutamide (an androgen-receptor blocker that will prevent virilization) reduced the rate of advancement of bone age and slowed weight and height velocity, as compared with a standard regimen of higher-dose hydrocorti-

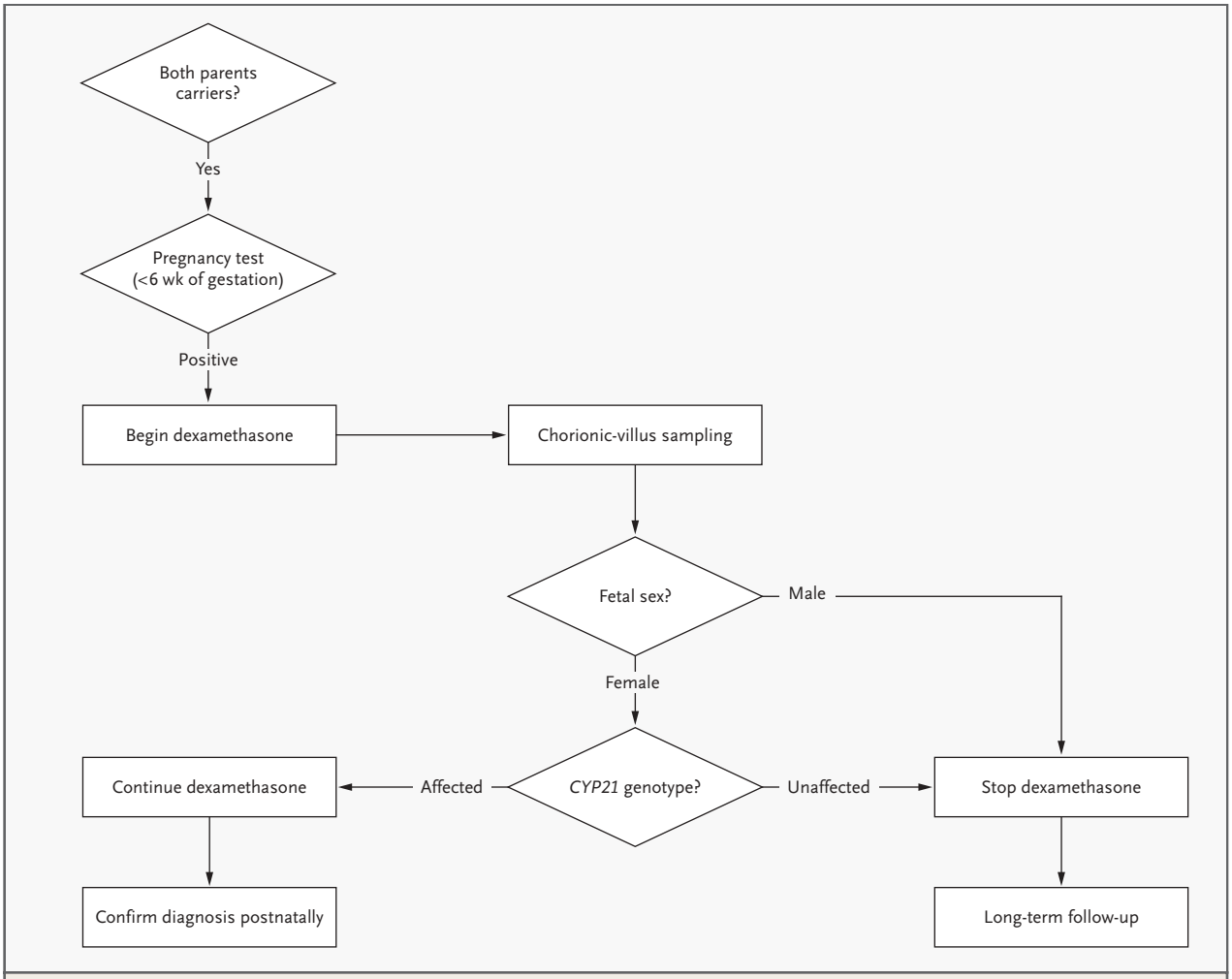

Figure 4. Algorithm for Decisions Pertaining to the Prenatal Diagnosis and Treatment of 21-Hydroxylase Deficiency.

