

# DIAGNOSIS AND MANAGEMENT OF CONGENITAL ADRENAL HYPERPLASIA

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## ABSTRACT

Congenital adrenal hyperplasia is a family of inborn errors of steroidogenesis, each characterized by a specific enzyme deficiency that impairs cortisol production by the adrenal cortex, and can lead to sexual ambiguity in both genetic males and females. The enzymes most often affected are 21-hydroxylase, 11 $\beta$ -hydroxylase, and 3 $\beta$ -hydroxysteroid dehydrogenase, and less often, 17 $\alpha$ -hydroxylase/17, 20-lyase and cholesterol desmolase. Decreased production of cortisol results in increased pituitary secretion of adrenocorticotrophic hormone. The elevated adrenocorticotrophic hormone stimulates both the accumulation of precursor steroids in the impeded pathways and excessive steroid synthesis in other adrenal biosynthetic pathways unaffected by the enzyme deficiency. Correct identification of the enzyme affected is achieved by the observation of clinical syndromes reflecting distinct hormonal patterns, and it is measured quantitatively as low levels of cortisol and other adrenal steroids, as well as increased levels of steroids proximal to the blocked step. Many of the corresponding genes for the described enzymes have been isolated and characterized, and specific mutations causing many cases of congenital adrenal hyperplasia have been identified. These advances have important implications for early prenatal diagnosis and prenatal treatment.

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## INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive disorders involving impaired enzymatic function at any of the various steps in the synthesis of cortisol from cholesterol by the adrenal cortex, with excessive secretion of adrenal androgens developing as a result of the defects. Blocks in cortisol synthesis impair the negative feedback control of adrenocorticotropin (ACTH) secretion, which leads to chronic stimulation of the adrenal cortex by ACTH. The enzyme deficiencies in CAH thus act as a dam behind which steroid precursors accumulate. They are then shunted through uninhibited pathways and result in excessive steroidogenesis.

The clinical symptoms of the different forms of CAH are caused by the particular hormones that are deficient and result in a wide range of abnormally low or high glucocorticoid, mineralocorticoid, progesterone, and/or sex steroid levels. Severe defects of any of the cortisol-synthesizing enzymes result in the classical forms of CAH, in which sex hormonal imbalances cause some degree of genital ambiguity. Adrenal androgen overproduction causes in females virilization at birth and in both sexes precocious development postnatally, whereas impairment of androgen synthesis in adrenals and gonads causes insufficient virilization of males at birth and failure of pubertal development in both sexes. Less severe, nonclassical forms of adrenal hyperplasia also occur, characterized by signs of postnatal androgen excess, and arise much more frequently than the classical forms.

The most common form of CAH is 21-hydroxylase deficiency, which can occur in a classical (simple virilizing or salt wasting) or a nonclassical form. Defects in the enzymes 11 $\beta$ -hydroxylase and 3 $\beta$ -hydroxysteroid dehydrogenase, in both classical and nonclassical forms, account for almost all of the remainder. The steroid 17 $\alpha$ -hydroxylase/17, 20-lyase and cholesterol desmolase deficiencies can have profound clinical consequences, though they are seen rarely. Understanding of the genetics of these disorders is growing rapidly (Table 1).

## ADRENAL STEROIDOGENESIS AND FETAL DEVELOPMENT

The adrenal cortex produces cortisol and aldosterone by specific and largely separate regulatory systems. The cortex is divided into three distinct zones—the outer zona glomerulosa, the middle zona fasciculata, and the inner zona reticularis—defined by their different cellular arrangements. They are functionally distinct zones, i.e. mineralocorticoids are synthesized in the zona glomerulosa, glucocorticoids are produced by the zona fasciculata/reticularis, and androgenic steroids are synthesized in the zona reticularis.

**Table 1** The forms of adrenal hyperplasia<sup>a</sup>

Deficiency	Syndrome	Ambiguous genitalia	Postnatal virilization	Salt metabolism	Steroids increased	Steroids decreased	Chromosomal location
Steroidogenic acute regulatory protein (StAR)	Lipoid hyperplasia	Males	—	No salt wasting	None	All	8p11.2
3 $\beta$ -Hydroxysteroid dehydrogenase	Classic	Males	Yes	Salt	DHEA, 17-OH-pregnenolone	Aldo, T, cortisol	1p13.1
	Nonclassic	No	Yes	Normal	DHEA, 17-OH-pregnenolone	—	?
17 $\alpha$ -Hydroxylase	—	Males	No	Hypertension	DOC, corticosterone	Cortisol, T	10q24-25
21-Hydroxylase	Salt wasting	Females	Yes	Salt wasting	17-OHP, $\Delta^4$ -A	Aldo, cortisol	6p21.3
	Simple virilizing	Females	Yes	Normal	17-OHP, $\Delta^4$ -A	Cortisol	6p21.3
	Nonclassic	No	Yes	Normal	17-OHP, $\Delta^4$ -A	—	6p21.3
11 $\beta$ -Hydroxylase	Classic	Females	Yes	Hypertension	DOC, 11-deoxycortisol	Cortisol, $\pm$ Aldo	8q21-22
	Nonclassic	No	Yes	Normal	11-deoxycortisol, $\pm$ DOC	—	8q21-22
Corticosterone methyl oxidase type II	Salt wasting	No	No	Salt wasting	18-OH-corticosterone	Aldo	8q21-22

