

Future Directions in the Study and Management of Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency

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Congenital adrenal hyperplasia describes a group of inherited autosomal recessive disorders characterized by an enzymatic defect in cortisol biosynthesis, compensatory increases in corticotropin secretion, and adrenocortical hyperplasia. 21-Hydroxylase deficiency is responsible for more than 95% of cases and is one of the most common known autosomal recessive disorders. The classic or severe type presents in the newborn period or early childhood with virilization and adrenal insufficiency, with or without salt loss; the mild or nonclassic form presents in late childhood or early adulthood with mild hyperandrogenism and is an important cause of masculinization and infertility in women. This wide range of phenotypic expression is mostly explained by genetic variation, although genotype-phenotype discrepancies have been described.

Reproductive, metabolic, and other comorbid conditions, including risk for tumors, are currently under investigation in both forms of the disease. A high proportion of patients with adrenal incidentalomas may be homozygous or heterozygous for 21-hydroxylase deficiency. Women with congenital adrenal hyperplasia often develop the polycystic ovary syndrome. Ectopic adrenal rest tissue is often found in the testes of men with congenital adrenal hyperplasia; characteristic clinical and radiologic findings help differentiate this tissue from other tumors. Levels of corticotropin-releasing hormone are elevated in patients with depression and

anxiety and are expected to be elevated in patients with congenital adrenal hyperplasia; it is unknown whether patients with 21-hydroxylase deficiency have an increased incidence of these psychiatric disorders. Abnormalities in both the structure and function of the adrenal medulla have been shown in patients with classic congenital adrenal hyperplasia, and the degree of adrenomedullary impairment may be a biomarker of disease severity.

The 21-hydroxylase-deficient mouse has provided a useful model with which to examine disease mechanisms and test new therapeutic interventions in classic disease, including gene therapy. Treatment of this condition is intended to reduce excessive corticotropin secretion and replace both glucocorticoids and mineralocorticoids. However, clinical management is often complicated by inadequately treated hyperandrogenism, iatrogenic hypercortisolism, or both. New treatment approaches currently under investigation include combination therapy to block androgen action and inhibit estrogen production, and bilateral adrenalectomy in the most severely affected patients. Other approaches, which are in a preclinical stage of investigation, include treatment with a corticotropin-releasing hormone antagonist and gene therapy.

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Dr. Deborah P. Merke (Warren Grant Magnuson Clinical Center, National Institutes of Health [NIH], Bethesda, Maryland): Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is one of the most common known autosomal recessive disorders (1). In this condition, impaired cortisol production leads to a lack of negative glucocorticoid feedback on the pituitary, hypothalamus, and suprahypothalamic centers, resulting in an increase in corticotropin, a buildup of cortisol precursors, and androgen excess (Figure 1). The carrier frequency of the classic or severe form of 21-hydroxylase deficiency is approximately 1 in 60 persons (2). The carrier frequency of the nonclassic or mild form ranges from 1 in 5 to 1 in 50 persons, depending on ethnicity (3); it is most common in Hispanic and Ashkenazi Jewish populations. Because congenital adrenal

hyperplasia has a high frequency, a variable presentation in children and adults, and potential complications, a thorough understanding of the disorder is of great importance to clinicians working in internal medicine, reproductive medicine, and pediatrics.

The classic form of congenital adrenal hyperplasia presents in infancy and early childhood as signs and symptoms of virilization with or without adrenal insufficiency. It is subcategorized as salt-losing or non-salt-losing, reflecting the degree of mineralocorticoid deficiency (4). In the early 1950s, cortisone therapy was found to be effective in treating adrenal insufficiency and excess androgen production in patients with congenital adrenal hyperplasia (5). Since the discovery of cortisone therapy and the addition of mineralocorticoid supplementation, the morbidity and mortality of pa-

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tients with classic disease have markedly decreased, and these patients now have a long life span. Thus, long-term consequences of current treatments are an important consideration.

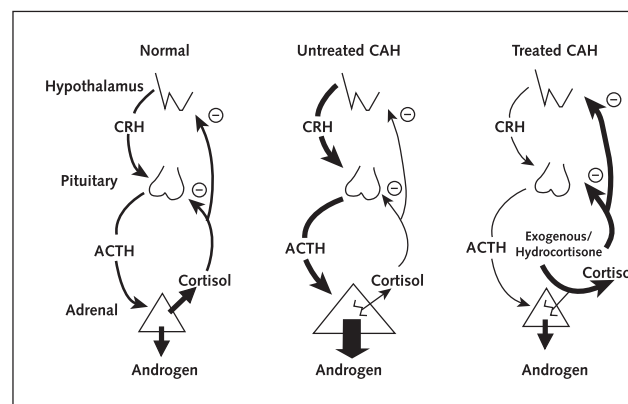
The 21-hydroxylase-deficient mouse, which was described in a Japanese study by Shiroishi and coworkers (6), has been a useful model for gaining new insights into the pathophysiology of the disease in humans and for developing new therapeutic strategies. The mouse model revealed an abnormal hypothalamic-pituitary-adrenal feedback mechanism (7), alterations in the structure and function of the adrenal medulla (8), and a good response to gene therapy (9). Further exploration of the disease process in this animal model is an essential aspect of the bench-to-bedside research approach to improving the human condition.

Abnormalities in both the structure and function of the adrenal medulla have been shown in patients with classic congenital adrenal hyperplasia (10). This finding may explain why some children with the severe form of 21-hydroxylase deficiency are prone to adrenal crises, hypoglycemia, and cardiovascular collapse in response to febrile illnesses or other stressful circumstances, despite adequate glucocorticoid replacement.

There are many unresolved clinical problems in the management of classic 21-hydroxylase deficiency in both males and females. Among the most critical are inadequate response to glucocorticoid and mineralocorticoid replacement therapy, iatrogenic Cushing syndrome (11), adult short stature (12, 13), and oligo-amenorrhea and infertility in women (14, 15). The new treatment approaches to classic congenital adrenal hyperplasia represent potential solutions to these unresolved issues. In a long-term randomized clinical trial, the NIH is testing a new treatment regimen consisting of reduced hydrocortisone dose, an antiandrogen, and an aromatase inhibitor (16, 17). Bilateral adrenalectomy is being performed in selected cases (18). Future therapies include a new class of drug called corticotropin-releasing hormone antagonists (19) and possibly gene therapy (9).

