

Review

Congenital Adrenal Hyperplasia – Current Insights in Pathophysiology, Diagnostics, and Management

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Abbreviations: 11KT, 11-ketotestosterone; 11OHD, 11-hydroxylase deficiency; 17OH-Preg, 17-hydroxypregnenolone; 17OHD, 17-hydroxylase deficiency; 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; AAV, adeno-associated virus; ACTH, adrenocorticotrophic hormone, corticotropin; BMD, bone mineral density; BMI, body mass index; CAH, congenital adrenal hyperplasia; cAMP, cyclic adenosine monophosphate; cIMT, carotid intima media thickness; COUP-TFII, Chicken Ovalbumin Upstream Promotor-Transcription Factor-2; CRF, corticotropin-releasing factor; CRH, corticotropin-releasing hormone; CYP21A2, 21-hydroxylase; CYP19A1, Aromatase; Dex, dexamethasone; DHEA, dihydroeioandrostene dione; DHEAS, dehydroeioandrosterone sulfate; DELFIA, dissociation-enhanced lanthanide fluoroimmunoassay; DHT, 5-dihydrotestosterone; DOC, 11 deoxycorticosterone; DSD, differences in sex development; ESC, embryonic stem cell; FSH, follicle-stimulating hormone; GC, gas chromatography; GnRH, gonadotropin-releasing hormone; HC, hydrocortisone; HOMA- β , homeostatic model assessment; HSD3B, 3 β -hydroxysteroid dehydrogenase; HSD17B, 17 β -hydroxysteroid dehydrogenase; IL, interleukin; iPSC, inducible pluripotent stem cell; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LH, luteinizing hormone; MC2R, adrenocorticotrophic hormone receptor; MLPA, multiplex ligation-dependent probe amplification; MR, mineralocorticoid receptor; MRI, magnetic resonance imaging; MS, mass spectrometry; NC, nonclassic; OMM, outer mitochondrial membrane; PGD, preimplantation genetic diagnosis; POR, P450 oxidoreductase; TARTs, testicular adrenal rest tumors; SF-1, steroidogenic factor-1; SRD5A1, 5 α -reductase type 1; StAR, steroidogenic acute regulatory protein; SV, simple virilizing; SW, salt wasting; TNXB, tenascin-X; WMD, weighted mean difference.

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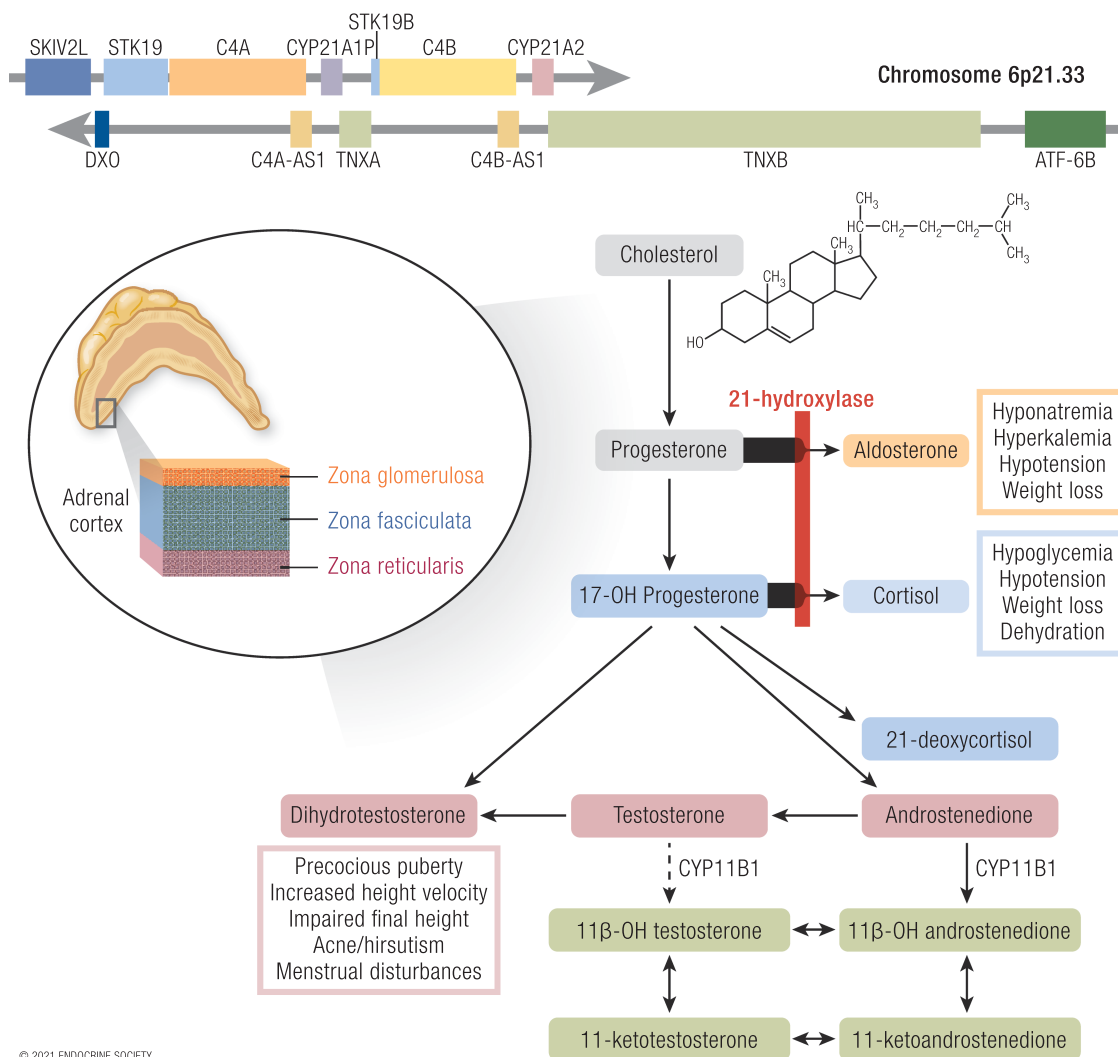
Abstract

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders affecting cortisol biosynthesis. Reduced activity of an enzyme required for cortisol production leads to chronic overstimulation of the adrenal cortex and accumulation of precursors proximal to the blocked enzymatic step. The most common form of CAH is caused by steroid 21-hydroxylase deficiency due to mutations in *CYP21A2*. Since the last publication summarizing CAH in Endocrine Reviews in 2000, there have been numerous new developments. These include more detailed understanding of steroidogenic pathways, refinements in neonatal screening, improved diagnostic measurements utilizing chromatography and mass spectrometry coupled with steroid profiling, and improved genotyping methods. Clinical trials of alternative medications and modes of delivery have been recently completed or are under way. Genetic and cell-based treatments are being explored. A large body of data concerning long-term outcomes in patients affected by CAH, including psychosexual well-being, has been enhanced by the establishment of disease registries. This review provides the reader with current insights in CAH with special attention to these new developments.

Key Words: Steroid biosynthesis, 21-hydroxylase deficiency, *CYP21A2*, glucocorticoid, mineralocorticoid, cortisol, aldosterone

Graphical Abstract

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency



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ESSENTIAL POINTS

- Congenital adrenal hyperplasia (CAH) is most often caused by deficiency of steroid 21 hydroxylase encoded by *CYP21A2*
- Allelic variants are associated with a spectrum of phenotypes
- CAH in its severe, classic form includes cortisol and aldosterone deficiencies, as well as androgen excess
- Newer concepts in steroid biosynthesis, hormonal and genetic diagnostic tools, and novel therapeutics have expanded our understanding of CAH
- Long-term sequelae of this disease have been reported in detail and strategies are being developed to improve quality of life for these patients

Congenital adrenal hyperplasia (CAH) is an inherited inability to synthesize cortisol. Approximately 90% to 99% of cases of CAH are caused by 21-hydroxylase deficiency (21OHD) caused by mutations in the *CYP21A2* gene (1,

2); the terms CAH and 21OHD will be used interchangeably in this article. The literature has historically described classic and nonclassic (NC) forms of this disorder, although current thinking views *CYP21A2* allelic variants

and their phenotypic manifestations as a continuum. The classic form, occurring in 1 in 14 000 to 18 000 based on newborn screening (Table 1), is defined by severely reduced or absent enzyme activity with impaired cortisol production manifesting clinically in the neonatal period. In the most severe, salt-wasting (SW) form of classic CAH, there is little or no residual enzymatic activity, resulting in cortisol and aldosterone deficiency. Lack of negative feedback on the hypothalamic–pituitary–adrenal axis leads to excess adrenal androgen production as elevated precursor steroids are shifted to the nonaffected androgen pathways. If not promptly treated, infants with this form of CAH quickly develop potentially fatal “salt-wasting crises” with hyponatremia, hyperkalemia, acidosis, and shock. Those infants who produce slightly more aldosterone are less likely to suffer acute SW crisis, but such patients still have severe cortisol deficiency and markedly elevated adrenal androgen production. They are said to have “simple virilizing” (SV) CAH, associated with residual enzymatic activity of 1% to 5% of normal. All infants affected with classic CAH benefit from glucocorticoid plus adjunctive mineralocorticoid treatment at least within the first year

of life, when there is relative renal tubular resistance to the salt-retaining effects of aldosterone in early infancy (28) and low sodium content of infant diets (29).

Whereas gonadal development is normal, severely increased prenatal adrenal androgen production leads to virilization of the female external genitalia (30), including variable degrees of clitoral enlargement and labial fusion. The genital appearance of affected 46,XX infants is occasionally indistinguishable from that of male genitals with penis and scrotum but empty of gonads. Müllerian duct development is normal, except for the formation of a urogenital sinus with conjoined urethra and vagina. Thus, reproductive potential exists in females despite atypical external genitalia. Males have normal external genitalia. Wolffian duct development is normal in males but absent in females, who continue to produce COUP-TFII (Chicken Ovalbumin Upstream Promoter-Transcription Factor-2), which induces Wolffian duct involution (31).

Adverse sequelae in CAH patients occur as a result of adrenal hormone imbalance and from chronic glucocorticoid therapy (32). Androgen excess can cause inappropriately rapid somatic growth, accelerated skeletal maturation,

Table 1. Incidence of CAH in different countries

| Country | Complete national data? | Sample size | 1/Incidence | PPV (term infants or overall) | Reference |
|----------------------------------|-------------------------|-------------|-------------|-------------------------------|-----------|
| Argentina (Buenos Aires) | No | 80 436 | 8937 | 50 | (3) |
| Australia* | Yes | | 18 034 | N/A | (4) |
| Australia (New South Wales) | No | 185 854 | 15 488 | 1.8 | (4) |
| Australia (Western Australia)* | No | 550 153 | 14 869 | N/A | (5) |
| Brazil | No | 748 350 | 14 967 | | (6) |
| Brazil (Goias state) | No | 82 603 | 10 325 | 28.6 | (7) |
| Brazil (Minas Gerais state) | No | 159 415 | 19 927 | 2.1 | (8) |
| Brazil (Rio Grande do Sul state) | No | 108 409 | 13 551 | 1.6 | (9) |
| China | No | 30 000 | 6084 | | (10) |
| China (Beijing) | No | 44 360 | 7393 | 3.0 | (11) |
| Croatia | Yes | 532 942 | 14 403 | | (12) |
| Cuba | Yes | 621 303 | 15 931 | 0.3 | (13) |
| Czech Republic | Yes | 888 891 | 12 520 | 1.6 | (14) |
| France | Yes | 6 012,798 | 15 699 | 2.3 | (15) |
| Germany (Bavaria) | No | 1 420,102 | 12 457 | 5 | (16) |
| India | No | 55 627 | 6334 | | (17) |
| Israel | Yes | 1 378,132 | 16 910 | 16.5 | (18) |
| Japan (Sapporo) | No | 498 147 | 20 756 | 8 | (19) |
| Japan (Tokyo) | No | 2 105,108 | 21 264 | 25.8 | (20) |
| Netherlands | Yes | 2 235,931 | 17 468 | 24.7 | (21) |
| New Zealand | Yes | 1 175,988 | 26 727 | | (22) |
| Sweden | Yes | 2 737,932 | 14 260 | 25.1 | (2) |
| Turkey | No | 241 083 | 15 067 | 1.9 | (23) |
| United Arab Emirates | Yes | 750 365 | 9030 | | (24) |
| United Kingdom* | Yes | | 18 248 | N/A | (25) |
| Uruguay | Yes | 190 053 | 15 800 | | (26) |

Data are from studies published in 2008 and later; Earlier studies are summarized by (27) and (2). Data are from newborn screening except those marked with an asterisk (*), which are from national case registries.

Abbreviation: PPV, positive predictive value.

and reduced adult height. A systematic review and meta-analysis for >1000 classic CAH patients found shorter than average stature for mid-parental heights (-1.03 standard deviations, corresponding to ~ 7 cm) (33), but many of these children were diagnosed before the implementation of neonatal screening and did not receive the benefit of early initiation of treatment.

Elevated levels of adrenal androgens affect the hypothalamic–pituitary–gonadal axis. Central precocious puberty is a risk in patients experiencing prolonged periods of poor hormonal control. Young women with well-controlled CAH usually experience normal menarche (34), but poor control is associated with acne, female hirsutism, male pattern baldness, altered body habitus, irregular menses, and subnormal fertility (35). Males with poor hormonal control may develop small testes and benign testicular adrenal rest tumors (TARTs) (see section “Long-term sequelae,” “Gonadal function in males,” “Testicular adrenal rest tumors”) (36).

Individuals affected with milder allelic variants (ie, NC CAH) tend to present to medical attention after infancy, hence the former term “late-onset” CAH. The associated alleles encode enzymes with residual activity of 20% to 50%. Thus, these individuals typically have normal basal cortisol and aldosterone production but mildly elevated levels of adrenal androgens; however, suboptimal cortisol levels after adrenocorticotropic hormone (ACTH) stimulation are reported in up to 30% of patients (37). Children may present with symptoms due to elevated adrenal androgens such as premature adrenarche, acne, and accelerated skeletal maturation but many, especially males, are asymptomatic. Adolescent girls or adult women may present with hirsutism, oligomenorrhea, acne, and subnormal fertility (37). Because NC CAH is not the primary target of neonatal screening and is rarely detected by that strategy, the true prevalence of this milder disorder is unclear. The estimated prevalence is ~ 1 in 200 in the Caucasian population (38).

Since the last publication summarizing CAH in Endocrine Reviews in 2000 (1), there have been numerous new developments. These include more detailed understanding of steroidogenic pathways, refinements in neonatal screening, improved diagnostic measurements utilizing high-throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled with steroid profiling, and improved genotyping methods. Clinical trials of alternative medications and modes of delivery have been recently completed or are under way, with the nearer prospect of genetic and cell-based treatments and a large body of data concerning long-term outcomes in patients affected by CAH, including psychosexual well-being, enhanced by the establishment of disease registries.

Much remains to be learned in several other domains spanning fetal life through adulthood. Both human and

animal studies have illuminated risks of antenatal dexamethasone (Dex) treatment. Noninvasive prenatal diagnosis of CAH in families with known *CYP21A2* pathogenic genotypes has been accomplished by analysis of circulating free fetal DNA in maternal blood in proof-of-concept studies, but is not yet widely available. Genital reconstructive surgery in affected females is no longer viewed as an emergency procedure, and indeed the practice of genital surgery in infancy has been questioned. Shared decision making among parents, patients, surgeons, endocrinologists, mental health providers, and support groups has been promoted as model for optimal care. Benefit-to-risk ratio for no surgery, or early or late genital surgery for females with CAH remains to be determined. Unfortunately, even in advanced societies, medical care for CAH is neglected, increasing the risk for cardiovascular or metabolic morbidities due to suboptimal corticosteroid therapy. Methods to improve transition of care from pediatric to adult healthcare, as well as patient and provider education, are important goals.