<sup>a</sup>Aldo, aldosterone; T, testosterone;  $\Delta^4$ -A,  $\Delta^4$ -androstenedione; DHEA, dehydroepiandrosterone; DOC, 11-deoxycorticosterone; 17-OHP, 17 $\alpha$ -hydroxyprogesterone.

The production of cortisol in the zona fasciculata occurs in five steps: cleavage of the cholesterol side chain by the cholesterol desmolase enzyme, cytochrome P450<sub>scc</sub>, to yield pregnenolone; conversion of pregnenolone by 3 $\beta$ -dehydrogenation (with accompanying  $\Delta^{5,4}$ -isomerization) to progesterone by the short-chain dehydrogenase family enzyme 3 $\beta$ -hydroxysteroid dehydrogenase; and successive hydroxylations at the 17 $\alpha$ , 21, and 11 $\beta$  positions, each mediated by a distinct cytochrome P450, resulting in cortisol (Figure 1) (1).

Cortisol is synthesized under the trophic control of ACTH, forming a negative feedback loop with high serum cortisol centrally inhibiting and low serum cortisol permitting further release of ACTH, which defines the hypothalamo-pituitary-adrenal axis (Figure 2). The central nervous system determines the

**ADRENAL STEROIDOGENESIS:**

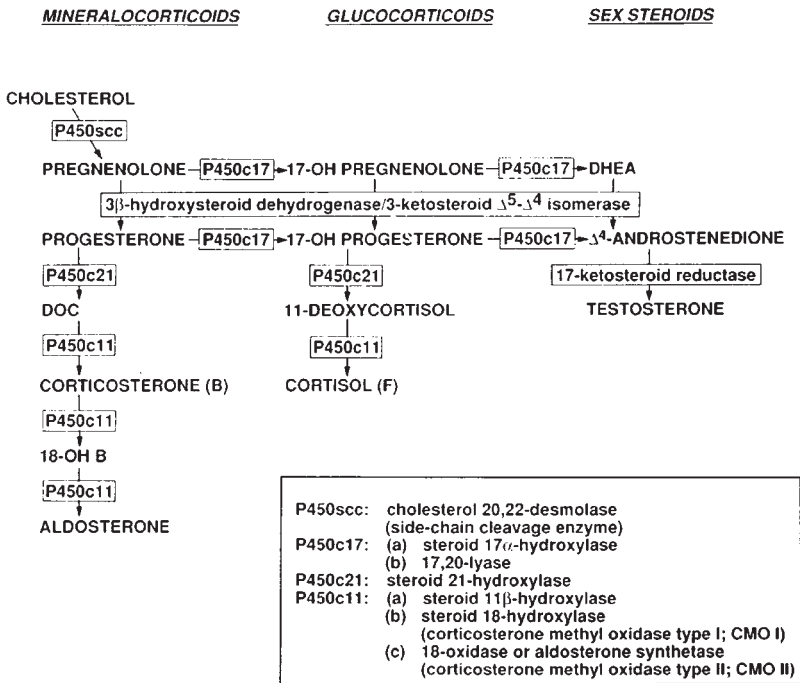


Figure 1 Pathways of steroid biosynthesis. DOC, deoxycorticosterone; CMO, corticosterone methyl oxidase.

hypothalamic setpoint for the expected plasma cortisol level. Plasma cortisol levels lower than the hypothalamic-pituitary setpoint will increase the rate and intensity of ACTH secretory pulses (net ACTH release has basal, diurnal, and stress-induced components). The adrenal enzyme deficiencies described above, causing impaired synthesis and decreased secretion of cortisol, thus lead to chronic elevations of ACTH, and to overstimulation and consequent hyperplasia of the adrenal cortex.