Nonclassic congenital adrenal hyperplasia, the mild form of the disease, is a common cause of hyperandrogenism in women. Although the same gene is involved in both the severe and mild forms, genetic mutations typically associated with the mild form of the disease only partially impair 21-hydroxylase activity. Thus, the patient with nonclassic congenital adrenal hyperplasia is in

Figure 1. Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency.



In a person with normal adrenal function (*left*), the adrenal gland produces both cortisol and androgen. The hypothalamic-pituitary-adrenal axis is controlled by negative feedback. In the untreated patient with CAH (*middle*), a block in cortisol biosynthesis leads to a buildup of cortisol precursors and lack of negative feedback. Corticotropin (*ACTH*) is oversecreted, and adrenal hyperplasia occurs. The combination of accumulated cortisol precursors and increased ACTH results in massive androgen production. In the treated patient with CAH (*right*), exogenous hydrocortisone replacement reduces androgen production. Supraphysiologic doses of hydrocortisone are often necessary to adequately suppress androgen production. CRH = corticotropin-releasing hormone.

a fully compensated state; she does not have cortisol deficiency but rather manifestations of hyperandrogenism, usually later in childhood, around puberty, or in early adulthood (20, 21). Nonclassic congenital adrenal hyperplasia is an important consideration in the differential diagnosis of female patients with symptoms or signs of hyperandrogenism, such as severe cystic acne, hirsutism, male pattern baldness, oligo-amenorrhea, or infertility. Nonclassic 21-hydroxylase deficiency, especially when it exists in conjunction with hyperinsulinemia, often results in the polycystic ovary syndrome, with its characteristic reproductive and metabolic comorbid conditions. Recognition of this disorder is crucial for family planning and management in women with hyperandrogenism. Men with nonclassic congenital adrenal hyperplasia are usually asymptomatic but may also present with early puberty or testicular adrenal rests.

Another recognized comorbid condition associated with congenital adrenal hyperplasia is activation of ectopic adrenal tissue resulting in adrenal rest tumors (22). These tumors are most commonly found in the testes of men with classic or nonclassic congenital adrenal hyperplasia and often result in oligo-azoospermia and infertil-

ity (23). New advances in the diagnostic evaluation and management of these tumors are presented in this paper.

GENETICS

The gene for 21-hydroxylase lies on chromosome 6 within the HLA locus of the major histocompatibility system; thus, 21-hydroxylase deficiency is an HLA-linked disorder (24). Two homologous genes result from ancestral duplication. *CYP21B* is the active gene; *CYP21A* is an inactive pseudogene.

The location of the *CYP21B* gene makes it vulnerable to relatively large genomic DNA exchanges with its homologous gene, *CYP21A*. The proximity of these genes and their location within the HLA region, which has a high rate of recombination, facilitate such exchanges. Therefore, the 21-hydroxylase locus shows an unusual degree of variability between individuals (25, 26). 21-Hydroxylase deficiency is unique because most mutations result from the transfer of sequences between pseudogenes and active genes (27). When deleterious sequences that normally present in the pseudogene are transferred to the active gene, they render the gene incapable of encoding a normal enzyme. The term *gene conversion* is used to denote the transfer of sequences between homologous genes; however, the mechanism is poorly understood.

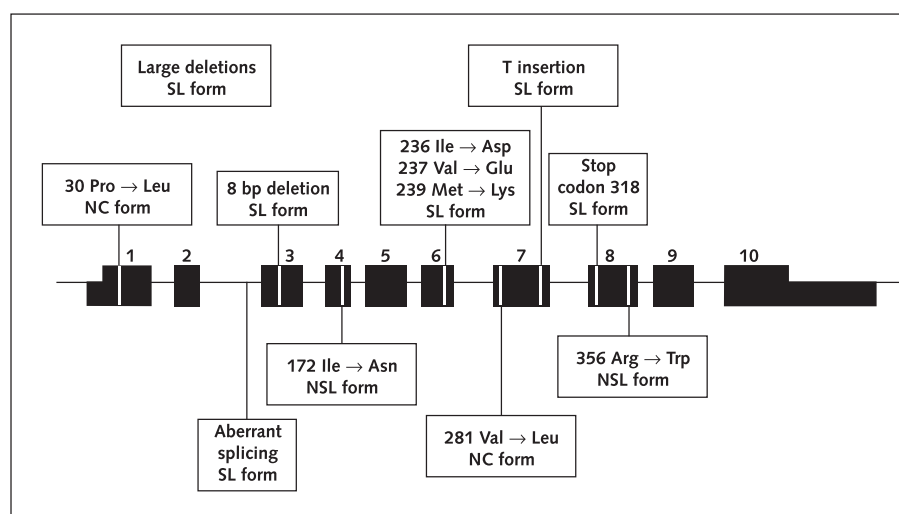
Specific mutations typically correspond to the three types of 21-hydroxylase deficiency: salt-losing, non-salt-losing (simple virilizing), and nonclassic congenital adrenal hyperplasia (Figure 2). In vitro studies have shown that mutations resulting in complete inactivation of 21-hydroxylase activity are associated with the salt-losing phenotype, those that reduce 21-hydroxylase activity to approximately 2% are associated with the non-salt-losing phenotype, and those that reduce 21-hydroxylase activity to 10% to 75% are associated with the nonclassic phenotype (28, 29) (Figure 2). Most patients are compound heterozygotes, and the severity of the disease is determined by the activity of the less severely affected allele.

The degree of functional impairment predicted by individual mutations usually corresponds to the clinical severity observed in a given patient. However, genotype does not always accurately predict phenotype. This disparity between genotype and phenotype may be due to androgen sensitivity or to other genes that cause differences in steroid metabolism and homeostasis.