sone and fludrocortisone.[78] During two years of follow-up, no serious adverse effects were observed in affected children who were treated with this four-drug regimen, although this group had a higher incidence of precocious central puberty. Antagonists of corticotropin-releasing hormone to reduce adrenal hyperstimulation and carbenoxolone to increase the levels of bioavailable cortisol represent other experimental modes of therapy.[79] Another small, brief clinical trial of growth hormone to promote growth with an analogue of gonadotropin-releasing hormone to delay central puberty showed some potential to increase adult height.[80] None of these experimental therapies can be recommended as the standard of care.

Laparoscopic adrenalectomy with low-dose steroid replacement has also been tried as an alternative to standard medical treatment.[81-84] Proponents argue that Addison's disease is easier to treat than congenital adrenal hyperplasia and does not interfere with growth and puberty. For female patients, elimination of the excess adrenal androgen might reduce hirsutism and other such symptoms. Opponents believe that adrenalectomy is too radical an approach for a medically treatable condition and leaves the patient more susceptible to sudden death. Adrenalectomy would not prevent the development of gonadal adrenal rests caused by high levels of corticotropin. Moreover, the loss of the secretion of adrenal dehydroepiandrosterone may have adverse effects on mood and increase fatigue.[85,86]

Finally, a genetically well-characterized disease

such as 21-hydroxylase deficiency might eventually be amenable to gene therapy. Early experiments using intraadrenal gene transfer in a mouse model of 21-hydroxylase deficiency resulted in transient gene expression and early death.[87] The implementation of such an approach for this as well as other metabolic diseases awaits a safe, effective, and stable tissue-specific delivery system.

Supported by a grant (R37 DK37867) from the National Institutes of Health.