Without these enzyme abnormalities, male genital differentiation in embryonic and fetal life is dependent on two functions of the testes (2): (a) the secretion of sufficient quantities of testosterone to direct the formation of the internal male genital structures (i.e. the epididymides, vasa deferentia, seminal vesicles, and ejaculatory ducts) from the wolffian (mesonephric) ducts; and (b) the secretion of the nonsteroidal anti-mullerian hormone glycoprotein to suppress development of the mullerian ducts, which would develop into female internal structures (i.e. the fallopian tubes, uterus, cervix, and upper vagina). Since there is no anomalous production of anti-mullerian hormone in the gonadally normal female, females presenting with even extreme virilization from androgen excess will have normal development of their internal repro-

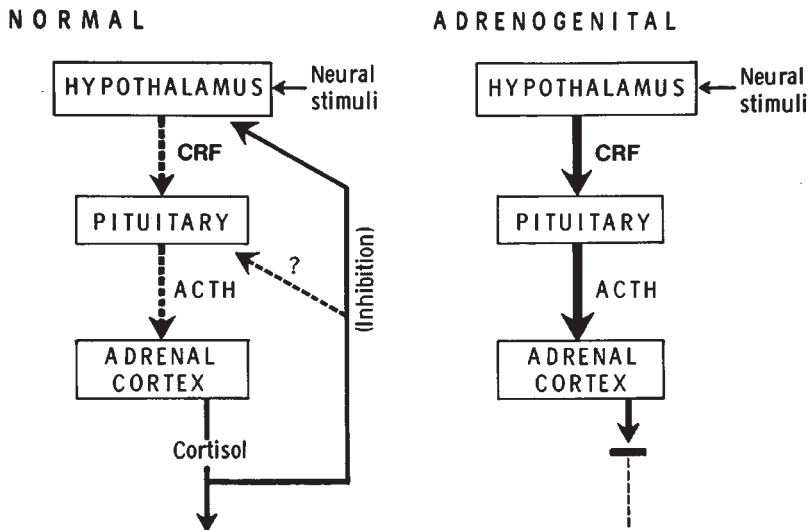


Figure 2 Regulation of cortisol secretion in normal subjects and in patients with congenital adrenal hyperplasia (From 1a). ACTH, Adrenocorticotropic hormone; CRF, corticotropin releasing factor.

ductive structures, allowing for childbearing if the condition is diagnosed and treated.

Testosterone, after it has undergone peripheral conversion to dihydrotestosterone, is also required for suppression of the breast anlage and indirectly for normal formation of the male external genitalia. Dihydrotestosterone is also responsible for midline closure of the genital folds, formation of the scrotum from the genital swellings, extension of the urogenital sinus by fusion along the ventral groove to form a penile urethra, and the elongation of the genital tubercle into the body of the phallus. Regarding the virilizing forms of CAH, progressive differentiation of genetic females toward the male type has been given a five-stage classification by Prader (3).

## ENZYME DEFECTS

### *21-Hydroxylase Deficiency*

**CLINICAL FEATURES** Decreased cortisol synthesis resulting from impaired steroid 21-hydroxylation is the most common biochemical cause of congenital adrenal hyperplasia. Complete and near-complete blocks of the 21-hydroxylase enzyme produce the classical form of CAH, in which the degree of androgen excess causes external genital ambiguity in newborn females, and progressive postnatal virilization in males and females, including precocious pubic hair, advanced somatic and epiphyseal development, and induced central precocious puberty in childhood.

Classical 21-hydroxylase deficiency (21-OHD) occurs in two forms: simple virilizing and salt wasting. In simple virilizing 21-OHD, approximately one third to one fourth of the cases, developmental genital anomalies in females are manifest in varying degrees of genital virilization. The extent of masculinization ranges from mild clitoral enlargement, through varying degrees of fusion of the labioscrotal folds (posterior to anterior), to the profound morphologic anomaly of a penile urethra. Because genital development in males is normal, the syndrome often goes unrecognized until signs of androgen excess appear in later childhood.

In two thirds to three fourths of classical cases, salt wasting also occurs (4). Renal salt wasting results from inadequate secretion of salt-retaining steroids, especially aldosterone, and may be manifested soon after birth by a shock-like state with low serum concentrations of sodium, depleted fluid volume, and high serum potassium levels. In females, these crises are anticipated because of the genital ambiguity that promotes their immediate diagnosis, but males may appear completely normal; such males are in jeopardy of entering a salt-wasting crisis at home. Salt losing that occurs in infancy from an aldosterone

biosynthetic defect may decrease with age (5–7); adjustments in sodium intake and mineralocorticoid replacement in patients labeled neonatally as salt wasters can be made on the basis of careful monitoring of plasma renin activity.