This multiauthored review is the result of a planned European CAH Symposium, which was postponed due to the Covid-19 pandemic. The large international group of authors contributed innovative approaches to understanding and managing this condition.

Basic Principles of Steroid Synthesis and Adrenal Enzymatic Defects

Physiology and Pathophysiology of Steroidogenesis

Steroidogenesis in the adrenal cortex takes place in 3 concentric zones: the outermost zona glomerulosa (mineralocorticoid biosynthesis), the zona fasciculata (glucocorticoid biosynthesis), and the innermost zona reticularis (sex steroid precursor biosynthesis). It entails conversion of cholesterol to active steroid hormones, and involves many enzymes, cofactors, and accessory proteins (Fig. 1). Most of these are expressed in the appropriate zones of the adrenal cortex, with others expressed in the gonads, placenta, and some “peripheral” tissues; these factors and the conditions caused by their mutations have been studied in detail (39). Mutations have been described in most of the genes encoding these proteins; those that disrupt cortisol synthesis with compensatory elevations in ACTH cause CAH, but in common parlance “CAH” refers to 21OHD. This section describes all enzymatic conversions required to synthesize cortisol.

Cholesterol side-chain cleavage

Steroidogenesis is initiated by the conversion of cholesterol to pregnenolone, catalyzed by the cholesterol

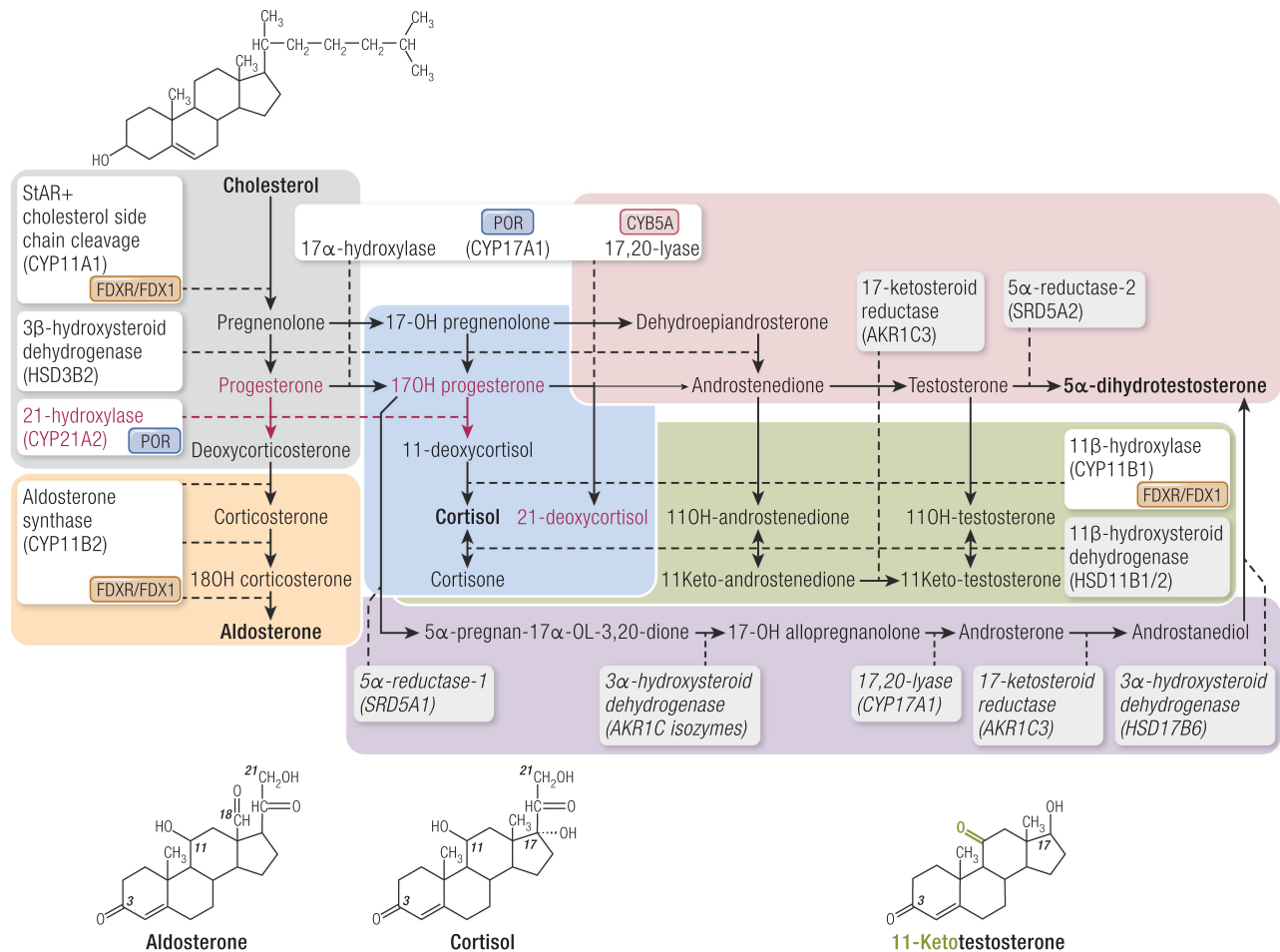


Figure 1. Adrenal steroidogenesis. Enzymes are boxed with dotted lines extending to arrows denoting each enzymatic conversion; 2 enzymes, CYP11B2 and CYP17, catalyze several successive enzymatic conversions. Accessory proteins required for activity of cytochrome P450 enzymes are shown next to each such enzyme: POR, P450 oxidoreductase, required by CYP enzymes in the endoplasmic reticulum; FDXR/FDX1, ferredoxin reductase and ferredoxin, required by mitochondrial CYP enzymes. Cytochrome B5 (CYP5A) is required for full 17,20-lyase activity of CYP17A1. There are 2 11β-hydroxysteroid dehydrogenase isozymes; HSD11B1, expressed mainly in the liver, catalyzes reduction (eg, cortisone to cortisol), whereas HSD11B2, expressed mainly in the kidney, catalyzes oxidation (eg, cortisol to cortisone). The steps affected by 21OHD, including steroids secreted in increased amounts in this disease, are denoted by red lines and red lettering. Steps taking place only in the adrenal glands are in unshaded boxes; steps taking place partly or predominantly outside the adrenal cortex are denoted by shaded boxes. Planar structures of cholesterol, aldosterone, cortisol, and testosterone are illustrated; the position of the 11-oxo (11-keto) group in 11-ketotestosterone is illustrated in green. Colored rectangles indicate the following: gray, early steps of steroidogenesis common to all zones of the cortex; orange, steps in the zona glomerulosa leading to aldosterone; blue, steps in the zona fasciculata leading to cortisol; magenta; steps in the zona reticularis and extra-adrenal tissues leading to androgens; purple, the “backdoor” or alternate pathway from 17-OH progesterone to dihydrotestosterone (for clarity, the alternative pathway from progesterone is not shown); green, conversions leading to 11-oxo androgens.

side-chain cleavage enzyme, CYP11A1 (P450_{scc}). To initiate steroidogenesis, cholesterol from cytoplasmic storage depots must reach CYP11A1 on the inner mitochondrial membrane; this cholesterol influx requires the steroidogenic acute regulatory protein (StAR), acting on the outer mitochondrial membrane (OMM) (40). The action of StAR requires its phosphorylation and interaction with some other proteins, but the exact mechanism of StAR's action remains under investigation (41, 42). Mutations in StAR cause another rare form of CAH, congenital lipoid adrenal hyperplasia, in which virtually no steroid hormones are made and 46,XY fetuses are phenotypically female due to impaired testicular

steroidogenesis (43, 44). CYP11A1 defects were once considered incompatible with term pregnancy; however, more than 30 cases of such defects have been reported (40). These 2 conditions are clinically and hormonally indistinguishable, but lipoid CAH is typically associated with very large adrenals, whereas CYP11A1 deficiency is not; gene sequencing is needed for definitive diagnosis. Milder “nonclassical” forms of these conditions have been reported with intermediate phenotypes (45-48). CYP11A1 is one of 7 human mitochondrial cytochrome P450 (CYP) enzymes, all of which require electron donation via ferredoxin and ferredoxin reductase (49). Mutations in ferredoxin have not been

reported, but several patients have been described with ferredoxin reductase mutations that disrupt synthesis of iron/sulfur centers, causing neuropathic deafness, optic atrophy, encephalopathy, and developmental delay (50–52); impaired steroidogenesis is to be expected but not yet reported.

3 β -Hydroxysteroid dehydrogenase

Once pregnenolone is produced, it may be converted to progesterone by 3 β -hydroxysteroid dehydrogenase (HSD3B, 3 β -HSD). There are 2 human *HSD3B* genes: *HSD3B1* encodes an isozyme found in the placenta, brain, liver, and elsewhere; *HSD3B2* encodes an isoenzyme found in the adrenals and gonads. Both of these isozymes can convert the Δ^5 -steroids (pregnenolone, 17-hydroxypregnenolone [17OHPreg], dehydroepiandrosterone [DHEA], and androstenediol) to the corresponding Δ^4 -steroids (progesterone, 17OH-progesterone [17OHP], androstenedione, testosterone) (53), but the placental/hepatic HSD3B1 has a low Michaelis–Menten constant (K_m), permitting it to act on low concentrations of steroids in the circulation (54), whereas the K_m for the adrenal/gonadal HSD3B2 is 10-fold higher (55), so it acts only on locally produced, intraglandular steroids. Mutations in *HSD3B2* cause a rare form of CAH, characterized by elevated ratios of Δ^5/Δ^4 steroids, notably 17OH-Preg/17OH-progesterone (17OHP), that are >8 SD above normal (56, 57). The low K_m of hepatic HSD3B1 permits it to convert some of the elevated 17OH-Preg to 17OHP, engendering false positives in newborn screening programs for 21OHD (58). HSD3B2 deficiency causes DSD in both sexes: genetic females are mildly virilized because some fetal adrenal DHEA is converted to testosterone by HSD3B1; genetic males synthesize some androgens by peripheral conversion of DHEA, but these are insufficient for complete male genital development (59).

17 α -Hydroxylase/17,20-lyase

Pregnenolone can also be converted to 17OH-Preg by 17 α -hydroxylase (CYP17A1, P450c17). CYP17A1 catalyzes both 17 α -hydroxylase and 17,20-lyase activities. The 17 α -hydroxylase activity converts pregnenolone to 17OHPreg and progesterone to 17OHP. The 17,20-lyase activity can convert 17OH-Preg to DHEA, but very little 17OHP is converted to androstenedione because the human enzyme catalyzes this reaction poorly (60, 61). The activities of CYP17A1 are expressed in a zone-specific fashion: the enzyme is absent in the adrenal zona glomerulosa, hence pregnenolone yields mineralocorticoids; only the 17 α -hydroxylase activity is found in the zona fasciculata, thus pregnenolone yields cortisol; both activities are present in the zona reticularis, hence pregnenolone yields 19-carbon (C19) precursors of sex steroids (Fig. 1). The principal

factor regulating 17,20-lyase activity is electron transport from NADPH via cytochrome P450 oxidoreductase (POR) with the assistance of cytochrome b_5 (b5). *CYP17A1* mutations causing 17-hydroxylase deficiency (17OHD) are rare except in Brazil and China. Lack of CYP17A1 prevents sex steroid biosynthesis, yielding a female phenotype in 46,XY males and sexual infantilism in both sexes; overproduction of 11-deoxycorticosterone (DOC) in the zona fasciculata typically causes mineralocorticoid hypertension; cortisol is not produced, but corticosterone substitutes for glucocorticoid requirements (62). Rare cases of apparently isolated 17,20-lyase deficiency may be attributable to mutations in CYP17A1, b5 (*CYB5* gene) or POR (63–65).

The enzymology of adrenal 21-hydroxylase (CYP21A2, P450c21, encoded by *CYP21A2* within the *HLA* locus), is discussed in section “Basic principles of steroid synthesis and adrenal enzymatic defects,” “Enzymology of CYP21A2.”

P450 oxidoreductase

All microsomal cytochrome P450 (CYP) enzymes, including CYP17A1, CYP21A2, CYP19A1 (aromatase, P450aro), as well as the drug-metabolizing CYP enzymes of the liver, require the activity of POR, a flavoprotein that transfers electrons from NADPH to all microsomal CYP enzymes (49). Mutations in POR cause POR deficiency; patients have been described with highly variable clinical and hormonal findings depending on the underlying mutations (66–72). Most POR mutations impair CYP17A1, especially 17,20-lyase activity (including the G539R POR variant with a phenotype simulating isolated 17,20 lyase deficiency) (63, 68, 73), with CYP21A2 and CYP19A1 being affected variably, depending on the POR mutation. It is difficult to reach definitive conclusions about phenotype–genotype correlations with such rare disorders, although there is a suggestion that compound heterozygotes carrying R457H in trans with null mutations tend to have a more severe phenotype (72). Findings range from severely affected infants with 46,XX and 46,XY disorders/differences of sex development (DSD), cortisol deficiency, and the Antley–Bixler skeletal malformation syndrome to mildly affected women who appear to have polycystic ovary syndrome, or mildly affected men with gonadal insufficiency. The skeletal phenotype probably results from diminished activity of CYP26B1, a POR-dependent enzyme that degrades retinoic acid (74). POR mutations also result in clinically relevant disruption of hepatic CYP enzyme activity (75). Patients with POR deficiency typically have normal electrolytes and mineralocorticoid function, nearly normal cortisol levels that respond poorly to ACTH stimulation, increased levels of 17OHP that respond variably to ACTH, and low levels of sex steroids. Impaired CYP21A2 activity may generate levels of 17OHP detected by newborn

screening for 21OHD (66, 76). Atypical genital development occurs in both sexes, with considerable variability. The 17,20-lyase activity of CYP17A1 is especially sensitive to disrupted electron transport (77), thus POR defects typically affect fetal testicular steroidogenesis. Virilization of 46,XX females has 2 causes. First, POR deficiency diverts steroids into the “backdoor pathway” of dihydrotestosterone biosynthesis (Fig. 1), contributing to the prenatal female virilization (69, 78-80). Second, as placental CYP19 (aromatase) requires POR, pregnant women carrying a fetus with the POR mutation R457H (but not POR A287P) may experience virilization during pregnancy (66-68), similarly to women carrying an aromatase-deficient fetus (81, 82). The POR polymorphism A503V, which mildly affects many P450 activities, is found commonly—from 19% among African Americans to 37% of Chinese Americans (83)—but does not affect the presentation of 21OHD (84).

11 β -Hydroxylase and aldosterone synthase

Steroid 11-hydroxylase (CYP11B1, P450c11 β) and aldosterone synthase (CYP11B2, P450c11AS, P450aldo) are closely related enzymes that catalyze the final steps in the synthesis of glucocorticoids and mineralocorticoids, respectively; they are encoded by duplicated genes (39, 85). Like CYP11A1, these are mitochondrial enzymes that require ferredoxin and ferredoxin reductase to receive electrons from NADPH. CYP11B1 is expressed abundantly in the zona fasciculata, where it converts 11-deoxycortisol to cortisol and DOC to corticosterone, and also in the zona reticularis, where it initiates the 11-oxo-pathway (see later) (86). CYP11B2 expression is less abundant and confined to the zona glomerulosa where it catalyzes the 11 β -hydroxylase, 18-hydroxylase, and 18-methyloxidase activities needed to convert DOC to aldosterone (87, 88). Mutations in *CYP11B1* cause 11 β -hydroxylase deficiency (11OHD), with deficient cortisol, increased adrenal sex steroids, female virilization, and increased DOC, causing mineralocorticoid hypertension; 17OHP may be elevated in the newborn, leading to misdiagnosis of 21OHD (85, 89). Mutations in *CYP11B2* selectively impair aldosterone synthesis, causing hyponatremia and hyperkalemia with normal cortisol production (39, 85). However, hyponatremia is typically less severe than in 21OHD because of continued DOC and cortisol secretion.