REFERENCES

1. White PC, New MI, Dupont B. Congenital adrenal hyperplasia. N Engl J Med 1987; 316:1519-24, 1580-6.
2. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 2000;21:245-91.
3. Therrell BL. Newborn screening for congenital adrenal hyperplasia. Endocrinol Metab Clin North Am 2001;30:15-30.
4. Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI. High frequency of nonclassical steroid 21-hydroxylase deficiency. Am J Hum Genet 1985;37:650-67.
5. Fitness J, Dixit N, Webster D, et al. Genotyping of CYP21, linked chromosome 6p markers, and a sex-specific gene in neonatal screening for congenital adrenal hyperplasia. J Clin Endocrinol Metab 1999;84: 960-6.
6. Oelkers WK. Effects of estrogens and progestogens on the renin-aldosterone system and blood pressure. Steroids 1996;61: 166-71.
7. Lamberts SW, Bruining HA, de Jong FH. Corticosteroid therapy in severe illness. N Engl J Med 1997;337:1285-92.
8. Merke DP, Chrousos GP, Eisenhofer G, et al. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. N Engl J Med 2000;343: 1362-8.
9. Fukushige J, Simomura K, Ueda K. Influence of upright activity on plasma renin activity and aldosterone concentration in children. Eur J Pediatr 1994;153:284-6.
10. Eugster EA, Dimeglio LA, Wright JC, et al. Height outcome in congenital adrenal hyperplasia caused by 21-hydroxylase deficiency: a meta-analysis. J Pediatr 2001;138: 26-32.
11. Jaaskelainen J, Voutilainen R. Growth of patients with 21-hydroxylase deficiency: an analysis of the factors influencing adult height. Pediatr Res 1997;41:30-3.
12. Yu AC, Grant DB. Adult height in women with early-treated congenital adrenal hyperplasia (21-hydroxylase type): relation to body mass index in earlier childhood. Acta Paediatr 1995;84:899-903.
13. Barnes RB, Rosenfield RL, Ehrmann DA, et al. Ovarian hyperandrogynism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. J Clin Endocrinol Metab 1994;79:1328-33.
14. Deneux C, Tardy V, Dib A, et al. Phenotype-genotype correlation in 56 women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 2001;86:207-13.
15. Mulaikal RM, Migeon CJ, Rock JA. Fertility rates in female patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N Engl J Med 1987;316:178-82.
16. Wilson JD. The role of androgens in male gender role behavior. Endocr Rev 1999;20:726-37.
17. Meyer-Bahlburg HF. Gender and sexuality in classic congenital adrenal hyperplasia. Endocrinol Metab Clin North Am 2001; 30:155-71.
18. Lo JC, Grumbach MM. Pregnancy outcomes in women with congenital virilizing adrenal hyperplasia. Endocrinol Metab Clin North Am 2001;30:207-29.
19. Premawardhana LD, Hughes IA, Read GF, Scanlon MF. Longer term outcome in females with congenital adrenal hyperplasia (CAH): the Cardiff experience. Clin Endocrinol (Oxf) 1997;46:327-32.
20. Urban MD, Lee PA, Migeon CJ. Adult height and fertility in men with congenital virilizing adrenal hyperplasia. N Engl J Med 1978;299:1392-6.
21. Cabrera MS, Vogiatzi MG, New MI. Long term outcome in adult males with classic congenital adrenal hyperplasia. J Clin Endocrinol Metab 2001;86:3070-8.
22. Stikkelbroeck NM, Otten BJ, Pasic A, et al. High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. J Clin Endocrinol Metab 2001;86:5721-8.
23. Srikanth MS, West BR, Ishitani M, Isaacs H Jr, Applebaum H, Costin G. Benign testicular tumors in children with congenital adrenal hyperplasia. J Pediatr Surg 1992; 27:639-41.
24. Murphy H, George C, de Kretser D, Judd S. Successful treatment with ICSI of infertility caused by azoospermia associated with adrenal rests in the testes: case report. Hum Reprod 2001;16:263-7.
25. Walker BR, Skoog SJ, Winslow BH, Canning DA, Tank ES. Testis sparing surgery for steroid unresponsive testicular tumors of the adrenogenital syndrome. J Urol 1997;157:1460-3.
26. Balsamo A, Cacciari E, Piazzi S, et al. Congenital adrenal hyperplasia: neonatal mass screening compared with clinical diagnosis only in the Emilia-Romagna region of Italy, 1980-1995. Pediatrics 1996;98:362-7.
27. Tajima T, Fujieda K, Nakae J, et al. Molecular basis of nonclassical steroid 21-hydroxylase deficiency detected by neonatal mass screening in Japan. J Clin Endocrinol Metab 1997;82:2350-6.
28. Therrell BL Jr, Berenbaum SA, Manter-Kapanke V, et al. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. Pediatrics 1998;101:583-90.
29. Moran C, Azziz R, Carmina E, et al. 21-Hydroxylase-deficient nonclassical adrenal hyperplasia is a progressive disorder: a multicenter study. Am J Obstet Gynecol 2000; 183:1468-74.
30. Knochenhauer ES, Cortet-Rudelli C, Cunnigham RD, Conway-Myers BA, Dewailly D, Azziz R. Carriers of 21-hydroxylase deficiency are not at increased risk for hyperandrogenism. J Clin Endocrinol Metab 1997;82:479-85.
31. Ohkubo S, Shimozawa K, Matsumoto M, Kitagawa T. Analysis of blood spot 17 alpha-hydroxyprogesterone concentration in premature infants — proposal for cut-off limits in screening for congenital adrenal hyperplasia. Acta Paediatr Jpn 1992;34:126-33.
32. Cutfield WS, Webster D. Newborn screening for congenital adrenal hyperplasia in New Zealand. J Pediatr 1995;126:118-21.
33. Allen DB, Hoffman GL, Fitzpatrick P, Laessig R, Maby S, Slyper A. Improved precision of newborn screening for congenital adrenal hyperplasia using weight-adjusted criteria for 17-hydroxyprogesterone levels. J Pediatr 1997;130:128-33.
34. Gruneiro-Papendieck L, Prieto L, Chiesa A, Bengolea S, Bossi G, Bergada C. Neonatal screening program for congenital adrenal hyperplasia: adjustments to the recall protocol. Horm Res 2001;55:271-7.
35. Mikami A, Fukushi M, Oda H, Fujita K, Fujieda K. Newborn screening for congenital adrenal hyperplasia in Sapporo City: sixteen years experience. Southeast Asian J Trop Med Public Health 1999;30:Suppl 2: 100-2.
36. Boudi A, Giton F, Galons H, et al. Development of a plasma 17alpha-hydroxyprogesterone time resolved-fluorescence immunoassay involving a new biotinylated tracer. Steroids 2000;65:103-8.
37. Minutti C, Magera MJ, Casetta BN, Zimmerman D, Rinaldo P, Matern D. Analysis of 17-OH progesterone (17OHP) by tandem mass spectrometry (MS/MS) for the detection of congenital adrenal hyperplasia (CAH) in newborn blood spots. J Inherit Metab Dis 2001;24:Suppl 1:10. abstract.
38. Zarkovic M, Ciric J, Stojanovic M, et al. Optimizing the diagnostic criteria for standard (250-microg) and low dose (1-microg) adrenocorticotropin tests in the assessment