ANIMAL MODEL FOR 21-HYDROXYLASE DEFICIENCY

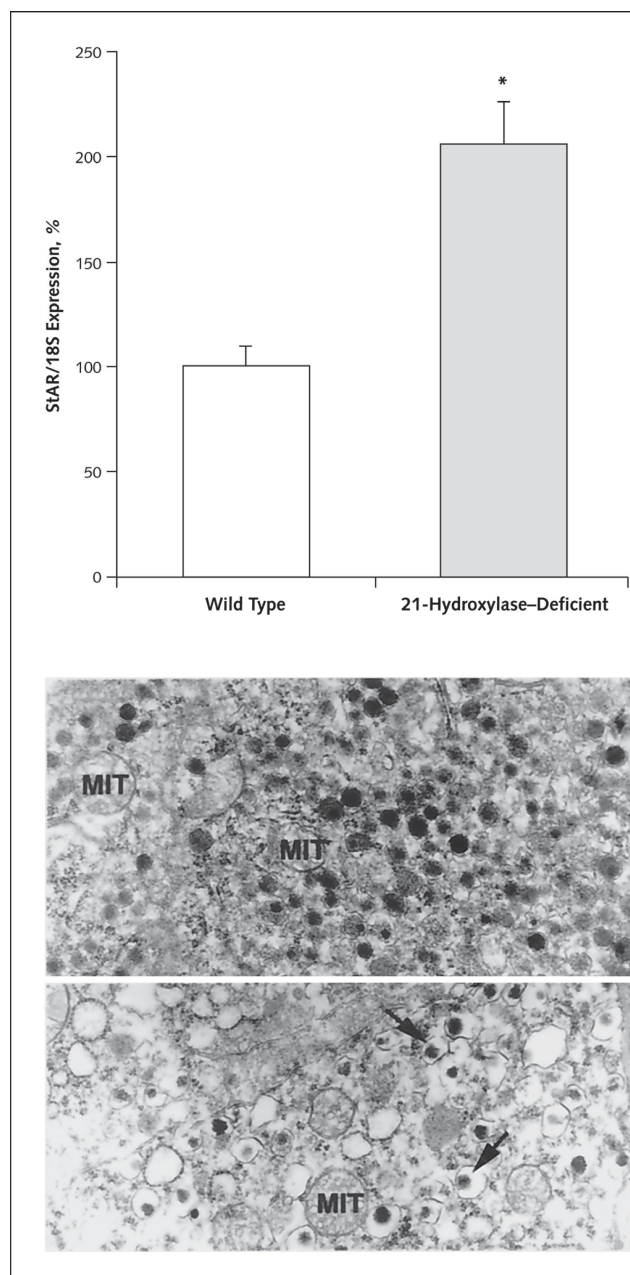
Dr. Stefan R. Bornstein (Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development [NICHD], NIH): In

Figure 2. The 10 most common genetic mutations found in 21-hydroxylase deficiency.



The location and nature of each microconversion and the expected clinical phenotype (salt-losing [SL], non-salt-losing [NSL], and nonclassic congenital adrenal hyperplasia [NC]) are shown. The 10 exons and 9 introns of *CYP21B* are drawn to scale. Arg = arginine; Asn = asparagine; Asp = aspartate; Glu = glutamate; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Pro = proline; Trp = tryptophan; Val = valine.

Figure 3. The 21-hydroxylase-deficient mouse.



Top. Steroidogenic acute regulatory protein (*StAR*) expression is increased in the adrenal glands of mice with 21-hydroxylase deficiency, as compared with wild-type animals. Quantitative steroidogenic acute regulatory protein was determined by TaqMan PCR Core Reagents Kit (Applied Biosystems, Foster City, California) ($n = 4$ adrenal glands; $P < 0.05$). Amounts of RNA were calculated with relative standard curves for both steroidogenic acute regulatory protein and 18S. The amount of messenger RNA was corrected by division by the amount of 18S RNA in each sample. **Middle.** Electromicrograph showing catecholamine-storing secretory vesicles in chromaffin cells of control animals (size range, 50 to 450 nm). **Bottom.** Electromicrograph of chromaffin cells.

the mouse, the 21-hydroxylase gene also lies within the major histocompatibility locus (30). Mice with spontaneous 21-hydroxylase deficiency have a deletion of the 21-hydroxylase gene (6, 7, 31). 21-Hydroxylase activity is completely absent in newborn mice homozygous for this deletion, and this absence is lethal in the early postnatal stage.

The deficiency of glucocorticoids results in adrenocortical hyperplasia and plasma accumulation of precursor steroids in both mice and humans with congenital adrenal hyperplasia. In mice, which normally lack adrenal 17- α -hydroxylase, the enzymatic blockade results mainly in accumulation of progesterone. Most of the affected mice, if not treated with glucocorticoids and mineralocorticoids, die within 1 week (6, 7, 31). Although the disease state of the 21-hydroxylase-deficient mouse is not completely comparable to human congenital adrenal hyperplasia, it has provided a useful model with which to examine molecular and cellular mechanisms of the disease and test new therapeutic interventions.

The adrenal glands of affected mice demonstrate a significant increase in expression of messenger RNA of steroidogenic acute regulatory protein (Figure 3), which is the rate-limiting step for steroidogenesis (32). Corticotropin regulates the expression of this protein, and the increase of messenger RNA reflects impaired negative feedback with increased corticotropin production. Moreover, one study showed that prenatal dexamethasone treatment (0.5 to 2 $\mu\text{g/d}$) failed to adequately suppress fetal adrenal hormones in mice, suggesting hyperactivity of the hypothalamic-pituitary corticotroph axis and insensitivity to glucocorticoid feedback inhibition (7). Intrauterine glucocorticoid deficiency may affect the sensitivity of feedback inhibition postnatally, thus blunting the central effects of treatment.

The animal model allowed a detailed analysis of morphologic changes of the adrenal glands in the setting of 21-hydroxylase deficiency (7, 8, 31). The adrenal cortex of mutant mice was markedly enlarged with adrenocortical cell hyperplasia and incomplete migration of chromaffin cells in the center of the gland. In addition,

chromaffin cells. Secretory granules are markedly reduced in chromaffin cells of 21-hydroxylase-deficient mice. The remaining granules are predominantly electron-dense, norepinephrine-containing vesicles, lying in large lucent vacuoles (arrows). For parts B and C, stain is uranyl acetate and lead citrate, and magnification is $\times 15\,000$. MIT = mitochondria.

Figure 4. Patients with salt-losing 21-hydroxylase deficiency.



secretory vesicles were depleted and adrenal catecholamine levels were reduced in chromaffin cells (Figure 3, middle) (8). Apparently, the sympathoadrenomedullary system, as well as the adrenal cortex, is affected in the 21-hydroxylase-deficient mouse.

In chromaffin cells, expression of phenylethanolamine-*N*-methyltransferase, the enzyme that converts norepinephrine to epinephrine, is stimulated by glucocorticoids (33–37). In concordance with this, epinephrine deficiency was reported in children with hypocorticotrophic hypopituitarism (38). Therefore, the absence of normal secretion of adrenocortical corticosterone is probably responsible for the impaired production of adrenal catecholamines in mice with 21-hydroxylase deficiency. However, exogenous replacement of glucocorticoid did not restore chromaffin cell function in 7-day-old mice. This supports the finding of reduced epinephrine levels in patients with Addison disease who are given adequate hydrocortisone replacement (39).