17 β -Hydroxysteroid dehydrogenases

The synthesis of sex steroids requires the action of 1 of the 17 β -hydroxysteroid dehydrogenases (17 β -HSD, HSD17B). These enzymes differ in their structures, co-factor requirements, reactions catalyzed, and tissue-specific expression (39). Several are important in steroidogenesis.

HSD17B1 is required for the synthesis of ovarian estradiol and placental estrogens (90-92). No genetic deficiency syndrome for HSD17B1 has been described. HSD17B2 inactivates estradiol to estrone and testosterone to androstenedione in the placenta, liver, small intestine, prostate, secretory endometrium, and ovary. Whereas HSD17B1 is found in placental syncytiotrophoblast cells, HSD17B2 is expressed in endothelial cells of placental intravillous vessels, consistent with a role in defending the fetal circulation from transplacental passage of maternal estrogens and androgens. No deficiency state for 17 β HSD2 has been reported. HSD17B3 is the testicular form of 17 β HSD that completes the synthesis of testosterone from androstenedione; its mutations cause a form of 46,XY DSD (93, 94). HSD17B5 (AKR1C3, an aldo-keto reductase enzyme), which is also a 3 α -hydroxysteroid dehydrogenase, reduces androstenedione to testosterone (95) in the ovary and several nonsteroidogenic tissues. AKR1C3 is expressed at low levels in the zona reticularis, accounting for the small amount of adrenally produced testosterone (96). HSD17B6, also known as RoDH for its homology to retinol dehydrogenases (97), is expressed at low levels in the fetal testes, where it appears to catalyze oxidative 3 α HSD activities in the alternative or “backdoor” pathway to 5 α -dihydrotestosterone (DHT) synthesis (79, 98)(see later).

Aromatase

Aromatase (CYP19A1) converts 19-carbon androgens to 18-carbon estrogens (99). Aromatase is abundantly expressed in the ovary and placenta and is slightly expressed in fat, but is only expressed in the adrenal in certain malignancies. Nevertheless, it is central to the pathophysiology of fetal development in CAH. The fetus with CAH is only virilized by its own adrenal androgens; even when maternal testosterone concentrations reach 300 ng/dL in a mother who herself has CAH, the female fetus is not virilized because placental aromatase inactivates the androgens from the maternal circulation (100).

Enzymology of CYP21A2

CYP21A2 (P450c21), like CYP17A1, is a microsomal or type II cytochrome P450, which catalyzes 2 essential reactions in adrenal steroidogenesis (39). The major substrate of CYP21A2 is 17OHP, which is converted to 11-deoxycortisol in the zona fasciculata during the biosynthesis of cortisol. In the zona glomerulosa, CYP21A2 21-hydroxylates progesterone to 11-deoxycorticosterone within the aldosterone pathway. Other hepatic cytochrome P450 enzymes have some 21-hydroxylase activity with progesterone as a substrate (101), but this activity does not rescue glucocorticoid deficiency in patients with classic CAH.

As with other microsomal P450s, CYP21A2 utilizes 2 electrons donated by POR to reduce molecular oxygen, producing a hydroxylated substrate and water. The enzymology of CYP21A2 is unusual for a cytochrome P450 in that the primary site of oxygenation is a methyl group, which is a kinetically disfavored site of hydrogen atom abstraction in the reaction cycle. The C-H bond breaking step is partially rate-limiting, and deuterium substitution at C-21 of progesterone shifts hydroxylation partially to the 16 α -hydrogen (102). The x-ray crystal structures of bovine (103) and human CYP21A2 (104) with 17OHP bound to the active site explain this activity profile. The steroid substrate is held perpendicular to the heme ring with the A-ring 3-keto oxygen hydrogen bonded to arginine-234 (R234) furthest from the reactive iron–oxygen complex, with C-21 dangling just close enough for the reaction to occur. On the side of the active site, valine-359 (V359) holds the steroid substrate with hydrophobic interactions in the geometry required for 21-hydroxylation and limits access of other reaction sites, principally the C-16 protons; mutagenesis of V359 to the smaller amino acids alanine and glycine progressively shifts progesterone hydroxylation to the 16 α -hydrogen (105). The crystal structures also contain a second molecule of steroid outside the active site where the F-G loop that forms the roof of the active site abuts the α -helical domain (103). Whether this second molecule reflects an intermediate state in substrate binding or simply a hydrophobic interaction that favors crystal formation is not known.

The common mutations that cause 21OHD have been compared with wild-type CYP21A2 as recombinant native enzymes in transfected mammalian cells (106), vaccinia-infected mammalian cells (107, 108) and yeast (109) or as purified proteins modified for expression in *Escherichia coli* and reconstitution in vitro (110). The catalytic activities of the mutants are reduced generally in proportion to the severity of the deficiency observed in patients with CAH. The studies of purified, reconstituted enzyme assays enable more detailed kinetic studies, which demonstrate that most mutations variably impair substrate binding, catalytic efficiency, and thermal stability in some combination. Extrapolation of these systems to the human adrenal in affected patients should be considered a good approximation but with limitations.

When using purified, reconstituted assay systems, investigators must add phospholipid and purified POR, in addition to the steroid substrate and NADPH. The phospholipid used does not exactly replicate the endoplasmic reticulum of adrenal cortex cells but does bring together CYP21A2 and POR in a proteoliposome to enable electron transfer and catalysis. The phospholipid composition is known to

influence the reconstituted activity of CYP17A1 and other steroidogenic P450 enzymes (111), although CYP21A2 has not been studied well in this regard.

New Pathways; New Steroids

The alternative or “backdoor” pathway to dihydrotestosterone

In addition to the classic pathway via DHEA, androstenedione, and testosterone, the most potent endogenous androgen, DHT, can also be synthesized via an alternative or “backdoor” pathway that bypasses the classical pathway intermediates (71, 79, 112–116). This alternative pathway is physiologically active during the major period of human sexual differentiation in the sixth to tenth week of human fetal development (79) and into the second trimester (117). To enter the alternative pathway to DHT, progesterone, or 17OHP are 5 α -reduced by steroid 5 α -reductase type 1 (SRD5A1) to yield 5 α -dihydroprogesterone and 17 α -hydroxydihydroprogesterone, respectively (for clarity, only the alternative pathway from 17OHP is shown in Fig. 1). These 3-ketosteroids are subsequently 3 α -reduced to allopregnanolone and 17 α -hydroxyallopregnanolone by isoforms of the AKR1C enzyme family. CYP17A1 converts allopregnanolone to 17 α -hydroxyallopregnanolone and then to androsterone by its 17,20-lyase activity, serving as its preferred substrate. Androsterone, which is also an inactive metabolite of androstenedione and testosterone, can then be activated to DHT by sequential 17 β -reduction and 3 α -oxidase reactions (118) (Fig. 1).

Because excessive 17OHP accumulation is a key characteristic of 21OHD, it is highly likely that the alternative pathway to DHT is a major contributor to fetal female virilization in 21OHD. Alternative pathway steroid metabolites can be detected in patients of all ages with 21OHD, most prominently in the neonate (119). These studies indicate that the high concentrations of 17OHP in individuals with 21OHD drive DHT production by the alternative pathway. The alternative pathway intermediate 17 α -hydroxydihydroprogesterone (also termed 5 α -17-hydroxypregnanolone) can be detected directly by urinary steroid profiling and indicates the activity of the alternative pathway (119, 120).

The role of 11-oxo-androgens in CAH

After cleavage of the side chain by 17,20-lyase activity of CYP17A1 in the zona reticularis, the major 19-carbon product of the human adrenal cortex is DHEA and its sulfate ester DHEAS. Whereas the latter is not a precursor to testosterone, DHEA is efficiently converted to androstenedione and also within the adrenal to lesser amounts of testosterone (121). Both androstenedione and testosterone are

good substrates for CYP11B1. Precursor steroids accumulate in the adrenals of patients with 21OHD, and CYP17A1 and CYP11B1 activities are high owing to chronic ACTH stimulation, hence the system can synthesize large quantities of 11OH-androstenedione, with concentrations exceeding that of androstenedione in both 21OHD patients and unaffected controls (122). 11-ketotestosterone (11KT) is primarily generated from circulating 11OH-androstenedione via the sequential action of 11 β -HSD type 2 (which converts the 11 β -hydroxyl to a keto group) and AKR1C3 (123). 11KT, which is in fact the major testicular androgen in teleost fishes (124), is nearly as potent as testosterone in transactivating the human androgen receptor (125). The intermediate 11-ketoandrostenedione—but not 11OH-androstenedione—is a much better substrate for AKR1C3 than androstenedione itself (126), which explains why 11KT is the second-most abundant circulating 11-oxo-androgen in both 21OHD patients and unaffected individuals. In addition, 11KT is a substrate for the 5 α -reductases (123), yielding 11-ketoDHT (11KDHT), which appears to be a more potent androgen than 11KT (reviewed in (86)), but is not detected in relevant concentrations in circulation.

In women with 21OHD, 11KT rises roughly proportionately to testosterone (122), reflecting the adrenal rather than gonadal origin of these androgens. Furthermore, 11-oxo-androgens are poor substrates for aromatase; whereas 11-oxo-androgens can be converted to 11-oxygenated estrogens, the latter do not contribute substantially to the circulating estrogen pool (127). In contrast, 11KT is inversely proportional to testosterone in men (122) and in boys Tanner stage 3 to 5 (128) with 21OHD. This is because men with poor disease control produce more 11OH-androstenedione, which is preferentially metabolized to 11KT, which then suppresses the hypothalamic–pituitary–testicular axis, thereby decreasing testicular secretion of testosterone. In men with good disease control, 11KT synthesis is low, whereas testicular testosterone synthesis is normal. Hence, a low 11KT/testosterone ratio in a man with 21OHD indicates both good disease control and good testicular function.

It is difficult to evaluate long-term disease control in adults with classic CAH. Assessing adrenal size, which might be the ultimate assessment, requires cross-sectional imaging with associated cost and radiation exposure. The 11-oxo-androgens (and 21-deoxycortisol), correlate better with adrenal size than traditional biomarkers of short-term disease control, such as androstenedione and 17OHP (128). Elevated 11-oxo-androgens are also predictive of menstrual irregularity in women and of TARTs in men with CAH (128). In contrast to DHEAS, androstenedione, and testosterone, 11KT does not decline with age in women from 20 to 80 years old, and 11KT declines very gradually

in men over the same age range (129). These data suggest that 11-oxo-androgens may be useful biomarkers of 21OHD control well into adulthood and in hypogonadal states. In patients with NC CAH, 11-oxo-androgens are elevated about 2-fold compared with women with clinical features of androgen excess, although 11-oxo-androgens alone cannot be used to establish the diagnosis of NC CAH (130). Finally, limited data suggest that 11-oxo-androgens are rather specific for 21OHD and are not elevated in other androgen excess forms of CAH such as 11 β -hydroxylase deficiency and 3 β HSD2 deficiency, because either CYP11B1 activity or intra-adrenal androstenedione production are low, respectively (86, 122).

In summary, androgens are generated in CAH patients via all 3 major pathways (131). First, classic pathway androgen synthesis is enhanced through increased conversion of accumulating 17OHP to androstenedione via the 17,20-lyase activity of CYP17A1, an ordinarily minor reaction compared with the preferred conversion of 17OH-Preg to DHEA (60). Second, the androstenedione so generated consequently drives increased substrate flow to the 11-oxoandrogen pathway, through conversion of androstenedione to 11 β -hydroxyandrostenedione. Third, while the alternative pathway to DHT contributes to excess androgen generation in 21OHD, its relative contribution appears to be more limited than that of classic and 11-oxo-pathways, as indicated by in vivo urinary steroid metabolite profiling in CAH patients during glucocorticoid therapy (132).

Biological activities of steroidal intermediates

Aside from defects in StAR and CYP11A1, in which essentially no steroids are secreted, a hallmark of inherited enzymatic defects in adrenal steroidogenesis is the accumulation of “upstream” steroids, proximal to the affected enzymatic step, which provide useful diagnostic markers. In 21OHD, 17OHP, the steroid before 21-hydroxylase, accumulates and is traditionally used to diagnose 21OHD (1, 133, 134). Besides 17OHP, several other “upstream” steroids such as pregnenolone, 17OH-Preg, and progesterone, and may also accumulate but are not diagnostically specific. In the absence of 21-hydroxylase activity, a substantial portion of 17OHP is converted into 21-deoxycortisol by CYP11B1 (Fig. 1). 21-deoxycortisol is a potentially useful marker for the diagnosis of 21OHD (135).

Some steroids that accumulate in 21OHD, including 21-deoxycortisol, progesterone and 17OHP, may also bind to glucocorticoid or mineralocorticoid receptors and act variously as either agonists or antagonists. In vitro, 21-deoxycortisol, corticosterone, 17OHP, and progesterone bind the glucocorticoid receptor with 24% to 43% of the affinity of cortisol. However, the transactivation activities of progesterone and 17OHP were only 0.2 to 0.8%

of that for cortisol, whereas the transactivation activity of 21-deoxycortisol was 8.5% and 17% in 2 different assays (136, 137). By contrast, 17OHP and progesterone inhibit aldosterone-mediated transactivation of the mineralocorticoid receptor in a dose-dependent fashion, explaining the strong antimineralocorticoid effect of 17OHP and progesterone in vitro (138, 139). Androstenedione and testosterone had no effect on mineralocorticoid receptor transactivation (139).

The clinical implications of these findings are not yet completely understood. Some adult classic CAH patients stop glucocorticoid medication without developing symptoms and signs of adrenal insufficiency (136, 140). Perhaps elevated levels of other steroids partially compensate for the low cortisol concentrations. Moreover, 21-hydroxylation of progesterone by hepatic cytochrome P450 enzymes other than CYP21A2 may permit some mineralocorticoid (11-deoxycorticosterone) synthesis (101). Clinical consequences of treatment lapses include androgen excess in women and

TARTs in men, adrenal hyperplasia, and/or tumors, as well as the theoretical risk of adrenal crisis in all patients.

Genetics in CAH

21OHD is caused by inactivating mutations in the gene coding for adrenal 21-hydroxylase (*CYP21A2*, older nomenclature *CYP21*, *CYP21B*, *P450c21B*; GeneID 1589).