of adrenal function. J Clin Endocrinol Metab 1999;84:3170-3.
39. New MI, Lorenzen F, Lerner AJ, et al. Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab 1983;57:320-6.
40. Fiet J, Gueux B, Gourmelen M, et al. Comparison of basal and adrenocorticotropin-stimulated plasma 21-deoxycortisol and 17-hydroxyprogesterone values as biological markers of late-onset adrenal hyperplasia. J Clin Endocrinol Metab 1988;66:659-67.
41. Nordenstrom A, Thilen A, Hagenfeldt L, Larsson A, Wedell A. Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 1999;84:1505-9.
42. Tajima T, Fujieda K, Nakae J, Mikami A, Cutler GB Jr. Mutations of the CYP21 gene in nonclassical steroid 21-hydroxylase deficiency in Japan. Endocr J 1998;45:493-7.
43. Day DJ, Speiser PW, White PC, Barany F. Detection of steroid 21-hydroxylase alleles using gene-specific PCR and a multiplexed ligation detection reaction. Genomics 1995;29:152-62.
44. Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y. Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a pseudogene and a genuine gene. Proc Natl Acad Sci U S A 1986;83:2841-5.
45. White PC, New MI, Dupont B. Structure of human steroid 21-hydroxylase genes. Proc Natl Acad Sci U S A 1986;83:5111-5.
46. White PC, Vitek A, Dupont B, New MI. Characterization of frequent deletions causing steroid 21-hydroxylase deficiency. Proc Natl Acad Sci U S A 1988;85:4436-40.
47. Cytochrome P450, subfamily XXIA (steroid 21-hydroxylase, congenital adrenal hyperplasia), polypeptide 2. Cardiff, Wales: Human Gene Mutation Database. 2003. (Accessed July 25, 2003, at http://archive.uwcm.ac.uk/uwcm/mg/search/120605.html.)
48. Cargill M, Altshuler D, Ireland J, et al. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 1999;22:231-8.
49. Tusie-Luna MT, White PC. Gene conversions and unequal crossovers between CYP21 (steroid 21-hydroxylase gene) and CYP21P involve different mechanisms. Proc Natl Acad Sci U S A 1995;92:10796-800.
50. Speiser PW, Dupont J, Zhu D, et al. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Invest 1992;90:584-95.
51. Higashi Y, Hiromasa T, Tanae A, et al. Effects of individual mutations in the P-450(C21) pseudogene on the P-450(C21) activity and their distribution in the patient genomes of congenital steroid 21-hydroxylase deficiency. J Biochem (Tokyo) 1991;109:638-44.
52. Mornet E, Crete P, Kuttenn F, et al. Distribution of deletions and seven point mutations on CYP21B genes in three clinical forms of steroid 21-hydroxylase deficiency. Am J Hum Genet 1991;48:79-88.
53. Owerbach D, Crawford YM, Draznin MB. Direct analysis of CYP21B genes in 21-hydroxylase deficiency using polymerase chain reaction amplification. Mol Endocrinol 1990;4:125-31.
54. Wedell A, Thilen A, Ritzen EM, Stengler B, Luthman H. Mutational spectrum of the steroid 21-hydroxylase gene in Sweden: implications for genetic diagnosis and association with disease manifestation. J Clin Endocrinol Metab 1994;78:1145-52.
55. Wilson RC, Mercado AB, Cheng KC, New MI. Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. J Clin Endocrinol Metab 1995;80:2322-9.
56. Higashi Y, Tanae A, Inoue H, Hiromasa T, Fujii-Kuriyama Y. Aberrant splicing and missense mutations cause steroid 21-hydroxylase [P-450(C21)] deficiency in humans: possible gene conversion products. Proc Natl Acad Sci U S A 1988;85:7486-90.
57. Tusie-Luna MT, Traktman P, White PC. Determination of functional effects of mutations in the steroid 21-hydroxylase gene (CYP21) using recombinant vaccinia virus. J Biol Chem 1990;265:20916-22.
58. Tusie-Luna MT, Speiser PW, Dumic M, New MI, White PC. A mutation (Pro-30 to Leu) in CYP21 represents a potential nonclassic steroid 21-hydroxylase deficiency allele. Mol Endocrinol 1991;5:685-92.