Nonclassical 21-OHD refers to the condition in which partial deficiencies of 21-hydroxylation produce late-onset, less-extreme hyperandrogenemia and milder symptoms. Females do not demonstrate genital ambiguity at birth, though both males and females may manifest signs of androgen excess at any phase of postnatal development. In pubertal-age girls, menarche may be delayed, and in adolescent and young adult females, secondary amenorrhea is common. In women, hirsutism, oligomenorrhea or amenorrhea, and/or polycystic ovary disease may be seen. In males, oligospermia has been found in some cases. For men and women, adult stature below genetic potential, insulin resistance, and reduced fertility are also seen in untreated groups.

**EPIDEMIOLOGY** Analysis of almost 6.5 million newborns screened in the general population worldwide has demonstrated a consistent overall incidence of 1:15,000 live births for the severe classic form of CAH (8–10), with certain ethnic groups exhibiting much higher ratios. The frequency is highest in Ashkenazic (East European) Jews (1 in 27), and is also increased in Hispanics (approximately 1/40), Slavs (1/50), and Italians (1/300) (11–13). The nonclassical form is much more common, occurring in approximately 1 in 100 births in the general population, and is known as the most common autosomal recessive disorder known to man (11).

**HORMONAL DIAGNOSIS** In classical 21-OHD, progesterone, 17-OH-progesterone (17-OHP), androstenedione, and testosterone are secreted in excess. The urinary excretion of the metabolites of these steroids is also increased (14, 15). Hormonal diagnosis of 21-OHD of any degree is best achieved by an ACTH stimulation test, which involves taking a blood sample to measure the serum 17-OHP concentration before and 60 min after intravenous administration of 0.25 mg of synthetic ACTH<sub>1-24</sub> (Cortrosyn) (16). In NC21-OHD, diagnosis is made during the diurnal peak of cortisol production, since serum 17-OHP concentrations are elevated at this time (measuring morning salivary also correlates with serum concentration). Random basal serum concentrations may not differ from normal. Nomogram plots of baseline versus stimulated 17-OHP concentrations result in three distinguishable groups (Figure 3): classical, nonclassical, and an overlap of heterozygotes and genetically unaffected. It should be noted that serum 17-OHP may be elevated in premature infants and infants under stress, which can result in false positives.

**MOLECULAR GENETICS** The molecular genetic basis of 21-OHD has been studied extensively. The gene encoding 21-hydroxylase (a microsomal cyto-

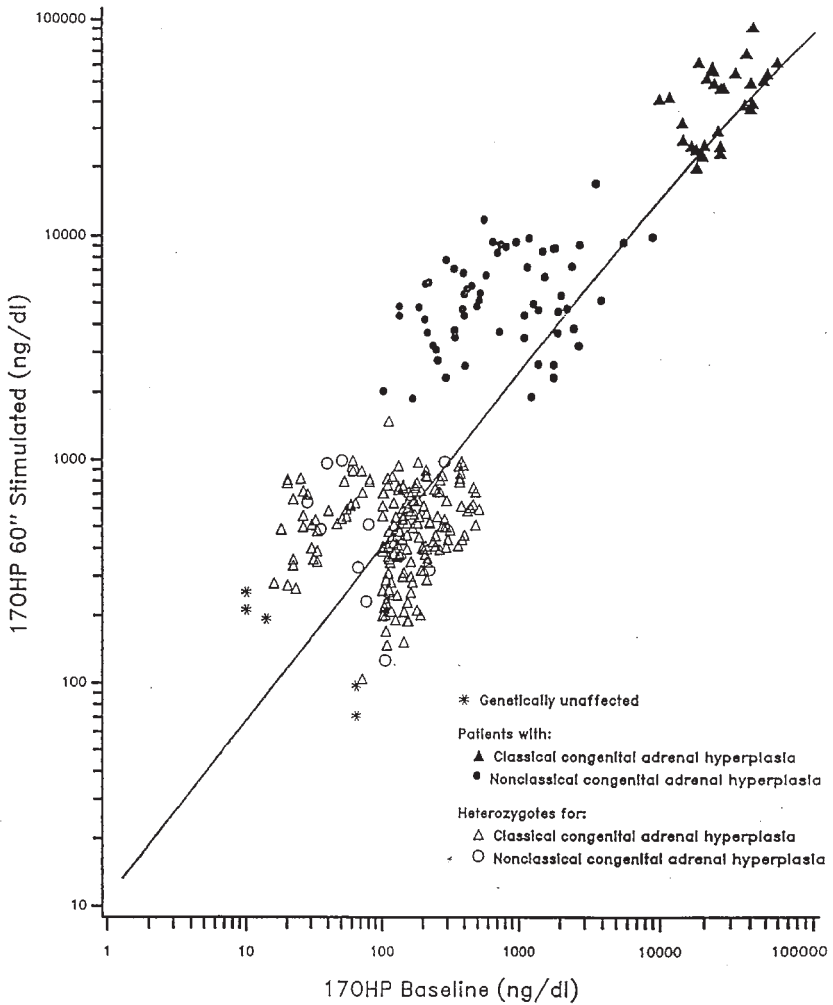


Figure 3 Nomogram relating baseline to adrenocorticotropic hormone-stimulated (60-min Cortrosyn stimulation test) serum concentrations of 17-OH-progesterone (17-OHP). Scales are logarithmic. A regression line for all data points is shown. (The data for this nomogram were collected between 1982 and 1991 at the Department of Pediatrics, New York Hospital-Cornell Medical Center, New York, NY 10021.)