The CYP21 Genes and the Surrounding Genetic Region

The *CYP21A2* gene encodes the microsomal P450 enzyme, 21-hydroxylase (*CYP21A2*, P450c21), a protein of 495 amino acids. *CYP21A2* is located in the Human Leukocyte Antigen (HLA) Class III region on the short arm of chromosome 6 β (6p21.3), approximately 30 kilobases apart from the nonfunctional *CYP21A1P* pseudogene (Fig. 2). *CYP21A2* and *CYP21A1P* both consist of 10 exons and share high

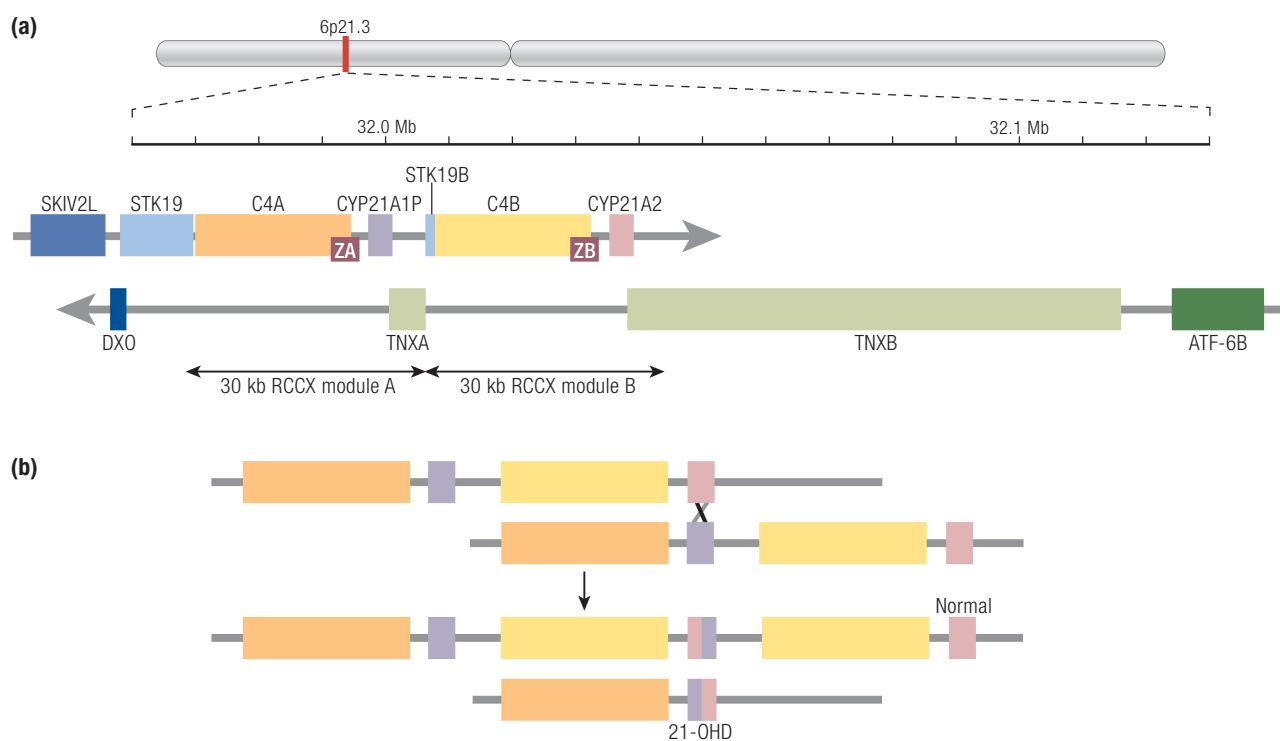


Figure 2. Genetics of the CYP21 genes. (A) the genetic region on chromosome 6p21.3, using data from the Human Genome database (<http://genome.ucsc.edu/>). The location of this region is indicated on a schematic of the entire chromosome. A scale is marked every 10 kb, with positions in the genome assembly numbered every 0.1 Mb. Genes transcribed in the telomeric-to- centromeric direction (left to right) are on the strand denoted by a right-facing arrow: *SKIV2L*, Ski2 like RNA helicase; *STK19*, serine/threonine kinase 19; *C4A*, complement component C4A; *CYP21A1P*, cytochrome P450 family 21 subfamily A member 1 (21-hydroxylase) pseudogene; *STK19B*, serine/threonine kinase 19 pseudogene; *C4B*, complement component C4B, *CYP21A2*, cytochrome P450 family 21 subfamily A member 2 (21-hydroxylase). Genes transcribed from the opposite strand (right to left in the figure) are immediately below: *ATF6B*, activating transcription factor 6 beta; *TNXB*, tenascin XB; *TNXA*, tenascin XA pseudogene; *DXO*, Decapping and exoribonuclease protein. ZA and ZB are adrenal-specific noncoding transcripts overlapping the C4 genes in the sense direction (141, 142); additional transcripts exist but are not shown. The 30 kb duplication of part of STK19, all of C4, all of CYP21, and part of TNX (a so-called RCCX module) is indicated. (B) An illustration of unequal meiotic crossing-over generating a deletion representing a salt-wasting 21-hydroxylase deficiency allele. The other chromosome has 3 copies of the RCCX tandem and is not associated with disease. The scale is expanded from Fig. 1A. For clarity, only the C4 and CYP21 genes are illustrated.

nucleotide homology of about 98% and 96% in exons and introns respectively (143, 144). *CYP21A1P* and *CYP21A2* are arranged in tandem with the *C4A* and *C4B* genes encoding the fourth complement factor (145). There are additional sense and antisense transcripts of unknown significance near or overlapping the *CYP21* genes (141, 142). The *C4/CYP21* unit is flanked by the serine-threonine kinase-19 (*STK19*, *RPI*) gene on the telomeric side and by the tenascin-X gene (*TNXB*, which encodes an extracellular matrix protein on the opposite DNA strand) (146) on the centromeric side, and their pseudogenes, *STK19B* and *TNXA*, forming a 30 kb tandem repeat sometimes referred to as an RCCX module (*RP-C4-CYP21-TNX*) (147). The *STK19*, *C4*, and *CYP21* genes are transcribed in the telomeric to centromeric direction, whereas *TNXB* is transcribed from the opposite strand. Most chromosomes have 2 copies of the module with a *CYP21A1P* pseudogene in the telomeric module and a *CYP21A2* gene in the centromeric module. However, this locus shows high structural variability with monomodular, trimodular, or even quadrimodular haplotypes detected (148, 149). The *TNXA* and *STK19B* pseudogenes were truncated during the duplication of the ancestral RCCX module. The last exons of *TNXA* and *TNXB* overlap the 3' untranslated regions of exon 10 of *CYP21A1P* and *CYP21A2*, respectively.

CYP21A1P is transcribed but its mRNA cannot encode a functional protein owing to at least 10 deleterious mutations (143, 144) including 2 frameshifts (8 bp deletion in exon 3, 1 bp insertion in exon 7, a nonsense mutation (p.Gln318stop; Q318X) (150), and a mutation in intron 2 that activates a cryptic splice site and causes an extra 19 nucleotides to be included in the mRNA (151). Missense mutations in the pseudogene include p.Pro30Leu (P30L) (107), p.Ile172Asn (I172N) (152), a cluster of missense mutations in exon 6, p.Ile236Asn, Val237Glu, Met239Lys (I236N, V237E, M239K), p.Val281Leu (V281L) (153), and p.Arg356Trp (R356W). Additionally, 4 single nucleotide differences in the 5' flanking region of *CYP21A1P* reduce its transcriptional activity to 20% of that of *CYP21A2* (see section "Genetics in CAH," "CYP21A2 gene expression," "Transcriptional control of *CYP21A1P* and *CYP21A2*") (141). Note that there is a polymorphism of no functional significance in the hydrophobic leader sequence at the amino terminus of *CYP21A2*, consisting of a single amino acid insertion. Consequently, some publications and databases list mutations with positions incremented by 1; eg, P31L instead of P30L.

CYP21A2 Gene Expression

Pattern of CYP21A2 expression

By immunohistochemistry, *CYP21A2* expression is first detected robustly at 50 to 52 days postconception within

the nascent inner fetal zone. Within the outer definitive zone, *CYP21A2* is more weakly detected and persists up to 14 weeks postconception. All other enzymes required for cortisol biosynthesis are present as well, and cortisol concentrations within the fetal adrenal are high during the first trimester (154). From 14 to 22 weeks, *CYP21A2* is expressed only in the fetal and transitional zones, but not the definitive zone, and cortisol secretion is relatively low; definitive zone expression is detected starting at 23 weeks and continuing through the remainder of gestation, as cortisol secretion increases (155). Cortisol secretion in the first trimester suppresses DHEA production and thus minimizes fetal androgen secretion until placental aromatase expression increases in the second trimester, by which time differentiation of the external genitalia is complete and cannot be affected by DHEA levels. Cortisol is again required in the third trimester to support lung maturation, including surfactant production (156). Low expression of *CYP21A2* during the second trimester partially explains the high incidence of false-positives in newborn screens for CAH in premature infants (see section "Diagnostics," "Neonatal screening") (157).

In normal adult adrenal glands, *CYP21A2* immunoreactivity is detected in all 3 cortical layers, particularly the zonae glomerulosa and reticularis, with variegated expression in the zona fasciculata. The immunoreactivity is more intense in adrenals from patients with Cushing disease and at sites of regeneration in normal adrenal glands (158).

In addition to the adrenal cortex, *CYP21A2* is detected in other tissues by RT-PCR. These include lymphocytes, which also express an additional 21-hydroxylase activity that is not mediated by *CYP21A2* (159). *CYP21A2* is expressed throughout the human heart at levels approximately 0.1% those in the adrenal cortex. Expression patterns of other steroidogenic enzymes suggest autocrine or paracrine roles for corticosterone and deoxycorticosterone, but not cortisol or aldosterone, in the normal adult human heart (160).

Regulation of CYP21A2 expression

Cortisol secretion is regulated mainly by ACTH, which acts via the $G\alpha$ -protein coupled MC2R receptor to increase activity of adenylyl cyclase and thus increase intracellular levels of cyclic adenosine monophosphate (cAMP). This in turn increases activity of protein kinase A. The main effect of corticotropin-releasing hormone (CRH) secreted by the hypothalamus is to increase ACTH secretion by the pituitary gland, but additionally, it may act directly on adrenocortical cells to increase cortisol secretion, and expression of *CYP21A2* and other steroidogenic enzymes (161). Infection, fever, and pyrogens stimulate the release of

interleukin (IL)-1 and IL-6, promoting secretion of CRH, and stimulate IL-2 and tumor necrosis factor promoting release of ACTH, increasing cortisol secretion during inflammation (162); IL-6 can also directly stimulate adrenal synthesis and release of cortisol (163).

In contrast, aldosterone secretion is regulated mainly by angiotensin II, which activates the Gq-protein coupled angiotensin 2 receptor (AT2R), which acts primarily through the protein kinase C pathway but also through Ca^{2+} signaling (164). Additionally, high extracellular potassium levels trigger voltage sensitive calcium channels that also increase intracellular calcium levels. Calcium then increases activity of protein kinase C (165).

Regulation of *CYP21A2* expression is consistent with these trophic stimuli. In the H295R human adrenocortical cell line (166, 167) and also in primary cultures of human adrenocortical cells (167, 168), mRNA, and/or protein expression of *CYP21A2* are induced by increases in cAMP analogs and by angiotensin II or tetradecanoyl phorbol acetate, which stimulate protein kinase C. Insulin and IGF-I are additional trophic stimuli (168). Additional hormone and environmental factors may regulate *CYP21A2* expression. These include orexins, which stimulate secretion of cortisol (169), and potential endocrine disruptors including brominated flame retardants (170, 171) and organic freshwater contaminants (172).

Transcriptional control of *CYP21A1P* and *CYP21A2*

The most important *CYP21A2* transcript begins 10 to 11 nucleotides before the initial AUG codon (143). *CYP21A1P* is also transcribed specifically in the intact adrenal cortex at a level 10% to 20% that of *CYP21A2* (141). However, the first 2 introns are inconsistently spliced out, and an uncertain proportion of transcripts include additional exons in the region between the end of *CYP21A1P* and the beginning of *C4B*. Some of these exons may overlap the truncated *TNXA* gene. Adrenal transcripts in the same direction as *CYP21A2* have also been detected overlapping *TNXB*; these are also of uncertain functional significance (173).

Similarly, *CYP21A1P* transcripts cannot be detected in primary cultures of human adrenocortical cells, whereas *CYP21A2* is appropriately expressed under the same conditions (168, 174). In cultured mouse Y-1 or human H295 adrenocortical cells, the 5' flanking region of human *CYP21A2* drives basal expression of reporter constructs at levels 2.5 to 8 times higher than the corresponding region of *CYP21A1P* (174-176). Sequences responsible for this difference have been localized to the first 176 nucleotides (174), although sequences upstream of this region are required for full expression. There are only 4 nucleotide differences (-126C>T, -113G>A, -110T>C, and -103A>G)

between *CYP21A1P* and *CYP21A2* in this region. The first 2 listed affect binding of the Sp1 transcription factor. In patients with 21OHD, gene conversions involving this region reduce but do not eliminate *CYP21A2* expression. In isolation with no additional mutations present, they can be associated with NC CAH (177). When the gene conversion extends to the P30L missense mutation (which is usually a Group C, ie, NC allele; see section "Genetics in CAH," "Genotype-phenotype correlation"), it becomes a SV (Group B) allele (178).

The most important transcription factor for adrenal-specific expression of *CYP21A2* is steroidogenic factor-1 (SF-1, Ad4BP, NR5A1). This protein is also required for development of the adrenal gland and gonads (179, 180). It interacts with specific DNA elements both within the proximal promoter and in intron 35 of the linked *C4B* gene (181).

Additional relevant transcription factors include nerve growth factor induced-B (NGFI-B, Nur77, NR4A1) (167, 182), and Nur-related factor 1 (NURR1) (NR4A2); they may overlap in their functions (183). NGFI-B is phosphorylated under basal conditions and dephosphorylated in response to ACTH, which activates it. Thus it may help to mediate ACTH regulation of *CYP21A2* expression. These transcription factors may also be important for mediating gene regulation by angiotensin II (165). A third closely related transcription factor, neuron-derived orphan receptor 1 (NOR1, NR4A3), is also expressed in the adrenal cortex and may function similarly (184).

Molecular Genetics of CAH

Over 90% of mutations causing 21OHD are the consequence of intergenic recombinations within the 30 kb tandem repeat (Fig. 2), promoted by the high recombination rate in the HLA region along with the nucleotide identity shared across the 30 kb repeat. These include both deletions generated by unequal meiotic crossing-over during gametogenesis, and gene conversions between *CYP21A2* and the *CYP21A1P*, generated by either meiotic or mitotic events (185). Unequal crossovers, owing to misalignment of the 30 kb tandem duplication, can occur with break points anywhere along the duplicated region. Breakpoints originating in *STK19* or *C4A* lead to a net deletion of 1 of the *C4* genes and *CYP21A1P* but leave *CYP21A2* unaffected. Such a configuration occurs on at least 5% of normal chromosomes (145). Breakpoints originating in *CYP21A1P* yield a deletion of *C4B* and a single chimeric *CYP21* gene that has a 5' end corresponding to *CYP21A1P* and a 3' end corresponding to *CYP21A2*. This chimeric gene usually (but not always (186)) includes *CYP21A1P* mutations that prevent synthesis of a

functional protein, so it represents a null allele and thus is usually referred to as a *CYP21A2* deletion. Occasionally a breakpoint occurs in the *TNX* genes, leading to complete deletion of *C4B* and *CYP21A2*, and a *TNXB/TNXA* chimeric gene (147, 148, 187). Homozygosity for such a chimera leads to a contiguous gene syndrome consisting of CAH and Ehlers–Danlos syndrome (188), which is rarely clinically reported in patients with severe CAH. However, 7% to 14% of patients with CAH have heterozygous *TNXB* mutations (189, 190). This extended phenotype has been termed the CAH-X syndrome. CAH-X is associated with joint hypermobility, chronic arthralgia, joint subluxations, hernias, and cardiac defects (191, 192). Deletions account for approximately 20% of mutant alleles in 21OHD (187, 193–211). *CYP21A2* gene duplications are relatively common in some populations (212, 213). Many of these alleles carrying a *CYP21A2* gene duplication have a p.Gln318X (Q318X) mutation in the duplicated *CYP21A2* gene next to the *TNXB* gene, and a wild-type *CYP21A2* gene next to the *TNXA* pseudogene. Importantly, such alleles are nondisease causing, but can be easily misinterpreted (212).

Approximately 70% to 75% of disease-causing *CYP21A2* mutations arise from the transfer of deleterious mutations from *CYP21A1P*, namely gene conversion (Fig. 3). In addition, over 200 pseudogene-independent mutations are listed in the Human Gene Mutation Database (HGMD, <http://www.hgmd.cf.ac.uk>) and the Pharmacogene Variation Consortium (<https://www.pharmvar.org/gene/CYP21A2>). Most of these rare mutations are sporadic. However, due to founder effects increased frequencies of some pseudogene-independent mutations are observed in some populations. Deletions, the splice site mutation in intron 2 (c.293-13A/C>G) and p.Ile172Asn (I172N) are the most common mutations in most populations (187, 193–211). The p.Val281Leu (V281L) mutation is by far the most common allele detected in patients with NC CAH and is highly prevalent in Ashkenazi Jews (153). Novel or rare mutations account for about 3% to 5% of detected mutations in large cohorts. The vast majority of these rare mutations have been identified in single families or small populations. Approximately 1% to 2% of *CYP21A2* disease causing mutations arise de novo.