59. Amor M, Parker KL, Globerman H, New MI, White PC. Mutation in the CYP21B gene (Ile-172→Asn) causes steroid 21-hydroxylase deficiency. Proc Natl Acad Sci U S A 1988;85:1600-4.
60. Speiser PW, New MI. Genotype and hormonal phenotype in nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab 1987;64:86-91.
61. Scriver CR, Waters PJ. Monogenic traits are not simple: lessons from phenylketonuria. Trends Genet 1999;15:267-72.
62. Dipple KM, McCabe ER. Phenotypes of patients with "simple" Mendelian disorders are complex traits: thresholds, modifiers, and systems dynamics. Am J Hum Genet 2000;66:1729-35.
63. Day DJ, Speiser PW, Schulze E, et al. Identification of non-amplifying CYP21 genes when using PCR-based diagnosis of 21-hydroxylase deficiency in congenital adrenal hyperplasia (CAH) affected pedigrees. Hum Mol Genet 1996;5:2039-48.
64. Metzger DL, Wright NM, Veldhuis JD, Rogol AD, Kerrigan JR. Characterization of pulsatile secretion and clearance of plasma cortisol in premature and term neonates using deconvolution analysis. J Clin Endocrinol Metab 1993;77:458-63.
65. Kerrigan JR, Veldhuis JD, Leyo SA, Iranmanesh A, Rogol AD. Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis. J Clin Endocrinol Metab 1993;76:1505-10.
66. Migeon CJ, Wisniewski AB. Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency: growth, development, and therapeutic considerations. Endocrinol Metab Clin North Am 2001;30:193-206.
67. Schnitzer JJ, Donahoe PK. Surgical treatment of congenital adrenal hyperplasia. Endocrinol Metab Clin North Am 2001;30:137-54.
68. Creighton SM, Minto CL, Steele SJ. Objective cosmetic and anatomical outcomes at adolescence of feminising surgery for ambiguous genitalia done in childhood. Lancet 2001;358:124-5.
69. Speiser PW. Congenital adrenal hyperplasia: transition from childhood to adulthood. J Endocrinol Invest 2001;24:681-91.
70. New MI, Carlson A, Obeid J, et al. Prenatal diagnosis for congenital adrenal hyperplasia in 532 pregnancies. J Clin Endocrinol Metab 2001;86:5651-7.
71. Pang SY, Pollack MS, Marshall RN, Immken L. Prenatal treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N Engl J Med 1990;322:111-5.
72. Chin D, Speiser PW, Imperato-McGinley J, et al. Study of a kindred with classic congenital adrenal hyperplasia: diagnostic challenge due to phenotypic variance. J Clin Endocrinol Metab 1998;83:1940-5.
73. Seckl JR, Miller WL. How safe is long-term prenatal glucocorticoid treatment? JAMA 1997;277:1077-9.
74. Newnham JP. Is prenatal glucocorticoid administration another origin of adult disease? Clin Exp Pharmacol Physiol 2001;28:957-61.
75. Pang S, Clark AT, Freeman LC, et al. Maternal side effects of prenatal dexamethasone therapy for fetal congenital adrenal hyperplasia. J Clin Endocrinol Metab 1992;75:249-53.
76. Clayton PE, Miller WL, Oberfield SE, Ritzen EM, Sippell WG, Speiser PW. Consensus statement on 21-hydroxylase deficiency from the Lawson Wilkins Pediatric Endocrine Society and The European Society for Pediatric Endocrinology. J Clin Endocrinol Metab 2002;87:4048-53.
77. *Idem.* Consensus statement on 21-hydroxylase deficiency from the European Society for Pediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. Horm Res 2002;58:188-95.
78. Merke DP, Keil MF, Jones JV, Fields J, Hill S, Cutler GB Jr. Flutamide, testolactone, and reduced hydrocortisone dose maintain normal growth velocity and bone maturation despite elevated androgen levels in children with congenital adrenal hyperplasia. J Clin Endocrinol Metab 2000;85:1114-20.
79. Merke DP, Bornstein SR, Avila NA, Chrousos GP. Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Ann Intern Med 2002;136:320-34.
80. Quintos JB, Vogiatzi MG, Harbison MD,