chrome P450 termed P450C21) is located on the short arm of chromosome 6 in the HLA complex (17). The gene locus for the 21-OH enzyme is termed CYP21 (18). CYP21 lies approximately 30 kb from an inactive cognate pseudogene, CYP21P. CYP21 and CYP21P, each of which contains 10 exons, are approximately 98% identical in exons and approximately 96% identical in introns (19, 20).

All the mutations causing 21-OHD described thus far are apparently the result of one of two types of recombination between CYP21 and CYP21P: (a) chromatid misalignment and unequal crossing over, resulting in large-scale DNA deletions; and (b) gene conversion events that result in the transfer to CYP21 of smaller-scale deleterious mutations normally present in the CYP21P pseudogene. Some CYP21 mutations have been linked to certain ethnic groups. It should be noted, however, that though the functional consequence of each DNA lesion generally corresponds to the clinical severity of the inherited disease, phenotype is not always as predicted by genotype (21–23). This remains to be explained.

### *11 $\beta$ -Hydroxylase Deficiency*

**CLINICAL FEATURES** The second most common cause of CAH is 11 $\beta$ -hydroxylase deficiency (11 $\beta$ -OHD), representing 5–8% of all cases in the general population, though it was found to be more common in an Israeli population of Moroccan Jewish origin (24). Like 21-OHD, 11 $\beta$ -OHD occurs late in cortisol synthesis, causing a shunting of accumulating precursor steroids into pathways of androgen biosynthesis, producing genital ambiguity in affected girls and postnatal hyperandrogenism in both sexes. Different types of imbalance in salt metabolism and fluid volume distinguish 21-OHD from 11 $\beta$ -OHD: In the former, deficient aldosterone synthesis causes salt wasting and hypovolemia, whereas in the latter, an excess of the mineralocorticoid deoxycorticosterone (DOC) causes expanded fluid volume and hypertension.

As with 21-OHD, cases of mild, late-onset forms of 11 $\beta$ -OHD have also been reported.

**HORMONAL DIAGNOSIS** The diagnosis of 11 $\beta$ -OHD is established by means of an ACTH test similar to that for 21-OHD, except that the important diagnostic hormones are DOC and 11-deoxycortisol, both of which are elevated, while plasma renin activity is markedly suppressed. There is also marked urinary elevation of the tetrahydro metabolites tetrahydro-S and tetrahydrodeoxycorticosterone, and complete absence of any 11-oxygenated C19 or C21 steroids in the blood or urine (25).

**MOLECULAR GENETICS** The human adrenal cortex expresses two closely related steroid 11 $\beta$ -hydroxylating enzymes: CYP11B1 (cytochrome P450c11) is

responsible for completing cortisol biosynthesis; and the CYP11B2 isoform, or aldosterone synthase (cytochrome P450c18, P450cmo, or P450aldo), completes aldosterone biosynthesis. CYP11B1 and CYP11B2 are encoded by two genes (26) on chromosome 8q21-q22 (27), located 30–40 kb apart (28, 29). The numerous mutations in CYP11B1 identified in cases of 11 $\beta$ -OHD appear to have arisen as independent, random events. Studies analyzing DNA from individuals of diverse ethnic backgrounds identified a series of distinct mutations: missense mutations, frameshift mutations (single-base deletions), and point mutations specifying premature termination (30–36).

### *3 $\beta$ -Hydroxysteroid Dehydrogenase*

**CLINICAL FEATURES** In the rare 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) deficiency, there is poor conversion of  $\Delta^5$  into  $\Delta^4$  steroids. Because of reduced 3 $\beta$ -HSD enzyme activity and the lack of  $\Delta^4$  steroid precursors in the gonads, genetic males are incompletely masculinized and exhibit genital ambiguity with hypospadias at birth. In affected females, very high levels of circulating dehydroepiandrosterone (DHEA) converted peripherally to active androgens produce a limited androgen effect. There is clitoral enlargement and, rarely, labial fusion. As with 21-OH and 11 $\beta$ -OH enzymes, the severity of the enzyme defect may not be deduced on the basis of the appearance of the external genitalia at birth. Deficient steroid production in 3 $\beta$ -HSD deficiency results in some cases in salt wasting (37, 38).