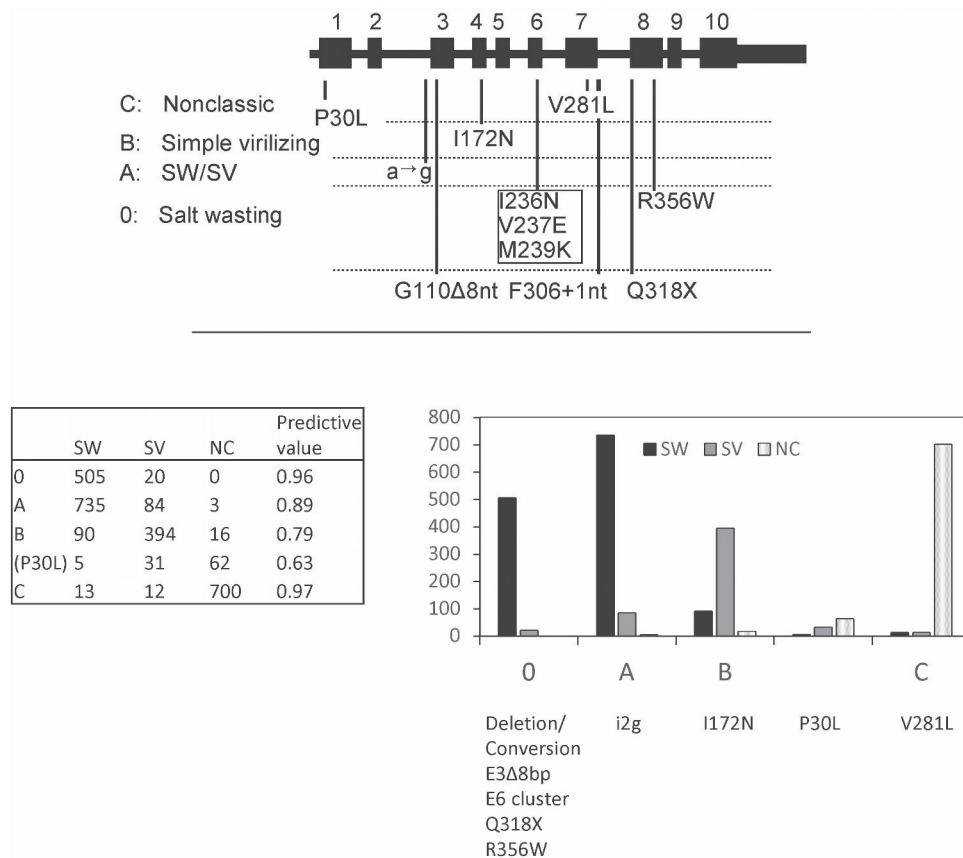


Figure 3. (A) Structure of the *CYP21* genes. Exons are numbered. Mutations affecting enzymatic function that are normally present in the *CYP21A1P* pseudogene are shown. They are positioned vertically to show the severity of CAH they cause when transferred to *CYP21A2* in gene conversion events. These are grouped into 4 mutation groups (O, A-C) and are associated with particular forms of CAH, as indicated. (B) Associations between mutation groups and forms of CAH. These are displayed in tabular form on the left and as histograms on the right.

Genotype–Phenotype Correlation

In descending order of compromised 21-hydroxylase activity, 4 general groups of *CYP21A2* mutations have been established to predict the phenotype (Fig. 3) (193, 194, 199, 201, 206, 211, 214). Deletions, large gene conversions, nonsense mutations, frameshifts, and missense mutations that totally abolish enzymatic activity are SW classic alleles, comprising mutation Group 0 (“null”). A single nucleotide mutation that alters splicing of intron 2 (c.293-13A/C>G, “intron 2 G” mutation) (151) is particularly common, comprising 20% to 25% of mutant alleles in most populations (Table 2). It has been seen in both SW and SV patients, suggesting that there is a small amount of normally spliced mRNA; it is generally considered its own separate Group A (in the first analysis of this sort (193), Groups 0 and A were referred to as Groups A1 and A2, respectively). A nonconservative amino acid substitution, p.Ile172Asn (I172N) (152) reduces enzymatic activity to <5% of normal and is associated with the SV form of the disorder (mutation Group B) (106, 108, 219). Finally, missense mutations such as p.Val281Leu (V281L) and p.Pro30Leu (P30L) (107) reduce enzyme activity to ~20% to 50% of normal (mutation Group C), and are associated with NC CAH. Although enzyme function in vitro appears to be similar (110), clinical observations suggest that patients carrying the P30L allele are somewhat more symptomatic, straddling the border between SV and NC forms of CAH (211, 220). As noted in section “Genetics in CAH,” “*CYP21A2* gene expression,” “Transcriptional control of *CYP21A1P* and *CYP21A2*,” in many cases this may be a consequence of gene conversions extending into the 5′ flanking transcriptional regulatory region, thus impairing gene expression (178).

CAH due to 21OHD is an autosomal recessive condition. About 65% to 75% of 21OHD patients are compound heterozygotes; ie, they carry different mutations on each allele. In cohort studies, the clinical phenotype of CAH strongly correlates with the less severely impaired *CYP21A2* allele (Fig. 3); 96% of individuals carrying 2 Group 0 alleles have SW CAH, whereas 97% of those with at least 1 Group C allele have NC CAH. The correlation is somewhat less strong for the groups with enzymatic impairment of intermediate severity (Groups A and B, and the P30L mutation). To some extent, this reflects the fact that the distinctions between SW and SV CAH, or SV and NC CAH, are a continuum and not absolute. For example, many SV CAH patients nevertheless require mineralocorticoid supplementation early in life and might be classified as SW, whereas the distinction between the SV and NC forms can be particularly challenging in males. Without exhaustive sequencing, it is difficult to rule out the existence of additional mutations in introns or flanking regions that might affect mRNA processing or gene expression; as noted in section “Genetics in CAH,” “*CYP21A2* gene expression,” “Transcriptional control of *CYP21A1P* and *CYP21A2*,” 1 transcriptional control region is several kilobases away from *CYP21A2*, in the *C4B* gene (181). Data correlating genotype with intermediate phenotypes are limited and often are not presented in a way that permits meta-analysis. In both American (193, 221) and German (199) data, median (interquartile range) Prader virilization scores (Fig. 4) for females in Groups 0, A, B, and C are 4 (3-5), 4 (3-5), 3 (2-4), and 0 (0-2) respectively (199). A similar correlation of severity with genotype is seen when evaluating genital appearance in adult women (222). No factors modulating androgen

Table 2. Allele frequencies in various regions

| | North America | South America | Europe | China | Total |
|---------------------|----------------------|---------------|----------------------|------------|-------|
| References | (193, 206, 211, 215) | (198, 216) | (195, 201, 210, 217) | (214, 218) | |
| Allele | | | | | |
| Deletion/conversion | 21.1% | 11.1% | 28.8% | 21.9% | 21.5% |
| P30L ^a | 2.4% | 1.0% | 1.2% | 1.1% | 1.8% |
| I2G | 23.1% | 20.6% | 26.7% | 33.8% | 25.3% |
| E3Δ8bp | 2.3% | 0.9% | 2.4% | 0.3% | 1.8% |
| I172N | 9.0% | 9.4% | 15.6% | 15.1% | 11.4% |
| E6 | 2.2% | 1.8% | 2.4% | 1.9% | 2.1% |
| V281L ^a | 22.2% | 24.5% | 6.2% | 1.4% | 15.7% |
| Q318X | 3.6% | 6.5% | 3.5% | 5.3% | 4.2% |
| R356W | 3.8% | 4.8% | 4.3% | 6.6% | 4.5% |
| Other | 10% | 10% | 8.9% | 12.4% | 10.2% |
| Alleles analyzed | 3527 | 1094 | 1338 | 1142 | 7101 |

Gene conversion mutations occur with similar frequencies in most populations (Table 2).

^aP30L and V281L are found mainly in patients with nonclassic CAH and therefore their allele frequencies depend on the proportions of nonclassic patients included in each study.

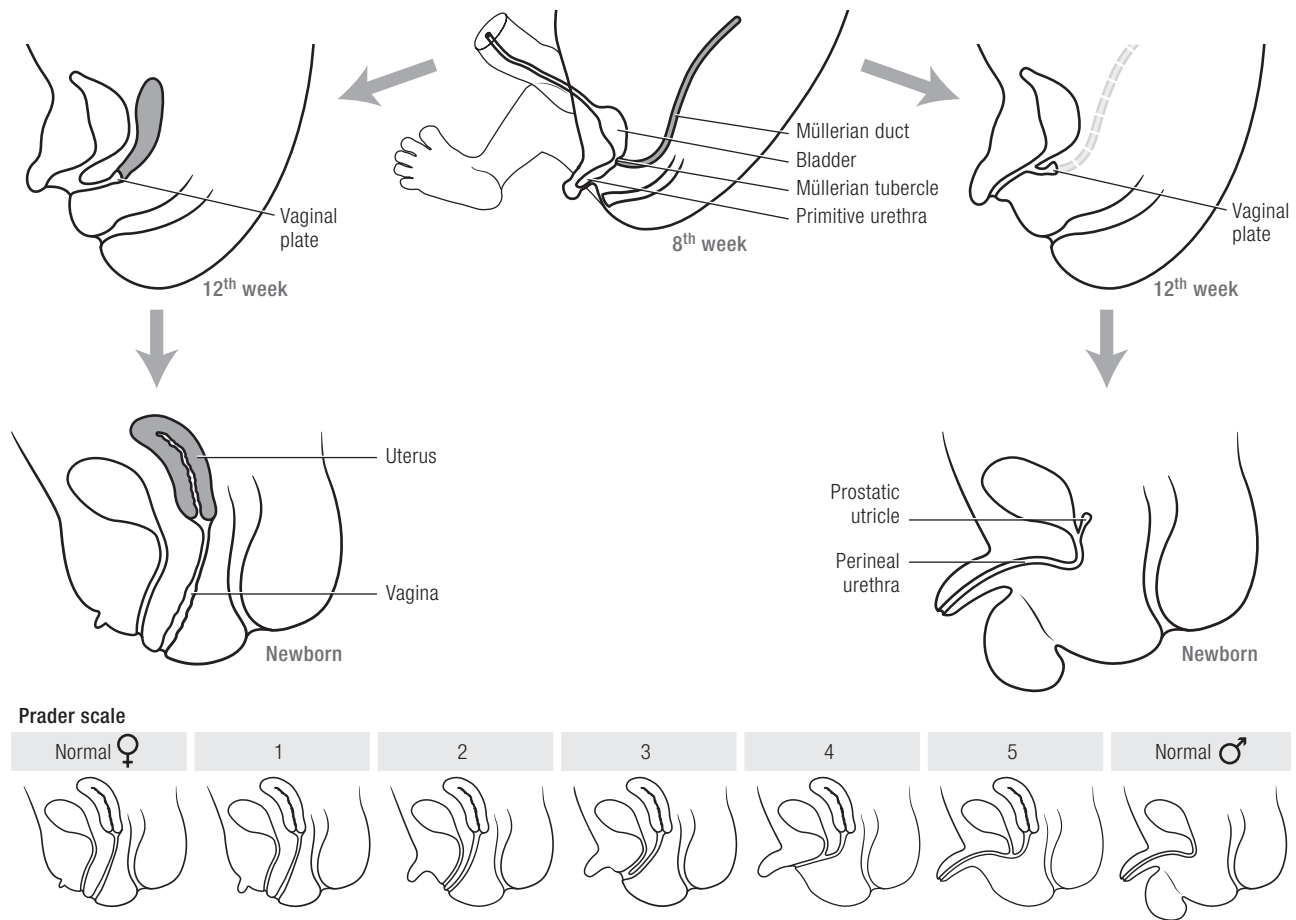


Figure 4. Genital development. Top, Differentiation of male and female reproductive systems are illustrated in schematic cross-section (not to scale). Bottom, the Prader scale of genital virilization.

effects have been demonstrated to influence the degree of virilization associated with each genotype group. Basal levels of 17OHP are also correlated with genotype (193, 198, 199, 223), with different studies reporting mean levels (in ng/dL) in Groups 0/A, B, and C of 23-41 000, 10-18 000, and 3-8000, respectively. However, there is substantial overlap in values between genotype groups. Adult height and mean hydrocortisone (HC) doses are also influenced by genotype (224). There are limited data directly correlating psychosexual functioning with genotype (222, 223), but gender dysphoria does tend to be most severe in women with SW CAH, which in turn is highly correlated with group 0 and A genotypes (225). Long-term health outcomes in adults do not correlate well with the genotype (210). However, girls and women with more severe *CYP21A2* genotypes appear to have an increased risk for psychiatric conditions (226) and variations of the complement component C4 may influence the risk of psychopathology (227). In summary, genotype-phenotype correlations are strong but not absolute, and clinical management should be based on clinical and hormonal data.

By analyzing the *CYP21A2* crystal structure, novel insights into the underlying molecular pathology have been gained (104, 228). Null and other severely deleterious mutations commonly disrupt heme and/or substrate binding domains, the anchoring of the protein to the membrane, or impair protein stability. Mutations categorized as group B partially impair membrane anchoring or affect conserved hydrophobic clusters within the protein. Milder mutations (group C) result in less severe alterations, often interfering with electron transfer from POR, salt bridge and hydrogen-bonding networks, and nonconserved hydrophobic clusters (104). However, other factors potentially influence enzymatic activity including mRNA expression, splicing and stability, and protein stability.

Diagnostics

Neonatal Screening

Benefits

Neonatal screening for classic CAH was introduced to prevent morbidity and mortality due to adrenal crisis. Currently, all 50 states in the United States, 35 other

countries, and portions of 17 additional countries screen for CAH (229, 230). Results of these screens indicate that the incidence of classic CAH in most populations is approximately 1:14 000 to 1:18 000. Table 1 summarizes data since 2008; data reviewed 1997-2004 are similar (231-233). Although newborn screening for CAH is now performed in an increasing number of countries, protocols and reported outcomes vary widely (234).

Screening markedly reduces the time to diagnosis of infants with classic CAH (89, 235-237), consequently reducing morbidity and mortality. Diagnosis is more likely to be delayed in males owing to the lack of genital ambiguity. Thus, a relative paucity of males in a patient population may be taken as indirect evidence of unreported deaths from SW crises. Females do outnumber males in some (12, 238, 239) although not all (240) retrospective studies in which CAH was clinically diagnosed without neonatal screening. Moreover, there is a greater preponderance of severe genotypes in screened infants than in those ascertained prior to screening, again suggesting extra deaths of severely affected infants prior to screening (2, 241). Nevertheless, infant deaths from CAH are now rare (0-4%) in advanced economies even without screening (242, 243).

Infants ascertained through screening have less severe hyponatremia and shorter hospitalizations (236, 240, 244, 245). The delay before correct sex assignment of severely virilized females is also markedly reduced (231). Moreover, males with SV CAH, and (mildly) virilized females, may otherwise not be diagnosed until later in childhood, at which time height may already be compromised. Although not an aim of screening, children with NC CAH are occasionally diagnosed. In some cases, the consequent close monitoring and, if necessary, treatment may improve adult height.

Initial screening methodology

First-tier screens for CAH due to 21OHD employ immunoassays to measure 17OHP in dried blood spots on the same filter paper (“Guthrie”) cards used for other newborn screening tests (236, 246, 247). Radioimmunoassay was the first method developed (248), but automated time-resolved dissociation-enhanced lanthanide fluoroimmunoassay (DELFA) has almost completely supplanted other immunoassays (249).