On the basis of these findings in mice with 21-hydroxylase deficiency, we investigated adrenomedullary function in humans with congenital adrenal hyperplasia and found a similarly severe impairment of both the structure and function of the adrenal medulla (10). This finding may have important clinical implications for the cardiovascular fragility that is often characteristic of such patients.

Mice with 21-hydroxylase deficiency have also proven useful in testing novel therapeutic strategies. In a model for genetic treatment of the disease in humans, a recombinant DNA fragment containing the murine genomic gene for 21-hydroxylase was successfully introduced into the adrenal glands of mutant mice (40). Although only 15% of newborns were typically rescued by synthetic steroid therapy, the efficiency of rescue was increased to 80% with the use of gene therapy (40).

We recently replaced the defective enzyme in the adrenal glands of the mouse model using an adenoviral vector encoding the genomic sequence of the human

The treatment outcome in classic congenital adrenal hyperplasia is often suboptimal because of incomplete suppression of hyperandrogenism (*top*), treatment-induced hypercortisolism (*bottom*), or both. At 16 years of age, a female patient with salt-losing 21-hydroxylase deficiency due to undertreatment with glucocorticoid and elevated androgen levels had hirsutism, acne, amenorrhea, and hyperpigmentation (*top*). Increased glucocorticoid treatment resulted in weight gain with cushingoid features and short stature in a male patient with classic 21-hydroxylase deficiency (*bottom*).

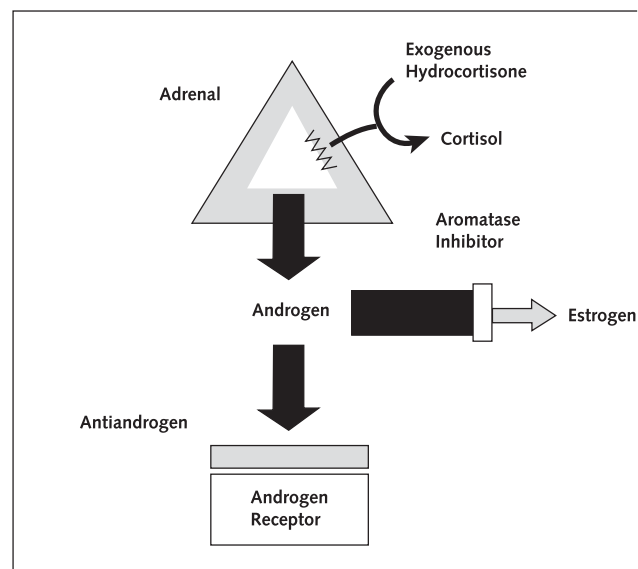
CYP21 gene (*AdCYP21*) (9). In homozygous 21-hydroxylase-deficient mice, intra-adrenal injections of *AdCYP21* allowed expression of human *CYP21* messenger RNA and 21-hydroxylase activity in the adrenal gland, as well as restored adrenal zonae, mitochondrial ultrastructure, and plasma corticosterone levels (9). Furthermore, adrenal gene transfer of the *CYP21* gene also corrected the abnormalities of the adrenal medulla; catecholamine secretory granules were restored (unpublished observation). The adenoviral vectors induced almost no inflammatory response in the adrenal glands, suggesting that high local glucocorticoid concentration suppresses the immune response caused by these vectors in other tissues. The adrenal gland may therefore be an ideal site for gene therapy, an observation that has been made elsewhere (41).

The adrenal cortex and medulla have a close functional relationship (42–47). Animal models with defined defects in the adrenal gland provide valuable and novel insights into the development, cross-talk, and functioning of the stress system and allow the testing of new therapeutic strategies (48). Unlike in exogenous hormone replacement therapy, adrenal gene transfer corrects the gene defect in 21-hydroxylase deficiency and thereby corrects abnormalities in both endocrine limbs of the stress system (9). Development of novel viral vectors (most likely retroviral vectors that allow stable long-term integration) with adrenal-specific promoters will be required to improve the efficiency and duration of gene transfer in the adrenal gland. With these advances, gene therapy may become a feasible option for treatment of congenital adrenal hyperplasia.

CLASSIC 21-HYDROXYLASE DEFICIENCY

Dr. Deborah P. Merke: Defects in the 21-hydroxylase gene, which significantly impair or even eliminate 21-hydroxylase activity, result in classic congenital adrenal hyperplasia, the most severe form of the disease. Females with classic congenital adrenal hyperplasia typically present at birth with ambiguous genitalia because of exposure to high levels of androgens in utero. Occasionally, genital ambiguity can be profound enough to cause incorrect sex assignment at birth. The age at diagnosis in males varies according to the severity of mineralocorticoid deficiency or salt loss. Males with salt loss typically present at 7 to 14 days of life with vomiting,

Figure 5. 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.

weight loss, lethargy, hyponatremia, and hyperkalemia. Males without salt loss present with precocious puberty, characterized by pubic hair and accelerated growth at 2 to 4 years of age (4).

A markedly elevated 17-hydroxyprogesterone level (>242.4 nmol/L [normal value < 8.9 nmol/L]) is a diagnostic indicator of classic 21-hydroxylase deficiency (28). A corticotropin stimulation test is unnecessary because random, unstimulated levels are markedly elevated. Typically, patients with salt loss have higher 17-hydroxyprogesterone levels than those without salt loss. Genetic analysis is also available and is often used for prenatal testing by chorionic villous sampling (49).

Treatment of classic congenital adrenal hyperplasia is intended to reduce excessive corticotropin secretion and replace both glucocorticoid and mineralocorticoid hormones. Glucocorticoid replacement in children is usually accomplished with oral hydrocortisone. In some patients with classic 21-hydroxylase deficiency, satisfactory control of androgens can be achieved with 12 to 15 mg/m² of hydrocortisone per day; however, higher doses are often needed to adequately reduce androgen produc-

tion (50). Because of the well-known risks of excessive glucocorticoid use, we are reluctant to exceed 25 mg/m² per day. Longer-acting glucocorticoids, such as prednisone (5 to 7.5 mg/d, divided into two doses) and dexamethasone (0.25 to 0.4 mg at bedtime), may be used in adults but are avoided in children because they may suppress growth.