New MI. Growth hormone therapy alone or in combination with gonadotropin-releasing hormone analog therapy to improve the height deficit in children with congenital adrenal hyperplasia. J Clin Endocrinol Metab 2001;86:1511-7.
81. Gunther DF, Bukowski TP, Ritzen EM, Wedell A, Van Wyk JJ. Prophylactic adrenalectomy of a three-year-old girl with congenital adrenal hyperplasia: pre- and postoperative studies. J Clin Endocrinol Metab 1997; 82:3324-7.
82. Gmyrek GA, New MI, Sosa RE, Poppas DP. Bilateral laparoscopic adrenalectomy as a treatment for classic congenital adrenal hyperplasia attributable to 21-hydroxylase deficiency. Pediatrics 2002;109:E28.
83. Meyers RL, Grua JR. Bilateral laparoscopic adrenalectomy: a new treatment for difficult cases of congenital adrenal hyperplasia. J Pediatr Surg 2000;35:1586-90.
84. Warinner SA, Zimmerman D, Thompson GB, Grant CS. Study of three patients with congenital adrenal hyperplasia treated by bilateral adrenalectomy. World J Surg 2000;24:1347-52.
85. Van Wyk JJ, Gunther DF, Ritzen EM, et al. The use of adrenalectomy as a treatment for congenital adrenal hyperplasia. J Clin Endocrinol Metab 1996;81:3180-90.
86. Hunt PJ, Gurnell EM, Huppert FA, et al. Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. J Clin Endocrinol Metab 2000;85: 4650-6.
87. Tajima T, Okada T, Ma XM, Ramsey W, Bornstein S, Aguilera G. Restoration of adrenal steroidogenesis by adenovirus-mediated transfer of human cytochrome P450 21-hydroxylase into the adrenal gland of 21-hydroxylase-deficient mice. Gene Ther 1999; 6:1898-903.