The main drawback to screening is that false positive rates are high, leading to substantial costs for evaluation and increasing parental concern. Several factors limit accuracy of these tests. First, premature, sick, or stressed infants tend to have higher levels of 17OHP than term infants; as studied by high-performance liquid chromatography, preterm infants have a functional deficiency of several adrenal steroidogenic enzymes with a nadir in function at 29

weeks of gestation (157). This “adrenal prematurity” can generate many false positives unless screening programs use higher screening cut-offs for preterm infants. For example, in 26 years of operation of the Swedish screening program, the positive predictive value for full-term infants was 25%, whereas it was only 1.4% for preterm infants, and it correlated very strongly with gestational age (250). There are no universally accepted standards for stratifying infants. Most laboratories use a series of birth weight-adjusted cut-offs (251, 252) but actual gestational age, or both, might be preferable, because gestational age correlates much better with 17OHP levels (27, 253).

Second, 17OHP levels are normally high after birth, decreasing rapidly during the first few postnatal days in healthy infants. In contrast, 17OHP levels increase with time in infants with 21OHD. Thus, diagnostic accuracy is poor in the first 2 days, and screening a second sample several days later improves both sensitivity and positive predictive value with the risk of delaying treatment (89, 247, 254). Moreover, a comparison of 1-screen vs 2-screen state programs found a higher incidence of 21OHD when a second screen was employed (255). It has been suggested that preterm infants should have additional samples rescreened at 2 and 4 weeks of age, most practical in a hospitalized population where potential SW can be monitored (252).

Multiple courses of antenatal corticosteroids might reduce 17OHP levels and thus potentially increase the likelihood of false negative screens. Studies have reported inconsistent effects of antenatal corticosteroid administration in practice (256, 257). Testing of later samples should minimize this problem, but the delay may increase the risk of SW crisis.

Female infants have lower mean circulating 17OHP levels than males, slightly reducing screening sensitivity (258). Because almost all females with SW CAH are virilized, most of them are diagnosed based on clinical symptoms, and therefore the reduced sensitivity is not usually problematic. However, even severely virilized girls can be missed as virilization is not always noticed at physical examination (2, 22, 259, 260). Finally, immunoassays may lack specificity; this is discussed in section “Diagnostics,” “Biochemical evaluation.”

Second-tier screening

Because 21OHD is a rare disease, the positive predictive value of neonatal screening is low, even though the specificity and sensitivity of the tests are very high (230). The positive predictive value might be improved with a second-tier screen.

Biochemical second screens. Direct biochemical analysis of steroids in blood samples using LC-MS/MS can obviate

the specificity problems of immunoassays (261-263) and both heel stick blood samples (264) and urine samples (265, 266) can also be analyzed by mass spectrometric methods. Measuring 21-deoxycortisol instead of 17OHP may improve diagnostic accuracy (135). Measuring steroid ratios further improves the screening specificity of LC-MS/MS. Such ratios have included (17OHP+androstenedione)/cortisol (261, 267, 268), 17OHP/11-deoxycortisol (269), and (17OHP+21-deoxycortisol)/cortisol (263). Some (270, 271) but not all (252) laboratories have reported markedly superior results with these approaches, with 1 recent report claiming a positive predictive value of 71% (268). A recent statistical approach using principal component analysis of 6 steroid levels (17OHP, both first and second tier, 11-deoxycortisol, androstenedione, 21-deoxycortisol, and cortisol) achieved a positive predictive value of 67% (272). Consistency of results might be improved by mandating participation in national proficiency testing programs (273). However, caution should be exercised in developing reference ranges for assays using dried blood spots that have been stored for prolonged periods at room temperature, because cortisol and 11-deoxycortisol are not stable past 4 weeks of such storage (274).

Molecular genetic second screens. *CYP21A2* mutations can be detected in DNA extracted from the same dried blood spots used for hormonal screening (see section "Diagnostics," "Neonatal screening," "Initial screening methodology"). Because >90% of mutant alleles carry 1 or more of a discrete number of mutations, we can assume with >99% confidence that samples that carry none of these mutations are unaffected. Several studies of genotyping of samples from screening programs have suggested that this is a potentially useful adjunct to hormonal measurements (275-280), but there has been no large-scale study of its efficacy as a second-tier screen in actual use.

Biochemical Evaluation

Determining levels of steroid hormones and their precursors is a mainstay of diagnosis and management of CAH. Currently, the determination of steroid hormones rests on analytical techniques either based on the principle of immunoassay or on chromatographic methods coupled to mass spectrometry (281).

The specificity of the antibody is crucial for the reliability of an immunoassay. Inefficient discrimination between the analyte and structurally closely related substances will lead to cross reactivity with consequent overestimation of the amount of analyte. The overestimation of 17OHP in serum or plasma of premature infants, neonates, or young infants by immunoassay techniques used in screening procedures or clinical routine with the consecutive risk of overdiagnosing

21OHD, presents a typical and important example of this phenomenon (282). Crossreactivity has been documented with 17-OH pregnenolone sulfate, a steroid originating in extremely high amounts from the fetal zone of the fetal adrenal (283), and 15 β -hydroxylated compounds apparently generated by gut bacteria and resorbed through the entero-hepatic circulation (284). There may be additional substances in dried blood spots that interfere with immunoassays (matrix effect) (285). The DELFIA was reformulated in late 2009 to make it less sensitive to crossreacting compounds in premature infants (286). This modification improved the positive predictive value from 0.4% to 3.7% for the first screen (247). The specificity of immunoassays may be further improved with organic extraction to remove crossreacting substances, such as steroid sulfates. Additional preparative steps such as extraction, chromatographic prepurification, or dilution can help to circumvent matrix effects.

Currently, MS represents the most versatile and exact of all analytical techniques for steroids. Initial separation by LC or gas chromatography (GC) can consistently improve specificity, and it also permits multicomponent analysis, namely the simultaneous determination of multiple analytes in a single run. This development laid the foundation for the field of metabolomics, which presents the unbiased and systematic study of small molecules present in a biological sample. If mass spectrometry records all ions of a particular mass range, this is called an "untargeted" mode. If operated in "targeted" mode, mass spectrometry only records preselected ions (281).

Of all separation techniques, GC provides the best resolution of steroids. In combination with MS as the detection method, GC-MS presents a robust analytical tool, unsurpassed in determining simultaneously a multitude of steroids including precursors or metabolites of progestins, glucocorticoids, mineralocorticoids (all C₂₁-steroids), androgens (C₁₉-steroids), and estrogens (C₁₈-steroids) (287, 288). GC-MS is particularly useful for urinary steroid metabolome analysis, but it can also be applied to the analysis of blood or tissues (289) or be used as a gold standard in quality assurance (290). As over two-thirds of steroid hormones and their metabolites are excreted into urine, the measurement of these urinary steroids provides an integrated picture of a patient's steroid hormonal status (steroidal fingerprint) and has enormous diagnostic power. Adrenal enzyme defects show unique metabolic patterns (disease signatures, metabotypes) (291). Usually, a spot urine sample is sufficient for diagnosing an adrenal enzyme defect (266, 292, 293). Timed samples (eg, 24-hr urine) provide additional information on hormonal production rates via determination of steroid excretion rates (294, 295). This information aids the diagnosis of hormonal

overproduction syndromes, such as Cushing syndrome or tumors, as well as in assessing compliance with hormonal therapy in CAH (296, 297). Moreover, this approach has been used to dissect the contribution of the 3 androgen biosynthesis pathways discussed in section “Basic principles of steroid synthesis and adrenal enzymatic defects,” “New pathways; new steroids” (132, 298, 299). Unbiased systems biology approaches allow for clustering and describing various metabotypes (300), reclassifying hitherto uncharacterized conditions (301) or improving metabolic monitoring of 21OHD (302, 303).

LC-MS is a more recent technique than GC-MS (301). MS/MS provides an extra level of filtering, thus improving the relatively poor separation properties of LC. Simple work up procedures and short run times permit much greater throughput than with GC-MS (281). Currently, determination of most clinically relevant steroid hormones in plasma or serum can be carried out by LC-MS/MS. It is the technique of choice for determining conjugated steroids (304). However, factors such as relatively low chromatographic resolution and lower ionization fraction, compared with electron impact in GC-MS, can impair the specificity of LC-MS/MS. Thus, GC-MS and LC-MS/MS should be considered complementary techniques.

Whatever analytical method is used, thorough method validation is a sine qua non. Important aspects of method validation comprise assessment of sensitivity, precision, reproducibility, accuracy, limits of quantification and detection, recovery, stability, carryover, and matrix effects. Recommendations have been published for the hormonal diagnosis of steroid related disorders (305).

Molecular Genetic Testing for *CYP21A2* Gene Mutations

Southern blot analysis was originally the gold standard for the detection of *CYP21A2* gene deletions but is no longer used clinically because it requires relatively large amounts of high-quality DNA, is labor intensive, and time consuming. Moreover, *CYP21A1P* duplications and certain other rearrangements at this locus may impede the detection of *CYP21A2* gene deletions or duplications (306). The most widely used current approach for gene dosage determination is multiplex ligation-dependent probe amplification (MLPA). MLPA requires only small amounts of DNA for detection of gene deletions, rearrangements, and fusion genes (210, 307-309). However, complex rearrangements can lead to challenges interpreting the correct genotype. The design of *CYP21A2*-specific primers for PCR-based amplification is crucial to avoid amplification of the pseudogene and allele dropout by nonamplifying PCR fragments. This represents a challenge due to the

high number of polymorphisms within *CYP21A2* and the high sequence identity with its *CYP21A1* pseudo-gene. A variety of targeted molecular genetic strategies for detecting the common mutations have been published and are established in diagnostic laboratories. However, direct sequencing of the amplified PCR products combined with a method for the detection of gene deletions and chimeric genes are the only available strategies that allow for the detection of close to 100% of *CYP21A2* mutated alleles. If possible, carrier testing should be performed in the parents to set phase (ie, confirm the parental origin of each mutation), which is required to determine compound heterozygosity, distinguish hemizyosity from homozygosity in the index case, and estimate the recurrence risk.

Prenatal Diagnosis

Prenatal diagnosis can be performed when both parents are carriers of *CYP21A2* mutations; most often this situation arises when they have a previous child with 21OHD. The possible methods for prenatal diagnosis have increased over the past decades. However, methods involving invasive sampling should only be performed if the results will lead to changes in approach or treatment (310).

Analysis of fetal hormones in amniotic fluid was the initial method available for prenatal diagnosis (311-313). Fetal cells obtained this way were originally used for HLA typing to determine the inheritance of maternal and paternal haplotypes (*CYP21A2* is located in the *HLA* complex) (314) but can also be used for genetic analysis, although cells must first be cultured, a time-consuming process.

Chorionic villus sampling to obtain fetal DNA can be performed as early as gestational week 10-11, compared with week 12-14 for amniocentesis (315). This method is available in many countries and centers today. Both amniocentesis and chorionic villus sampling are associated with a small but increased risk of fetal loss (316).

Noninvasive methods

Cell-free fetal DNA can be isolated from maternal plasma (317). Unlike fetal cells, it disappears from the maternal circulation shortly after delivery (317, 318) and therefore does not confound prenatal genetic investigations in subsequent pregnancies (319, 320). Prenatal sex typing (SRY detection) can be performed using PCR amplification of cell-free fetal DNA as early as week 6-9 (321) and may be useful in decisions regarding prenatal treatment with Dex to minimize treatment of male fetuses (see section “Management,” “Prenatal treatment”) (322). Next generation sequencing of cell-free fetal DNA can ascertain mutations, but it is challenging to detect *CYP21A2* mutations in

this manner because the vast majority of such mutations are already present in the *CYP21A1P* pseudogene. Instead, targeted massive parallel sequencing of cell-free fetal DNA in maternal plasma can identify SNPs flanking *CYP21A2* that are specific for the mother, father, and proband (previous child), thus constructing haplotype blocks to determine the maternal and paternal alleles inherited by the fetus (323). The technique is promising but costly; it requires specific resources and personnel and is not yet available as part of routine clinical care.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is available in many countries for families at risk of having a child with a severe genetic condition including 21OHD. PGD requires an in vitro fertilization approach and enables implantation only of embryos without the specific genetic disorder. It may present ethical challenges beyond the scope of this review (324). The preferred approach to obtain DNA for PGD is a biopsy at day 5-6 from the trophoctoderm of the blastocyst when it comprises about 120 cells. The inner cell mass that will develop into the fetus, from which 5 to 10 cells are required, can then be separated from the trophoctoderm (310). If the first polar body of the oocyte is used in PGD, the procedure is performed before fertilization occurs, and the analysis offers the unique possibility of preconceptional diagnosis. The disadvantage for autosomal recessive disorders is that only the oocyte is assessed and the paternal allele is not included in the assessment. The rate of fetal anomalies is not increased with PGD, rather it is thought that if damage occurs during the procedure it is lethal to the embryo (325).

Management

Hormonal Treatment of Classic CAH

Treatment of classic CAH is intended to replace both glucocorticoid and if necessary, mineralocorticoid hormones to prevent adrenal and SW crisis and to reduce excessive corticotropin driving adrenal androgen secretion (133). Clinical goals are normal growth and development and pubertal maturation from birth to adolescence, and prevention of adrenal crisis, virilization, and other long-term complications discussed below (133, 326, 327). The levels of evidence for management guidelines are detailed in The Endocrine Society's Clinical Practice Guideline published in 2018 (133) and are not repeated here. There are no large-scale prospective randomized trials for any therapies discussed here. As such the evidence is generally of low or moderate quality and rests to some extent on expert opinion, values, and preferences. Glucocorticoid

replacement in CAH faces 3 particular challenges. First, it aims to replace cortisol, but current treatment strategies cannot completely mimic the circadian rhythm of cortisol with a typical early morning rise of cortisol leading to a peak concentration at 6 to 8 AM and a nadir at midnight (328). Second, it aims to mimic the adaptation to stress (329). These distinctive features of physiological cortisol biosynthesis could only be closely mimicked by an infusion pump (330, 331) which, however, is neither practical nor cost-effective and thus not routinely available. Third, it aims to restore negative feedback on pituitary ACTH drive thereby controlling adrenal androgen excess (332). Normalizing ACTH levels in CAH patients requires supraphysiological glucocorticoid doses compared with the mere replacement doses of cortisol required in other forms of adrenal insufficiency. Treatment in classic CAH therefore constantly struggles to prevent overtreatment with multiple adverse side effects on growth and on metabolic, cardiovascular, and bone health, or undertreatment, which carries risks life-threatening adrenal crises and virilization or, in children, accelerated skeletal maturation with consequently reduced adult height. Both over- and undertreatment also affect reproductive function in both sexes.

Treatment in the neonatal period and early infancy

In growing children with classic CAH, the preferred glucocorticoid is the synthetic form of cortisol, HC, because its shorter half-life minimizes the adverse side effects typical of longer-acting, more potent glucocorticoids, especially growth suppression (133). As cortisone must be converted to cortisol for bioactivity (333, 334) and differences in conversion rates may influence drug effectiveness, cortisone acetate is not recommended (335, 336). HC should be given in 3 to 4 divided doses totaling 10 to 15 mg/m² daily (Table 3), a supraphysiological dose under which many patients show satisfactory control of adrenal androgen production. Although there are data to suggest that 4 daily doses are preferable (337), this may not be practical for many patients or their families. Data remain inconclusive regarding morning vs evening dose weighting (338, 339). The total dosage should be individualized based on adequate monitoring (see below) and may need to be increased for short periods in certain circumstances; such increased needs are described below. Therefore, all children with CAH should be under the care of a pediatric endocrinologist (340).