Mineralocorticoid replacement is accomplished with fludrocortisone. We encourage patients to use salt freely to satisfy salt cravings and adjust mineralocorticoid dose to maintain a normal plasma renin activity for the level of salt intake. A typical oral dose of fludrocortisone ranges from 100 to 200 µg/d; rarely, a patient may require a higher dose. The dose of fludrocortisone is relatively independent of body size from childhood to adulthood. Insufficient replacement with fludrocortisone results in hypovolemic stimulus of corticotropin. Many patients with non-salt-losing disease have elevated plasma renin activity. Therefore, fludrocortisone therapy in such patients allows management with lower doses of glucocorticoid (50).

At physiologic doses, hydrocortisone prevents adrenal insufficiency but does not suppress corticotropin and androgen production. Higher doses of hydrocortisone are necessary to adequately suppress androgens (50). Clinical management of classic congenital adrenal hyperplasia is often a difficult balancing act between two undesirable states: hyperandrogenism and hypercortisolism (Figure 4). The signs of hypercortisolism are characteristic of iatrogenic Cushing syndrome: obesity, short stature, osteoporosis, carbohydrate intolerance, and dyslipidemia. The symptoms and signs of hyperandrogenism include virilization of females, precocious virilization of males, early puberty, and adult short stature in both sexes.

New Treatment Approaches

Androgen Antagonism

Androgen antagonist approaches are designed to optimize hormonal control and adult outcome in patients with classic congenital adrenal hyperplasia. These goals entail controlling the effect of excess adrenal androgen and reducing glucocorticoid dose. At the NIH, a long-term randomized study is evaluating a new treatment approach (17). Instead of using high-dose hydrocortisone to suppress androgens, we use lower doses of hy-

drocortisone and allow levels of corticotropin and androgens to remain mildly elevated. We then antagonize the effects of androgens with an androgen-receptor antagonist (flutamide) and block the conversion of androgen to estrogen with testolactone, an aromatase inhibitor (Figure 5). Blocking estrogen production is important because estrogens advance bone age and induce early puberty.

In this NIH study, half of the participating children received reduced doses of hydrocortisone and appropriate doses of fludrocortisone, as well as flutamide and testolactone; the other half received conventional doses of hydrocortisone and fludrocortisone. On average, children in the investigational group received half the dosage of hydrocortisone compared with the children in the control group (mean dosage [$\pm$ SD], 8.6 $\pm$ 0.6 vs. 13.3 $\pm$ 0.6 mg/m² per day) (17). As expected, after 2 years of therapy, we found significantly higher levels of 17-hydroxyprogesterone, androstenedione, and testosterone in the investigational group than in the control group (17). Despite these elevated hormone levels, however, children receiving the new treatment regimen had normalized growth velocity and bone maturation. Thus, this new regimen represents a promising new treatment approach for children with classic 21-hydroxylase deficiency.

Bilateral Adrenalectomy

Bilateral adrenalectomy has been proposed recently as a surgical treatment option for severe classic congenital adrenal hyperplasia (18, 51). To date, five such operations have been reported (52–56); all were done in difficult-to-control cases, and one was performed laparoscopically (51, 54). The argument for adrenalectomy is that concern about progressive virilization is eliminated once the adrenal glands are removed. Arguments against the procedure include risks of surgery and anesthesia; its irreversibility, which eliminates the option of new medical approaches; activation of adrenal rest or ectopic adrenal tissue; and theoretical loss of possibly protective adrenomedullary function. As we observed in the mouse model, the adrenal medulla does not function normally in patients with this disorder (10). There are two approaches to adrenalectomy: 1) Perform a bilateral adrenalectomy in severely affected patients with difficult-to-control disease, or 2) perform an adrenalectomy

in the first year of life, concurrent with genitoplasty in females (18). However, predicting disease severity in the first year of life is difficult, and genotyping is not sufficient because of genotype–phenotype variation (27, 28).

Adrenomedullary Function in Congenital Adrenal Hyperplasia

Evaluation of the adrenal medulla may be useful in predicting disease severity. We evaluated adrenal morphologic characteristics and adrenal function in patients with 21-hydroxylase deficiency (10). Similar to the mouse model, our study found incomplete formation of the adrenal medulla in three patients with 21-hydroxylase deficiency who had bilateral adrenalectomy. Of interest, we also found that patients with 21-hydroxylase deficiency who had very low, even undetectable levels of 24-hour urinary epinephrine were categorized as salt-losing and were hospitalized for adrenal crises.

The use of an antiandrogen in combination with an aromatase inhibitor and a reduced hydrocortisone dose is a promising new treatment approach to optimize growth, development, and adult height in patients with classic congenital adrenal hyperplasia. Adrenalectomy should be considered in selected cases. Adrenomedullary function is impaired in patients with 21-hydroxylase deficiency and may be a biomarker for disease severity.

NONCLASSIC CONGENITAL ADRENAL HYPERPLASIA

Dr. George P. Chrousos (Pediatric and Reproductive Endocrinology Branch, NICHD, NIH): The nonclassic form of 21-hydroxylase deficiency is mild and is most often recognized in late puberty and early adulthood. One of the first families diagnosed with nonclassic congenital adrenal hyperplasia was studied at the NIH in the early 1980s (57). Until that time, the existence of this condition was in doubt, yet it is quite common in some populations and has characteristic reproductive, metabolic, and other comorbid conditions. Our first patient with nonclassic congenital adrenal hyperplasia was 1 of 2 from a sample of 40 women consecutively admitted to the NIH with hirsutism (57). All of the patients had serial 24-hour sampling for measurement of plasma 17-hydroxyprogesterone concentrations and a corticotropin stimulation test. In the 24-hour sampling, 17-hydroxyprogesterone was secreted in both a circadian and pulsatile fashion with elevations in the early morn-

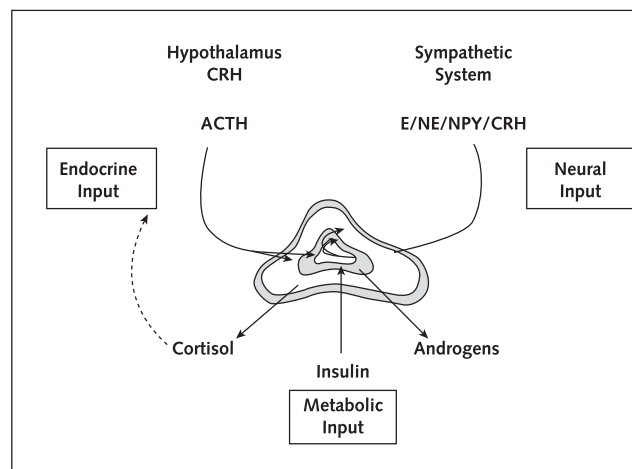
ing and most values within the normal range. These findings were later confirmed in a larger study, which found elevated levels of adrenal androgens occurring primarily during the early morning hours (21, 58).