Again, like 21-OHD, 3 $\beta$ -HSD deficiency exists in a nonclassical form as an attenuated enzyme defect with no major developmental abnormalities (39, 40). Signs of virilization appear in females after adrenarche or at the time of puberty.

**HORMONAL DIAGNOSIS** A high ratio of  $\Delta^5$ : $\Delta^4$  steroids is diagnostic for 3 $\beta$ -HSD deficiency. An ACTH stimulation test highlights this abnormality (39). Serum pregnenolone, 17 $\alpha$ -hydroxypregnenolone, and DHEA concentrations are elevated, and the levels of the  $\Delta^5$  metabolites pregnenetriol and 16-pregnenetriol are elevated in the urine. A dexamethasone suppression test is indicated to rule out an adrenal or ovarian steroid-producing tumor.

**MOLECULAR GENETICS** Analysis of DNA from patients with classic 3 $\beta$ -HSD deficiency has confirmed a monogenic autosomal recessive mode of transmission for the HSD3B2 gene (41, 42). Patients carry nonsense or frameshift mutations that have been shown to destroy enzymatic activity completely in cultured cells (42). In several families known to have classical 3 $\beta$ -HSD, some of the family members were found to have nonclassical 3 $\beta$ -HSD. In these rare cases, genetic mutations in HSD3B2 have been linked to the nonclassical form of 3 $\beta$ -HSD deficiency (43–45).

### *17 $\alpha$ -Hydroxylase/17, 20-Lyase*

Steroid 17 $\alpha$ -hydroxylase deficiency is an infrequent cause of CAH, accounting for about 1% of cases overall (1). Defects of the CYP17 gene produce 17 $\alpha$ -hydroxylase deficiency CAH, which is a combined 17 $\alpha$ -hydroxylase/17, 20-lyase deficiency (46). Patients have a decreased ability to synthesize cortisol; the consequently elevated ACTH causes increased serum levels of DOC and especially corticosterone. Both adrenal glucocorticoids and sex steroids are diminished. Isolated 17, 20-lyase has also been observed, which results in deficient C19 sex steroids in the adrenals and gonads.

A deficiency in 17 $\alpha$ -hydroxylase activity results in low-renin hypertension, hypokalemia, and metabolic alkalosis. Affected females have normal genitalia at birth, but at pubertal age they present with primary amenorrhea, lack of axillary and pubic hair, and hypoplastic breasts. Affected males can be born with external female genitalia due to their deficient gonadal testosterone production; however, a uterus and fallopian tubes do not develop since anti-mullerian hormone secretion by Sertoli cells inhibits mullerian duct development. The wolffian ducts are incompletely developed as well.

Hormonal diagnosis is made by the presence of very high serum corticosterone levels and high DOC levels, in addition to high levels of their metabolites. Because of the excess DOC and, therefore, suppressed renin and hypokalemia, aldosterone levels may be low, even though the aldosterone pathway is intact. Patients with defective 17, 20-lyase activity have increased levels of pregnanetriolone, a metabolite of 17-OHP.

A single gene, CYP17, encodes the 17 $\alpha$ -hydroxylase enzyme (also a cytochrome P450); it exists in a single active copy and is identically transcribed in both the adrenals and testes. CYP17 is located on chromosome 10, region q24-q25, and to date about 17 mutations have been described (47). These mutations appear to be random and hence do not appear to be due to a predisposing mechanism.

### *Cholesterol Desmolase*

Lipoid congenital adrenal hyperplasia (LCAH) is extremely rare. The biochemical defect responsible for LCAH resides in the cholesterol side-chain cleavage system, causing a deficiency in the conversion of cholesterol to pregnenolone. This profoundly impairs synthesis of all steroids and results in massive accumulations of cholesterol and cholesterol esters. Males are born with female-appearing external genitalia but have undescended testes, and females have a normal genital phenotype at birth but remain sexually infantile without treatment (1).

If not detected and treated, LCAH is fatal in newborn infants: Death can occur within days to weeks of birth. Severe fluid and electrolyte disturbances, low levels of all steroids in plasma and urine, and addisonian pigmentation are present. Poor stress response and high susceptibility to infection are also seen.

It was originally thought that a defect in the cholesterol side-chain cleavage enzyme, cytochrome P450<sub>scc</sub>, was responsible for this disease, as P450<sub>scc</sub> produces pregnenolone from cholesterol. However, this enzyme was shown to be normal in patients with LCAH. Recently a new enzyme has been discovered that is required for steroidogenesis, steroidogenic acute regulatory protein (StAR). StAR is involved in the transfer of cholesterol from the outer to the inner mitochondrial membrane, where it is then converted to pregnenolone. The gene encoding StAR is on chromosome 8, region p11.2, and a StAR pseudogene is located on chromosome 13. Mutations found in the gene encoding StAR were found to cause LCAH (48).