In the neonatal period, some clinicians exceed the recommended glucocorticoid dose in order to reduce elevated androgen levels as quickly as possible. If this treatment strategy is adopted, more frequent monitoring is necessary to rapidly reduce the dose when target levels of monitored steroids are achieved, to avoid adverse effects of high doses of glucocorticoids (341). After a few months,

Table 3. Maintenance therapy in patients with CAH classic CAH

| Drug | Recommended total daily dose | Divided dosing frequency (times daily) |
|---|----------------------------------|---|
| Children | | |
| Hydrocortisone | 10-15 mg/m ² | 3-4 |
| Fludrocortisone | 0.05-0.2 mg | 1-2 |
| Sodium chloride supplements | 1-2 g (17-24 mEq/day) in infancy | Several |
| Adults | | |
| Glucocorticoids | | |
| Hydrocortisone | 15-25 mg | 2-3 |
| Prednisone | 5-7.5 mg | 2 |
| Prednisolone | 4-6 mg | 2 |
| Methylprednisolone | 4-6 mg | 2 |
| Modified-release hydrocortisone (Plenadren®) | 15-25 mg | No published data in CAH patients, clinical experience shows that in addition to the morning dose a second GC dose is required in the evening |
| Modified- and delayed-release hydrocortisone (Chronocort®) ^a | 15-25 mg | 2 (2/3 of dose at 2300 and 1/3 of dose at 0700) ^a |
| Dexamethasone ^b | 0.25-0.5 mg | 1 |
| Fludrocortisone | 0.05-0.2 mg | 1 |

^aNot yet launched, currently only available within the extension phase of the phase III study.

^bAvoid if possible or limit to a short time. Adapted from Speiser et al. (133).

maintenance daily totals of about 3 to 4 mg HC divided in 3 doses (ie, 1-2 mg 3 times daily) are usually sufficient. Infants have low sensitivity to androgens, and completely suppressed adrenal androgens should not be the main goal in the first year of life. Commercial HC tablets, which may be extemporaneously crushed and mixed into food or suspended, are preferred as there have been reports of variable dose accuracies in compounded preparations (342, 343). A suspension would be preferable for small children, but the commercial suspension was withdrawn 20 years ago owing to unreliable therapeutic effects, although in some countries reliable solutions are now available (344). An immediate-release granule formulation of HC (Infacort®/Alkindi®, Diurnal Ltd) available in 0.5, 1, 2, and 5 mg preparations has been approved for use in the EU and the United States (345, 346).

Mineralocorticoid replacement is achieved with fludrocortisone. Monitoring is discussed in section “Management,” “Hormonal treatment of classic CAH,” “Monitoring.” Subclinical or overt aldosterone deficiency is present in all forms of classic CAH (347, 348). Therefore, fludrocortisone is given to all newborns with classic CAH detected in neonatal screening programs even before hyponatremia develops. Due to relative mineralocorticoid resistance and the antimineralocorticoid effects of elevated 17OHP in this period, neonates and young infants require higher fludrocortisone doses than older children, typically 100 to 200 µg/day but occasionally more, divided in 1 or 2 oral doses. However, this treatment requires frequent monitoring of electrolytes, plasma renin, and blood pressure and

tapering the fludrocortisone dose in order to avoid iatrogenic hypertension. Because of a lower glomerular filtration rate, immature renal tubules, and the low sodium concentration in breast milk and infant formula, infants often require additional supplemental sodium chloride to maintain sodium balance. The recommended dosage is approximately 1 to 2 g (4-8 mEq/kg/day) of NaCl given in divided doses ideally using a standard saline solution (349) or crushed, aliquoted sodium chloride tablets. In patients receiving high doses of fludrocortisone, NaCl supplementation may not be needed (350). Moreover, 0.1 mg of fludrocortisone has the glucocorticoid potency of ~1 mg of HC, so high fludrocortisone doses may permit (or require) a reduction of the HC dose in young children.

Treatment during childhood

Children younger than 18 months should be monitored at least every 3 months, while older children should be monitored every 4 to 6 months or more frequently after a change in dosing. The suggested target 17OHP range is 100 to 1200 ng/dL (3-36 nmol/L) when measured in the early morning before medication (335, 336), and/or age-appropriate androstenedione levels. Attempts to normalize 17OHP levels should be avoided because of the risk of HC overdosing causing iatrogenic Cushing syndrome. Because prepubertal children normally have low circulating sex steroid levels, adequate androgen suppression is important to achieve normal growth and puberty. Table 3 provides suggested dosing guidelines (133). HC dosing requirements may vary and depend on differences in HC absorption

and half-life (351). Long-acting glucocorticoids should be avoided in growing children except for short intervals when necessary to restore hormonal control, or if HC is unavailable (Table 4). If used, care must be taken to avoid overdosing, which will suppress growth (352-354), and the dose should be decreased as quickly as possible once hormonal control is achieved.

Aldosterone deficiency is described in up to 75% to 90% of all classic CAH patients, now viewed as a continuum of phenotypes rather than strict divisions between SV and SW disease. In classic CAH after infancy, fludrocortisone is usually given in doses ranging between 50 and 200 µg. Fludrocortisone has a biological half-life of approximately 18 to 36 hours. Therefore, low doses can be administered once a day, although doses above 0.1 mg may still be divided to be given twice daily. In hot and humid weather conditions, some endocrinologists suggest a seasonal increase in fludrocortisone, although increased salt intake may suffice. In contrast to glucocorticoid treatment fludrocortisone does not need to be increased during illness (section “Management,” “Hormonal treatment of classic CAH,” “Management of adrenal emergency in CAH”).

Treatment during puberty and adolescence

Puberty is often associated with difficult hormonal control, even if the replacement dose seems adequate and there is good adherence to the medication regimen. During puberty, the pharmacokinetics of HC may be altered by increased clearance due to decreased activity of 11β-HSD1. Therefore, higher glucocorticoid doses are necessary during puberty (355). However, as adult height of patients with CAH correlates negatively with the glucocorticoid dose administered in early puberty (354), HC doses exceeding 17 mg/m² per day should be used with care. Treatment should be continued with the lowest effective dose to achieve treatment goals, prioritizing height attainment over arbitrary steroid measurements. At or near the completion

of growth, long-acting glucocorticoids might be considered but are not preferred.

Management of CAH during adolescence and the transition from pediatric to adult healthcare is challenging (356). CAH patients may have poorer health, beginning in adolescence and persisting into adulthood, highlighting the importance of this period in patient care (357). Multidisciplinary transition clinics involving pediatric and adult endocrinologists, gynecologists, urologists, and psychologists may promote good medical adherence among adults with CAH (133). Uninterrupted glucocorticoid and mineralocorticoid administration at the transition from adolescence to adulthood is required to prevent increased morbidity and mortality, particularly from adrenal crises. Treatment regimens should be reassessed and adapted to the recommendations for adult CAH patients. Importantly, mineralocorticoid requirements, which change from birth to adolescence, should be reassessed in adolescence/young adulthood to avoid mineralocorticoid over- and under-replacement (358). Transition, however, is more than just prescribing steroid replacement for primary adrenal insufficiency and must address sex- and gender-specific issues (359, 360). In females, obesity and hyperandrogenism are common problems leading to menstrual irregularities and hirsutism (357). Gynecologic evaluation should be offered to all adolescents with CAH at transition, particularly in cases of blocked menstrual flow, planned penetrative vaginal intercourse, or desired pregnancy (133, 361). Boys should have a testicular ultrasound upon completion of puberty and regular examination for TARTs (358). All patients should be aware of the risk of reduced fertility with poor medical adherence (133). Psychosexual and genetic counseling of the adolescent patient are strongly recommended during transition (362).

Treatment of adults

Treatment of adults with classic CAH aims to replace the missing cortisol and aldosterone, and ameliorate adrenal

Table 4. Indications for different glucocorticoid (GC) preparations

| Steroid | Clinical indication | Pros | Cons |
|------------------------------|---|---|---|
| Hydrocortisone | Preferred option for GC replacement. | Best long-term outcome with regard to metabolic, cardiovascular, and bone health | Short half-life. Needs to be given 3 times daily. Adrenal androgen suppression overnight may escape. |
| Prednisolone (Prednisone) | Might be a preferred option for regulation of menstrual cycles or fertility induction, or if patient adherence is poor with thrice daily hydrocortisone | Longer half-life, twice daily regimen. Potentially better patient adherence compared with 3 times daily regimen | Potential higher rate of adverse effects on metabolic, cardiovascular, and bone health compared with hydrocortisone |
| Dexamethasone | Fertility induction TART treatment | Strong adrenal suppressive effect, longest half-life, once daily regimen often possible | Highest rate of adverse effects on metabolism, bone health. Traverses placenta barrier |

androgen excess (332). Optimal hormone replacement theoretically should enable normal quality of life and life expectancy. However, this aim is not always achieved, and adults with classic CAH suffer from multiple disease-associated comorbidities (363-365), reduced health-related quality of life (358), and increased mortality (366).

The decision of which preparation to use for glucocorticoid treatment in adults with classic CAH is based on the clinical experience of the individual physician and on the needs of each patient (Table 4). In general, the lowest possible doses should be prescribed that minimize risk of adrenal crises and control androgen excess. HC is associated with better bone mineral density (BMD) and better metabolic and cardiovascular outcome than Dex in both sexes; prednisolone and prednisone have adverse effect profiles intermediate between HC and Dex (364, 367). Therefore, immediate-release HC remains the preferred option for glucocorticoid treatment in adulthood. Due to its short half-life of 4 to 6 hours, however, it needs to be taken 3 to 4 times per day and requires reliable adherence.

When patient adherence to a 3 times daily regimen is difficult, a twice daily regimen with prednisolone or prednisone (eg, 1-5 mg per dose, for a total daily dose of 20% to 25% of the previous HC dose) might be used instead (358, 368, 369). Prednisolone/prednisone also has been used for fertility induction when it might be more effective and can be continued throughout pregnancy (370). Dex also effectively helps to establish regular menstrual cycles, but it is long-acting and associated with more adverse metabolic side effects (371). In contrast to prednisolone, prednisone, or HC, Dex traverses the placenta and therefore should be avoided during pregnancy (the use of Dex for prenatal treatment of a possibly affected fetus is discussed in section "Management," "Prenatal treatment") (133, 372-375). Due to its strong adrenal-suppressive effect, Dex is preferred in the treatment of TARTs (376, 377). For this purpose, it needs to be given in supraphysiological doses, and should be given for short duration to avoid adverse metabolic effects such as weight gain, striae, edema, and glucose intolerance.

Sustained-release HC preparations have been developed as an alternative to longer-acting synthetic corticosteroids such as prednisone/prednisolone or Dex. A modified-release HC formulation Plenadren® (Shire Services BVBA, Belgium), is approved in Europe for treatment of adrenal insufficiency in adults. When given once daily to patients with primary adrenal insufficiency, it significantly improves metabolic variables such as body weight, body mass index (BMI), and HbA1c compared with conventional HC replacement of the same daily dose (378, 379). However, data on its use in CAH patients are lacking. Clinical experience shows that once daily HC therapy fails to control early

morning rise of ACTH with subsequent excess of adrenal androgens in CAH, requiring an additional glucocorticoid dose in the evening. Excessive nighttime glucocorticoid administration has potential adverse metabolic consequences (369, 380). Another modified-release preparation (Chronocort®, Diurnal, UK) addressing this CAH-specific challenge is currently under regulatory review for the treatment of CAH. It exerts a delayed (4 hours following intake) and sustained action (381). If taken at 23.00 (11 PM), the delayed release mimics the overnight rise and following morning peak of cortisol (381, 382). A second dose is given in the morning (7 AM) ensuring cortisol supply during the day. A phase III trial including 122 patients with classic CAH revealed superior hormonal control during the early morning and early afternoon compared with patients receiving standard glucocorticoid therapy (336, 383).

Monitoring

Regular follow-up should include measurement of height, weight, blood pressure, and physical examination. In children, special attention should be paid to accelerated or reduced height velocity, rapid weight gain, skin and mucosal hyperpigmentation, signs of virilization, pubic hair onset, development of apocrine odor, and signs of central precocious puberty such as breast development or testicular enlargement. Medical history concerning symptoms of salt craving, phases of unusual fatigue during the day, irregular menstrual cycles in girls, and skin hyperpigmentation point to the need for medication titration (Table 5).

Laboratory monitoring traditionally relies on consistently timed serum 17OHP, androstenedione, and plasma renin levels, whereas ACTH measurements are superfluous (Table 6). Plasma renin activity and direct renin levels are extremely variable and should be used along with standing blood pressure and electrolytes to titrate mineralocorticoid dosing (133, 384). Other hormonal monitoring approaches have been suggested but are not yet used routinely. Adrenal-specific metabolites such as 21-deoxycortisol (385) and 11-oxygenated androgens (86) may provide more direct evidence for adrenal androgen production in CAH. Steroids can be measured in blood, urine (266, 303), saliva (386) and dried filter paper blood samples (387, 388) and fluctuate with both the circadian rhythm and the timing of glucocorticoid intake (296, 389-391).

Regular bone age X-rays in growing children beyond 2 years of age are helpful to detect unwanted bone age advancement as a result of cumulative exposure to excess adrenal androgens. The clinician should be alert to signs of central precocious puberty (eg, testicular enlargement in boys, breast development in girls) because elevated adrenal androgens may activate the hypothalamic-pituitary-gonadal axis (133). The decision to adjust HC and fludrocortisone doses

Table 5. Biochemical monitoring of glucocorticoid replacement in children and adults

| Sample | Variable | Goals and Comments ^a |
|------------------------|---|---|
| Serum | Androstenedione | Normal values for sex and age (<i>often useful to assess together with testosterone in males</i>) |
| | Testosterone | Normal values for sex and age (<i>assess in the context of gonadotropins and androstenedione</i>) |
| | Sex hormone-binding globulin | For calculation of free and bioavailable testosterone |
| | DHEAS | Low to suppressed, not a good marker of disease control, but can be used to check for compliance/adherence |
| | 17OHP | Normal values indicate overtreatment, aim at ULN to 400-1200 ng/dL (12-36 nmol/L) |
| | ACTH | Not a useful parameter for disease control; normal values indicate overtreatment |
| | Androstenedione/Testosterone ratio | Healthy woman: <2 Women with CAH: >4 indicates testosterone mainly of adrenal origin Healthy males: <0.2 Men with CAH: >0.5 indicates testosterone mainly of adrenal origin Men with CAH: >1.0 + LH, FSH suppressed indicates testosterone only of adrenal origin due to poor disease control |
| Progesterone (females) | | Goal is <2 nmol/L (<0.6 ng/mL) in follicular phase for women trying to conceive |
| | 11-oxygenated C19 steroids (11-ketotestosterone, 11-hydroxytestosterone, 11-hydroxyandrostenedione, 11-ketoandrostenedione) | Translational method; not yet established in clinical care |
| Saliva | Androstenedione | Normal values for sex and age |
| | 17OHP | Up to ~3 times upper normal limit |
| Urine | GC-MS urinary steroid metabolome analysis (C ₂₁ -, C ₁₉ -, C ₁₈ -steroids) | Translational method; not yet established in clinical care |

^aThese goals are derived from clinical experience and based on expert opinion as there are no established optimal biomarkers nor target values for treatment monitoring.

should consider clinical symptomatology and should not solely rely on laboratory data. Monitoring for reproductive complications are discussed below including reduced fertility in females (see section “Long-term sequelae”), and TARTs in males (see section “Long-term sequelae,” “Gonadal function in males,” “Testicular adrenal rest tumors”).

Management of adrenal emergency in CAH

The overwhelming majority of patients with CAH survive into adulthood, but with shortened life expectancy. Adrenal crises were responsible for 42% of deaths in 588 patients with CAH in a Swedish population-based study; those with the SW form were especially at risk, as they had the lowest cortisol and aldosterone reserves (366). In a retrospective matched-cohort study in the UK, all-cause mortality rates were higher in patients with CAH, with a mean age at death of 54.8 years vs 72.8 years in controls (392). The incidence of adrenal crisis in adults with adrenal insufficiency is estimated to be 5 to 10 crises/100 patient years with a mortality rate of 0.5/100 patient years (393). Studies of children

report similar findings. Two German studies estimated the incidence of adrenal crisis after the neonatal period to be 4.9 to 6.5 adrenal crises/100 patient years (394, 395). In an American series, 55/155 children with SW CAH were hospitalized a total of 105 times over a 14-year period (396). In an Australian population-based study of children and adolescents with CAH, both hospital admission and the risk of adrenal crisis decreased with age (397). A large multicenter international study of 518 children from low- and middle-income countries as well as high-income countries reported an adrenal crisis rate of 2.6/100 patient years (398).