Results of a random test of plasma 17-hydroxyprogesterone level may be normal in a patient with nonclassic congenital adrenal hyperplasia. A 30- or 60-minute corticotropin test with a 17-hydroxyprogesterone level greater than 45.5 nmol/L is usually necessary to diagnose this disorder, although cutoff levels reported for the diagnosis of nonclassic congenital adrenal hyperplasia vary from 30.3 nmol/L (59) to 60.6 nmol/L (60). Early-morning basal 17-hydroxyprogesterone level can be used for screening (61), but it is not as sensitive or specific as a corticotropin test. Heterozygote carriers of congenital adrenal hyperplasia often have mildly elevated 17-hydroxyprogesterone levels (<30.3 nmol/L) in a corticotropin stimulation test (28), but they do not have symptoms or signs of the disease. In the rare case of a patient whose corticotropin-stimulated 17-hydroxyprogesterone level is between 30.3 nmol/L and 45.5 nmol/L, genetic analysis may be necessary to distinguish a heterozygote carrier from a homozygously affected patient with a mildly deleterious mutation in the 21-hydroxylase gene.

A glucocorticoid, a birth control pill, an antiandrogen, or combinations of these medications are used to treat women with nonclassic congenital adrenal hyperplasia. Spironolactone and flutamide, which are not formally approved for this indication, are the typical antiandrogens used. Birth control pills protect the ovaries from becoming polycystic, sclerotic, and androgen-secreting and prevent the birth of undermasculinized male children in women taking antiandrogens. In studies of female transsexuals, up to 70% developed the polycystic ovary syndrome secondary to exogenous hyperandrogenism (62). Thus, it is extremely important to diagnose nonclassic congenital adrenal hyperplasia and start therapy as early as possible. Early treatment prevents the vicious cycle of progressive deterioration and sclerosis of the ovaries, a process that is accelerated and worsened by the concurrent presence of insulin resistance (63). When fertility is desired, glucocorticoid therapy (usually prednisone) is often necessary and combination therapy with the antiandrogen and birth control pill is discontinued.

Men with nonclassic congenital adrenal hyperplasia typically do not require treatment. Occasionally, how-

Figure 6. Mechanisms of adrenal hyperandrogenism.



Elevations in adrenocorticotrophic hormone, increases in neural–adrenomedullary input, and presence of insulin-mediated metabolic input may lead to adrenal hyperandrogenism and premature adrenarche. In turn, adrenal hyperandrogenism, insulin resistance, or both may lead to full-blown polycystic ovary syndrome in a woman with an inherent ovarian vulnerability. ACTH = adrenocorticotrophic hormone; CRH = corticotropin-releasing hormone; E = epinephrine; NE = norepinephrine; NPY = neuropeptide Y.

ever, males with nonclassic congenital adrenal hyperplasia present with slightly advanced puberty and bone age. These boys may be at risk for early epiphyseal fusion and adult short stature and might benefit from glucocorticoid therapy during childhood. Rarely, glucocorticoid treatment is indicated in adult men to suppress the development of testicular adrenal rest tissue.

REPRODUCTIVE, METABOLIC, AND OTHER COMORBID CONDITIONS ASSOCIATED WITH CONGENITAL ADRENAL HYPERPLASIA

Reproductive Comorbid Conditions

Females with classic congenital adrenal hyperplasia are born with varying degrees of genital virilization. Exposure to androgens in early fetal life causes labial fusion and the formation of a urogenital sinus, pigmentation and rugation of the labia, and clitoromegaly. Occasionally, genital ambiguity can be severe enough to cause the wrong sex assignment at birth. This severe virilization can now be repaired surgically with a single procedure in the first few months of life (64). However, other surgical approaches often involve several procedures during childhood and early adulthood. Scarring may cause vaginal stenosis, which requires dilatation before a patient is

able to have intercourse. Affected women therefore often face physical and psychological barriers to sexual function (65).

Despite these complex psychosexual and biological issues, many women with classic congenital adrenal hyperplasia, particularly the non-salt-losing form, marry and have children. However, women with congenital adrenal hyperplasia may have decreased fertility if their adrenal androgens are not suppressed adequately. The polycystic ovary syndrome may develop secondary to adrenal hyperandrogenism. The effect of ovarian exposure to adrenal androgens prenatally and during childhood is unknown, but it may predispose these women to the polycystic ovary syndrome.

In children and women with nonclassic congenital adrenal hyperplasia, hyperandrogenism is the key morbidity factor. Nonclassic congenital adrenal hyperplasia accounts for about 5% to 10% of children with premature adrenarche and 5% to 10% of women with hirsutism (57, 66–68). The clinical spectrum includes early adrenarche in both girls and boys, menstrual irregularities (mostly oligo-menorrhea), adolescent and postadolescent cystic acne, hirsutism, male pattern baldness, polycystic ovaries, oligo-anovulation, and infertility. In theory, women with nonclassic disease should have increased risk for cancer of the reproductive system as a result of anovulation and unopposed estrogen action, but this has not been shown.

In men, growth of adrenal rest tissue in the testes may lead to testicular tenderness and pain, oligospermia or azospermia, and infertility. Rarely, men with nonclassic congenital adrenal hyperplasia present with a markedly enlarged and tender testis and oligospermia due to testicular adrenal rests (69). Oligospermia is caused by destruction of the tubuli efferentes, which bring incapacitated spermatozoa from the spermatic tubules to the epididymis. With the advances in fertility technologies, affected patients can father children by using sperm obtained directly from the testis to fertilize the mother's ova in vitro.