## TREATMENT

Treatment of CAH due to 21-hydroxylase deficiency began in 1950 (49, 50). Glucocorticoid replacement therapy not only replaces the deficient hormone but also reduces the overstimulation of the adrenal cortex by reducing the release of ACTH, thereby suppressing the overproduction of adrenal androgens. Hydrocortisone (cortisol) is the most frequently used compound for replacement therapy for 21-OHD, 11 $\beta$ -OHD, and 17 $\alpha$ -hydroxylase deficiency. Proper replacement therapy in 21-OHD and 11 $\beta$ -OHD ameliorates the effects of oversecretion of adrenal androgens, preventing further virilization, slowing accelerated growth and bone-age advancement to a more normal rate, and allowing a normal onset of puberty. In 11 $\beta$ -OHD and 17 $\alpha$ -hydroxylase deficiency, glucocorticoid treatment suppresses the oversecretion of DOC, leading to the remission of hypertension. Excessive glucocorticoid administration can cause cushingoid facies, growth retardation, and inhibition of epiphyseal maturation.

Hydrocortisone is the physiologic hormone and therefore minimizes complications. Oral administration is the usual mode of treatment, conventionally given daily in divided doses. It is believed that divided doses better suppress the production of adrenal androgens. Hydrocortisone given in two equally divided doses of 10–20 mg/m<sup>2</sup> daily is adequate for an otherwise healthy child. The dosage may have to be increased for a few days to two to three times that of the normal daily dosage during times of non-life-threatening illness or stress. Families are given injection kits of hydrocortisone (50 mg for young children; 100 mg for older patients) for times of emergency. Up to five to ten times the daily dosage may be required during surgical procedures.

Patients who show poor response to the standard dosage of hydrocortisone may have their dosage increased to 20–30 mg/m<sup>2</sup>/day, or their regimen may have to be changed to a synthetic hormone analog such as prednisone or dexamethasone. Because they are more potent and longer acting, use of these analogs requires critical dosage adjustment.

Patients with salt-wasting CAH may also require mineralocorticoid replacement. A cortisol analogue, 21-acetyloxy-9 $\alpha$ -fluorohydrocortisone (Florinef: 9 $\alpha$ -FF), is used for its potent mineralocorticoid activity. A combination of hydrocortisone and Florinef has proved quite effective in treatment of patients with salt-wasting 21-OHD.

In simple virilizing patients, it is common to find elevated plasma renin activity because of the interaction of the renin-angiotensin-aldosterone system and the hypothalamopituitary-adrenal axis. In these cases, Florinef reduces the plasma renin activity, which in turn lowers ACTH levels further, resulting in better control of androgens without increased glucocorticoid dose.

Patients with NC21-OHD and nonclassical 3 $\beta$ -HSD deficiency are treated with low doses of dexamethasone. Excess ovarian androgen production may have to be suppressed by using progestational and estrogenic agents to suppress the release of gonadotrophin. Other anti-androgen agents that may help include spironolactone, cyproterone acetate, and the androgen receptor blocker flutamide. The aim of treatment in these patients is to minimize symptoms without giving rise to glucocorticoid side effects.

In lipoid congenital adrenal hyperplasia, as all steroidogenic enzymes are normal, a substrate for steroidogenesis can be used for effective treatment of patients. Freely diffusable 20 $\alpha$ -hydroxycholesterol is recommended, which must be implemented as a lifelong treatment plan. In 1985 it was reported that successful management was maintained for one patient for 18 years using replacement glucocorticoids and mineralocorticoids in physiologic doses; estrogen replacement induced a pubertal growth spurt.

## PRENATAL DIAGNOSIS AND TREATMENT

Prenatal diagnosis of 21-OHD has been possible for several decades (51). Initially, diagnosis by 17-OHP levels and HLA serotyping was attempted but found to be inaccurate. After mutations in the CYP21 gene were found to be the cause of 21-OHD, prenatal diagnosis by direct molecular analysis was initiated. Fetal DNA is obtained from either cultured amniocytes or cultured cells obtained by chorionic villus sampling and is used for specific amplification of the CYP21 gene utilizing the polymerase chain reaction (PCR). The PCR products are dot blotted, followed by hybridization with radiolabeled, allele-specific probes. We recently developed a method that only requires PCR with