Metabolic Comorbid Conditions

Patients with congenital adrenal hyperplasia who are exposed to excessive doses of glucocorticoids may develop iatrogenic Cushing syndrome and all its metabolic comorbid conditions. Nonclassic congenital adrenal hy-

perplasia affects approximately 0.5% of the female population. Patients with simultaneous mild enzymatic deficiency of 21-hydroxylase and insulin resistance may develop premature adrenarche or the full-blown polycystic ovary syndrome (Figure 6). Insulin resistance may be genetic, constitutional, or both and may be further aggravated by obesity. The polycystic ovary syndrome affects approximately 5% to 7% of women of reproductive age (63), and at least one quarter of patients with nonclassic congenital adrenal hyperplasia have full-blown polycystic ovary syndrome associated with the metabolic syndrome X and its long-term sequelae, atherosclerosis and cardiovascular disease (Figure 7). Our first patient with nonclassic congenital adrenal hyperplasia had already developed full-blown polycystic ovary syndrome at the time of her diagnosis because she was also insulin resistant (57). To conceive her two children, she required treatment with dexamethasone and clomiphene.

Adrenal Tumors

Congenital adrenal hyperplasia may be associated with increased incidence of adrenal adenomas or incidentalomas. On magnetic resonance imaging of the adrenal glands, patients with both classic and nonclassic congenital adrenal hyperplasia frequently have nonsecretory adrenocortical adenomas (70). One of 10 patients with congenital adrenal hyperplasia had an adenoma greater than 5 cm in diameter, which indicates surgical removal (43, 71). Of interest, carriers of classic and nonclassic congenital adrenal hyperplasia, who are not expected to have any endocrine or other abnormalities, also develop such tumors in their adrenal glands. One in 20 of these patients has tumors that exceed 5 cm in diameter (70). Rough calculations indicate that a significant proportion of all patients with incidentalomas have nonclassic congenital adrenal hyperplasia or are carriers for congenital adrenal hyperplasia (43, 71).

EVALUATION AND MANAGEMENT OF ADRENAL REST TISSUE

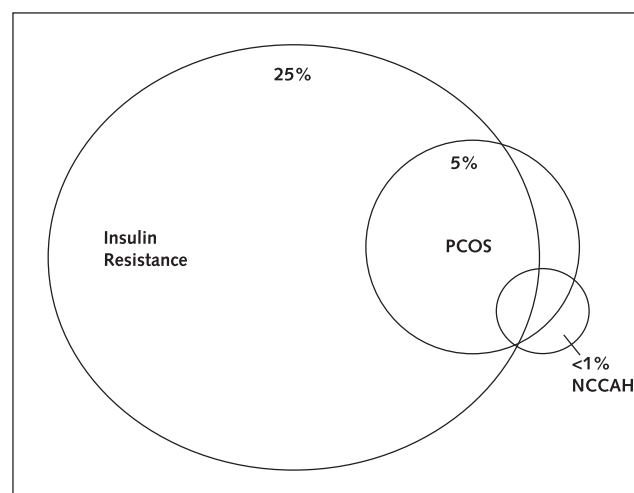
Diagnosis and Radiologic Evaluation

Dr. Nilo A. Avila (Diagnostic Radiology Department, Warren Grant Magnuson Clinical Center, NIH): Ectopic adrenal rest tissue is biochemically identical to that of the adrenal cortex (72–74) and can function like

adrenal tissue; its function is increased by corticotropin and decreased by high doses of glucocorticoids. Persistent virilization in selected patients with congenital adrenal hyperplasia has been attributed to activated adrenal rest tissue (75). Embryologically, both the gonads and the adrenal glands originate from the mesoderm of the urogenital ridge; therefore, adrenal rest tissue is most frequently found in the gonads, although it may occur in other parts of the body. It is most often found in the testes, where it is called testicular adrenal rest tissue (22). It has also been described in the celiac plexus, broad ligaments, and ovaries (76–79). Characteristic radiologic features of testicular adrenal rest tissue have been described (22, 80, 81). The rare diagnosis of ovarian adrenal rest has been based on biopsy and pathologic examination, with such nonspecific radiologic findings as ovarian enlargement (79).

At the NIH, we performed testicular ultrasonography on 42 patients with classic congenital adrenal hyperplasia and found that approximately 30% had testicular masses (81). Only two of the patients with positive findings (5%) had palpable masses. Thus, physical examination misses most of the masses found on testicular ultrasonography. The clinical significance of small (<5 mm) testicular adrenal rest tissue, which is detected only by screening ultrasonography, is unknown.

Figure 7. Venn diagram including the overlapping populations of women with insulin resistance, the polycystic ovary syndrome (PCOS), and nonclassic congenital adrenal hyperplasia (NCCAH).



Several ultrasonographic features are characteristic but not pathognomonic of testicular adrenal rest tissue (Figure 8A and 8B). The bilateral nature of these masses is important because other testicular tumors, including testicular cancer, tend to be unilateral. If blood flow is present, color Doppler will demonstrate undisturbed blood flow through testicular adrenal rest tissue, whereas solid testicular carcinoma tends to divert vascular structures (22).

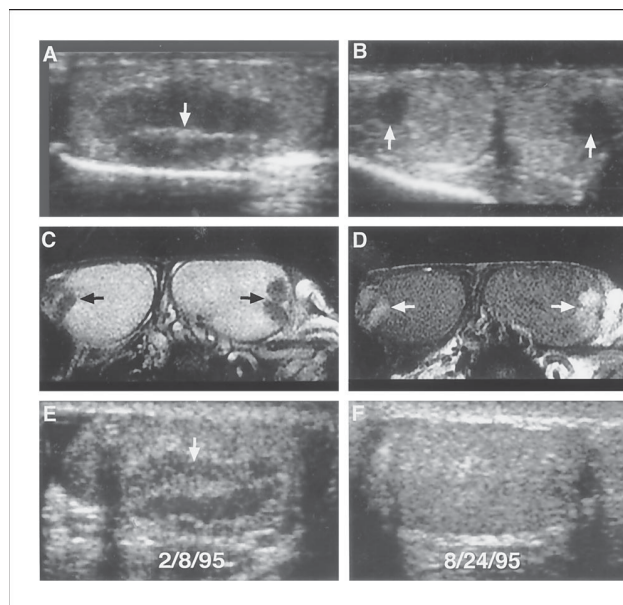
The features of testicular adrenal rest tissue on magnetic resonance imaging are similar to those of normal adrenal glands (80). Most of these masses are isointense on T1-weighted images, hypointense on T2-weighted images (Figure 8C), and are diffusely enhanced on post-contrast images (Figure 8D). Because ultrasonography is more available and less expensive than magnetic resonance imaging, it should be the method of choice in the diagnosis and follow-up of testicular adrenal rest tissue.