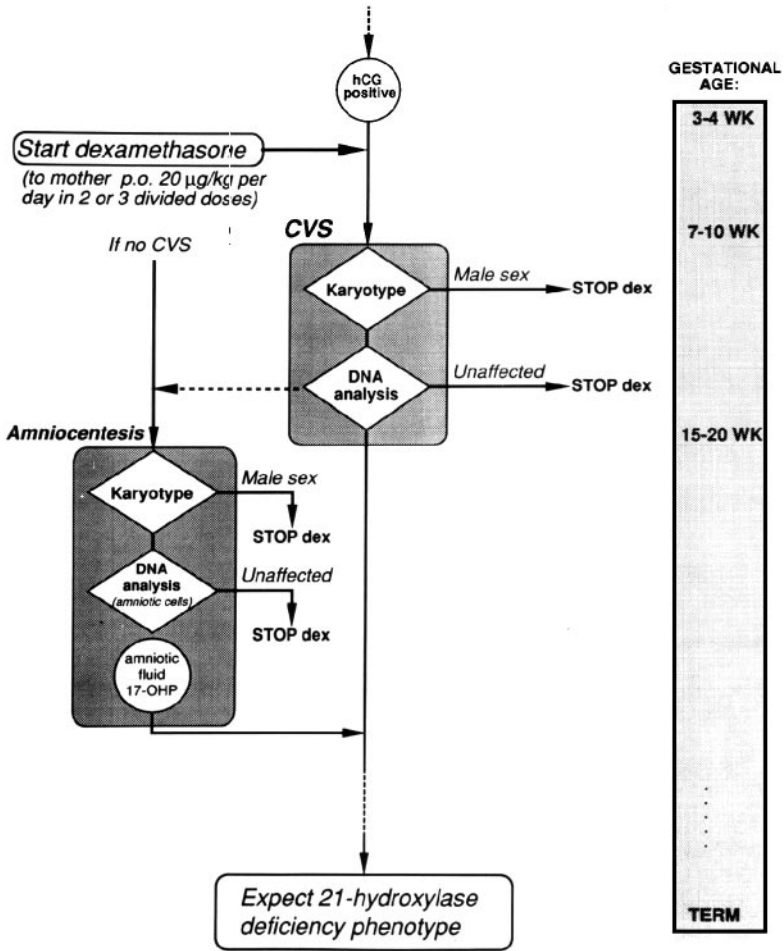


Figure 4 Algorithm depicting prenatal management of pregnancy in families at risk for a fetus with 21-hydroxylase deficiency. CVS: Chorionic villus sampling; 17-OHP, 17-OH-progesterone.

allele-specific primers (52), which reduces the time for prenatal diagnosis from approximately two weeks to only a few days, thus allowing unnecessary prenatal treatment to be terminated promptly (see below).

It has been shown that the prenatal treatment (Figure 4) of female fetuses affected with 21-OHD can greatly reduce or prevent virilization of external genitalia, preventing the need for later genital surgery (53, 54). For this outcome, prenatal treatment must be initiated before 10 weeks of gestation. Dexamethasone (20  $\mu\text{g}/\text{kg}/\text{day}$ ) given orally to mothers at risk, blind to the status of the fetus, suppresses fetal adrenal androgen secretion. Depending on which procedure is available, either chorionic villus sampling (10–12 weeks of gestation) or amniocentesis (15–18 weeks of gestation) is performed. Fetal cells are cultured for karyotyping and DNA analysis. If the fetus is either male or an unaffected female, prenatal treatment is terminated. Prenatal treatment is continued to term for affected female fetuses.

Prenatal treatment for the fetus with low doses of dexamethasone does not appear to have any side effects (53, 54). This is in spite of the findings in rodents that high doses of dexamethasone resulted in cleft palate formation in utero and placental degeneration with fetal death. Some patients have been followed past 10 years of age without reported side effects.

Maternal side effects due to prenatal dexamethasone treatment can include mood changes, weight gain, pedal and leg edema, striae, elevated blood pressure, and general discomfort. All complications disappear upon discontinuation of treatment, and because of the positive genital outcome of their prenatally treated daughters, almost all women with complications reported that were they to become pregnant again, they would again undergo dexamethasone treatment (53). Therefore, the risk-benefit ratio is highly favorable for prenatal dexamethasone treatment of female fetuses affected with 21-OHD.

Prenatal diagnosis of 11 $\beta$ -OHD in at-risk pregnancies has been reported, testing amniotic fluid concentration of 11-deoxycortisol and maternal urinary tetrahydro-S levels (55). In some cases molecular genetic techniques have been used to make the diagnosis (31). Dexamethasone prenatal treatment for 11 $\beta$ -OHD-affected fetuses has been attempted, and it has been found to be as effective as prenatal treatment for 21-OHD (56).

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