Treatment and Follow-up

It is important to recognize adrenal rest tissue as a cause of intratesticular masses in men with congenital adrenal hyperplasia because glucocorticoid therapy, rather than surgery, is the appropriate treatment. Higher-dose glucocorticoid therapy may be warranted if the patient is symptomatic or infertile, while standard treatment is indicated for small testicular adrenal rest tissue. The clinician should balance the need for shrinkage of testicular adrenal rest tissue with the iatrogenic side effects of increased glucocorticoid therapy, especially because the significance of small testicular adrenal rest tissue is unknown. If the mass rapidly increases in size or is unresponsive to glucocorticoid therapy, further evaluation, such as testicular venous sampling or a testicular biopsy, may be performed to confirm the diagnosis (82, 83). Orchiectomy is the treatment of choice for testicular neoplasms but is rarely indicated for testicular adrenal rest tissue (72, 84, 85). Suspicion of carcinoma or unrelieved pain and tenderness are the major indications for an orchiectomy. If orchiectomy is performed, a testicular prosthesis is recommended.

Although the cause of testicular adrenal rest tissue is unknown, large symptomatic masses tend to occur in patients with very high levels of corticotropin and typically respond to glucocorticoid therapy (Figure 8E and 8F). In the past, many physicians did not treat men with classic congenital adrenal hyperplasia in adulthood un-

Figure 8. Characteristic radiologic features of testicular adrenal rest tumors.



Testicular adrenal rest tissue masses often surround the mediastinum testes (arrow) (A), and are bilateral, intratesticular, and hypoechoic (B). Testicular adrenal rest tissue masses are seen equally as well on ultrasonography and magnetic resonance imaging. Most of these masses are hypointense on T2-weighted images (C) and isointense on T1-weighted images, with diffuse enhancement post-contrast (D). Large testicular adrenal rest tissue (E) typically shrinks or disappears (F) with higher-dose glucocorticoid therapy.

less treatment was required for extreme salt loss. There is now enough evidence of risk for testicular adrenal rest tissue and infertility (23, 73, 86) to warrant lifetime glucocorticoid therapy in men with classic congenital adrenal hyperplasia.

CONCLUSIONS AND FUTURE DIRECTIONS

Dr. George P. Chrousos: Congenital adrenal hyperplasia is a disease of widely varying severity. In the classic form, the therapeutic spectrum of cortisol is very narrow and the addition of antiandrogens may help with optimizing control (11). The addition of an aromatase inhibitor may be a necessary adjunct to moderate the conversion into estrogens of androgens diverted from the androgen receptor (17). Bilateral adrenalectomy may be necessary in some patients who cannot be controlled medically (18, 52). Adrenal rest tumors are often present in the testes of males with classic or nonclassic congenital adrenal hyperplasia. Characteristic radiologic fea-

tures help differentiate these tumors from testicular carcinoma. Undiagnosed nonclassic congenital adrenal hyperplasia should be considered in patients with testicular tumors (69), and nonclassic congenital adrenal hyperplasia, the congenital adrenal hyperplasia carrier state, or both should be considered in patients with adrenal incidentalomas (70).

Several important questions will have to be answered in the next few years. Increased secretion of hypothalamic corticotropin-releasing hormone is expected in patients with congenital adrenal hyperplasia. Elevated levels are seen in patients with melancholic depression and anxiety disorders and have been considered a pathogenic mediator in such disorders (87). The incidence of these psychological disorders in patients with congenital adrenal hyperplasia needs to be studied.

The heterozygote state for 21-hydroxylase deficiency has been found in increased frequency in children with premature adrenarche (66). In a recent study of Greek children with premature adrenarche, the incidence of heterozygotic 21-hydroxylase gene mutations was 35% (67). We do not know the incidence of the congenital adrenal hyperplasia carrier state in women with hyperandrogenism, in men with testicular tumors, or in men and women with adrenal incidentalomas. We will need to determine whether and why such a mild defect is or may be associated with these conditions.

It is possible that a heterozygotic defect is subclinical in most instances but becomes apparent when it is associated with other defects in patients' genetic and constitutional backgrounds. A person who is heterozygous for congenital adrenal hyperplasia could also have genetically and constitutionally determined mild insulin resistance and a mild gene defect that makes her ovaries vulnerable to the polycystic ovary syndrome. The latter two states (insulin resistance and gene defect) may potentiate the effects of the heterozygosity on the adrenal glands, ovary, or both and lead to overt adrenal or ovarian hyperandrogenism manifested as premature adrenarche or the polycystic ovary syndrome (42). Thus, a single defect that is too small in itself to cause disease may do so when it combines with one or more defects of genes or functions with epistatic effects on its action.

What are the new avenues for treatment? A corticotropin-releasing hormone antagonist, in combination with glucocorticoid and mineralocorticoid therapy, could in theory obviate the need for an antiandrogen–

aromatase inhibitor or bilateral adrenalectomy. Our early preclinical results with a corticotropin-releasing hormone antagonist are promising (19, 88, 89). Such an antagonist could be used until gene therapy becomes safe and effective. A prototype corticotropin-releasing hormone receptor antagonist, antalarmin, binds to corticotropin-releasing hormone receptor type I and blocks the effects of corticotropin-releasing hormone on that receptor (88). It acutely and chronically decreases corticotropin and cortisol secretion without causing adrenal insufficiency (19).

There is now convincing evidence from studies of the fetal adrenal gland in humans that corticotropin-releasing hormone of placental origin stimulates the secretion of androgens by the fetal adrenal zone (90). In the adult adrenal gland, the adrenomedullary sympathetic system, through such locally produced peptides as corticotropin-releasing hormone, stimulates the zona reticularis (42). It is therefore possible that antalarmin, acting also at the level of the zona reticularis, blocks the production of androgens by this zone, possibly adding further benefit. We are currently testing this hypothesis.

In the past 50 years, since the discovery of cortisone therapy as an effective treatment for congenital adrenal hyperplasia, many advances have been made in the study and management of 21-hydroxylase deficiency. The discovery of the 21-hydroxylase gene was followed by the discovery of the 21-hydroxylase–deficient mouse, which has provided a useful model in which to test new treatment approaches. As our knowledge of this disease expands, new therapies are being developed and tested in both humans and the animal model.

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