Adrenal crisis is most often triggered by infectious illness (395, 399, 400). A population-based retrospective cohort study (drug prescriptions and clinical diagnoses) in the UK reported increased rates of infectious illnesses in patients with CAH (399). Gastrointestinal illnesses and upper respiratory tract illnesses are the most common precipitants of adrenal crises in both children and adults (395, 396, 399, 400). Socioeconomic factors influence risk; in the United States, patients with government insurance (reflecting low family

Table 6. Monitoring glucocorticoid replacement by history and clinical/technical examination (generally every 4-6 months in adults, every 3-4 months in children >18 months old)

| Parameter | Goals and Comments |
|---|--|
| History | |
| Symptoms of adrenal insufficiency (fatigue, headache, nausea, abdominal pain, postural dizziness, frequent stress dosing) | No signs of adrenal insufficiency |
| Adrenal crisis prevention | Well-educated and equipped patient with knowledge of sick day rules, and possession of steroid emergency card and injection kit; medical alert identification worn at all time |
| Menstrual cycle | Regular menstrual cycles |
| Libido, erections (males) | Normal |
| Sexual health (females) | Pain-free intercourse |
| Physical examination | |
| Height (children) | Linear growth within target range |
| Pubertal development/Tanner stage (children and adolescents) | Normal pubertal development |
| Blood pressure | Within age- and sex-dependent reference range |
| BMI | Within age- and sex-dependent reference range |
| Cushingoid features, Striae distensae | No clinical signs of hypercortisolism |
| Gynecological assessment only if indicated | |
| Imaging | |
| Bone age yearly (children >2 years old/adolescents) | Bone age within 2 SD |
| Scrotal ultrasound every 2-5 years | No gonadal masses |
| Ovarian ultrasound only indicated in unexplained hyperandrogenism | |
| Bone mineral density every 3-5 years (adults treated with high GC doses) | Within age- and sex-dependent reference range |
| Others | |
| Semen analysis if indicated, ie, presence of TARTs (males) | Normal results (WHO guideline) |
| Genetic assessment and counselling | Confirmation diagnosis CAH; counselling for family planning |

income) were twice as likely to be hospitalized as patients with commercial insurance (396). Preschool children, adolescents, males, and those with SW CAH were more likely to experience sick days requiring stress dosing. Patients treated with higher glucocorticoid doses were less likely to suffer illness requiring stress dosing. The frequency of adrenal crises has decreased over time, perhaps due to greater awareness of this risk during sick days. None of the adrenal crises reported in a multicenter study were fatal (398).

Hypoglycemia can occur unexpectedly (394), may be associated with seizures, and can occasionally result in permanent neurologic sequelae, especially in children (400, 401). Patients with SW CAH have adrenomedullary dysfunction with epinephrine deficiency (402) and this contributes to the risk of hypoglycemia especially in young children.

Protocols for the prevention and treatment of adrenal crisis are based on expert opinion and clinical experience (133, 403-405). "Sick day rules" aim to prevent acute deterioration and a life-threatening adrenal crisis. However, the definition and reporting of sick days is more variable than that of adrenal crises, with evidence of systematic variation between centers (398). Adverse outcomes in children are related more to hypoglycemia than to electrolyte disturbances (394, 400, 406); thus, frequent intake of carbohydrates is important (400). Oral stress doses (2-3 times usual

doses) of glucocorticoid cannot always prevent the progression to adrenal crisis and the occurrence of hypoglycemia (406, 407). Increased HC doses are suggested with infectious illnesses (Table 7). HC sodium succinate for intramuscular injection should be prescribed with instructions for home use if oral medication is not tolerated during episodes of major stress (eg, febrile illness with vomiting), especially for patients residing far from medical facilities. Once brought to emergency care, intravenous HC and isotonic fluids should be given. Continuous intravenous infusion of HC sodium succinate might have a theoretical advantage over intermittent bolus administration because of lower variability and avoidance of regular troughs in plasma cortisol levels (383, 408), but these 2 approaches have not been compared directly and clinical outcomes are likely similar. Stress dosing is indicated for pregnant women in active labor, similar to that used in major surgical stress (403). Stress dosing is not recommended for everyday mental and emotional stress, minor illness, or before routine exercise (133, 403, 404). Serum cortisol did not exceed 10 µg/dL (276 nmol/L) in healthy children undergoing minor surgical procedures; therefore, stress dosing for minor procedures (eg, brief medical or dental procedures performed under local anesthetic with or without light sedation) should be individualized (247, 409).

Table 7. Suggested management and glucocorticoid stress dosing for patients with adrenal insufficiency due to congenital adrenal hyperplasia (393, 396, 397, 400)

| Clinical Scenario | Glucocorticoid Management | Additional considerations |
|--|--|--|
| At home | | |
| Major illness or high-grade fever (>39°C children) | Children: Three times the usual dose of hydrocortisone divided into 4 doses (given every 6 hours). Adults: 20 mg of hydrocortisone orally 3 times daily in addition to usual glucocorticoid or triple usual glucocorticoid. | Drink regularly and increase fluid ^a intake for concentrated (dark) urine Eat regularly simple and complex carbohydrates. 15 g (children) or 30 g (adults) Adults with severe infections should divide dose every 6 hours |
| Gastroenteritis with diarrhea ± vomiting (with or without fever) | Children: 3 times the usual dose of hydrocortisone divided into 4 doses (given every 6 hours); Adults: 10-20 mg of hydrocortisone 3 to 4 times daily in addition to usual glucocorticoid or double or triple usual glucocorticoid; dose depends on severity of diarrhea Repeat oral dose if vomiting occurs within 1 hour of medication. If vomiting reoccurs, parenteral hydrocortisone 100 mg (children 50-100 mg/m ²) | Consider early parenteral hydrocortisone If unable to tolerate fluids, call emergency services for evaluation following glucocorticoid injection Return to usual dose within 1-2 days of recovery with return to usual diet |
| Minor illness or low-grade fever (>38°C in children) | Children: 2 to 3 times the usual dose of hydrocortisone divided into 3-4 doses (given every 6-8 hours) Adults: 10 mg of hydrocortisone orally 3 times daily in addition to usual glucocorticoid, or double usual glucocorticoid | Drink regularly and increase fluid ^a intake for concentrated (dark) urine Eat regularly simple and complex carbohydrates. 15 g (children) or 30 g (adults) Return to usual dose within 1 day of recovery |
| Exhausting physical exercise | Add 1 usual dose (children) or 10 mg hydrocortisone (adults) 30 to 60 minutes before exercise | For unusual activities beyond normal routines. Not for routine use Can repeat dose(s) if extended time period of strenuous exercise (eg, marathon) |
| Procedures | | |
| Major surgery | Hydrocortisone intravenous bolus 50-100 mg (children 50-100 mg/m ²) followed by continuous intravenous hydrocortisone infusion 100-200 mg (children 100 mg/m ²) over 24 hours. Alternatively, divided doses every 6 hours, intravenous hydrocortisone 100-200 mg/day (children 100 mg/m ² /day) | Taper over 2-3 days with return to usual dose |
| Short surgeries | Hydrocortisone intramuscularly or intravenous bolus 50-100 mg (children 50 mg/m ²) just before general anesthesia. Alternatively, give triple the usual morning dose before oral intake is held | Rapid return to oral regimen |
| Labor and delivery | As for surgical procedures | |
| Bowel procedures requiring overnight laxative | Double or triple usual glucocorticoid dose prior to laxative and repeat every 6 hours if oral medication tolerable and allowed. Alternatively, hydrocortisone 50-100 mg (children 50 mg/m ²) intramuscularly with laxative Hydrocortisone 50 mg (children 50 mg/m ²) intramuscularly or intravenous prior to procedure | |
| Dental surgery | Extra morning dose 1 hour prior to surgery | Can repeat dose depending on recovery No additional doses for routine dental procedures |
| Minor procedures with no sedation | No adjustment needed | |

Table 7. Continued

| Clinical Scenario | Glucocorticoid Management | Additional considerations |
|-------------------|--|---|
| Acute emergency | Rapid infusion of intravenous fluids: 1000 mL of 0.9% sodium chloride (children 20 mL/kg normal saline, repeat up to 60 mL/kg) during the first 60 minutes, further fluid administration guided by individual patient needs | Measurement of glucose and electrolytes |
| | Hydrocortisone bolus 100 mg (children 50-100 mg/m ²) followed by continuous intravenous infusion 200 mg over 24 hours or 50 mg every 6 hours (children 50-100 mg/m ² /day divided every 6 hours). Reduce to 100 mg (children 50 mg/m ² /day) over 24 hours the following day | Cardiac monitoring Consider antibiotics |
| | For hypoglycemia: dextrose 0.5-1g/kg dextrose or 2-4 mL/kg of D25W (maximum single dose 25 g) infused slowly at rate of 2 to 3 mL/minute Alternatively, 5-10 mL/kg of D10W for children <12 yrs old | Rapid hydrocortisone tapering and switch to oral regimen depending on clinical status |

^aElectrolyte- and sugar-containing fluids recommended. If hydrocortisone sodium succinate is unavailable, another parenteral glucocorticoid, such as dexamethasone, methylprednisolone, or prednisolone, may be used in equivalent doses. Fludrocortisone replacement is not required if hydrocortisone doses exceed 50 mg every 24 hours but is generally administered, in those normally on fludrocortisone, when oral hydrocortisone is started.

Approximately one-third of patients with NC CAH have mild but clinically silent cortisol impairment (410, 411) and the risk of adrenal crisis is unknown. Adrenal crisis has only been reported in NC CAH patients receiving glucocorticoid therapy in the setting of iatrogenic tertiary hypothalamic–pituitary–adrenal axis suppression (133, 400). Thus, stress dosing for the prevention of adrenal crisis is recommended for glucocorticoid-treated patients with NC CAH. The Endocrine Society Clinical Practice Guideline (133) suggests HC stress dosing in the case of severe illness, major surgery, major trauma, or childbirth for untreated individuals with a suboptimal ACTH test (in adults, cortisol below 14 to 18 µg/dL, <400-500 nmol/L).

In general, prevention of adrenal crisis in patients with known adrenal insufficiency is best accomplished through repeated structured patient education regarding “sick day rules” (412, 413). All patients should wear medical alert identification tags or have an emergency card (and/or emergency information on their mobile phones) indicating adrenal insufficiency. A medical card developed by the European Society of Endocrinology is downloadable and includes guidance for healthcare providers as well (<https://adrenals.eu/emergency-card/>). A UK version including a QR code rapidly linking emergency personnel to instructions on adrenal crisis treatment is available (<https://www.endocrinology.org/media/3652/steroid-nhs-card.jpg>).

Treatment of NC CAH

In NC CAH the estimated residual enzymatic activity of CYP21A2 is about 20% to 50% based on in vitro or in

silico studies, resulting in a generally mild but highly variable phenotype (210, 215, 414). In contrast to classic CAH, no general guidelines exist for the management and follow-up of these patients and the overall evidence of recommendations for NC CAH is low (133, 340, 415-417). Decisions about starting treatment should be individualized and based mainly on clinical symptoms; the Endocrine Society guidelines do not recommend routine treatment with glucocorticoid in asymptomatic individuals (133). The general treatment goals in children are to maintain normal growth and pubertal development and to minimize risk of therapies; children should be regularly monitored clinically for height, weight, signs of androgen excess, puberty, and bone age advancement (418).

When glucocorticoid treatment is required, HC is preferred, as with classic CAH. Patients receiving glucocorticoid therapy require stress dosing per guidelines (see section “Management,” “Hormonal treatment of classic CAH,” “Management of adrenal emergency in CAH”). Mineralocorticoid supplementation with fludrocortisone is not required.

Growth

In contrast to untreated children with SV CAH, children with NC CAH may not have increased growth velocity although bone age maturation can be accelerated, potentially leading to reduced adult height (419, 420). However, most studies describe nearly normal adult height in NC CAH patients (415, 421-423). Glucocorticoid treatment should be reserved for patients who suffer from androgen excess, although criteria for deciding when symptomatology

warrants treatment are not well defined. Supraphysiological dosages of glucocorticoids similar to those used to treat classic CAH patients may be necessary to suppress adrenal androgen production (424). Treatment will suppress the hypothalamic–pituitary–adrenal axis requiring stress dosing in case of illness. In many cases glucocorticoid treatment may be discontinued after reaching adult height, if the individual is otherwise asymptomatic (133). Adverse effects such as excess weight gain may make continued glucocorticoid treatment less desirable.

Puberty

Children with NC CAH can present with signs of increased adrenal androgen production such as premature pubarche, acne, mild hirsutism, and menstrual disturbances that can progress over time (425), but in contrast to classic CAH, central precocious puberty is infrequently observed (37). Glucocorticoids can lower adrenal androgen concentrations ameliorating signs of hyperandrogenism, but prolonged glucocorticoid treatment may have long-term adverse effects. Alternative treatment options in adolescent and young adult females to induce menstrual cycles and improve acne and hirsutism include oral contraceptives containing progestins with low androgenic activity such as desogestrel (426). Antiandrogens can be considered as an add-on for patient-important hirsutism that persists despite oral contraceptives (see the next section).

NC CAH in adult women

Most patients diagnosed with NC CAH are females suffering from mild adrenal androgen excess without clinically relevant deficiencies of gluco- and mineralocorticoids (133, 427). Typical symptoms in affected women are hirsutism, oligo- and amenorrhea, acne, alopecia, and sub- or infertility (425). Sometimes the diagnosis is made within the course of evaluation for adrenal incidentalomas (428–430). The main treatment goal is to reduce adrenal androgens and symptoms of androgen excess. Clinical studies comparing different treatment approaches in adults with NC CAH are lacking; treatment should only be started in symptomatic patients desiring treatment (133). The risks, benefits, and effectiveness of various treatment options should be discussed. Fertility and childbearing in women with NC CAH are discussed in the section “Long-term sequelae,” “Reproductive function in women”.

Additional treatments for signs of androgen excess

Hirsutism is the most prevalent symptom in women with NC CAH, but also the most difficult to treat (431). Clinical experience suggests that a combination of oral contraceptives, topical eflornithine, and cosmetic treatment (shaving, chemical depilatories, plucking, tweezing, threading,

waxing or epilation therapy, electrolysis, and intense pulsed light) might be the most effective treatment approach (432). For the treatment of acne and androgenic alopecia a dermatologist should be consulted.

Oral contraceptives act on the production, transport (increase of sex hormone binding globulin) and action of androgens. Antiandrogenic oral contraceptives containing cyproterone acetate, chlormadinone acetate, dienogest, or drospirenone effectively reduce androgenic symptoms. If hirsutism is the leading symptom, oral contraceptives are the preferred treatment (133). One randomized study in 30 women with NC CAH found cyproterone acetate to be more effective than HC for isolated hirsutism (433).

Spironolactone, flutamide, and finasteride can be used to treat hirsutism (431), acne, and androgenic alopecia (434) but are teratogenic and not approved for this use. Eflornithine hydrochloride cream is used as topical therapy for facial hirsutism (431). It prevents hair growth by inhibiting the anagen phase of hair production. Eflornithine irreversibly binds to ornithine decarboxylase and thus prevents the natural substrate, ornithine, from accessing the active site. It is most effective when combined with physical means of hair removal, such as topical lasers.

Treatment of adult men with NC CAH

As androgen production in the testis far outweighs adrenal androgen production, men generally do not experience symptoms of androgen excess requiring treatment, and therefore remain undiagnosed. In rare cases, severe acne, reduced fertility, or adrenal incidentaloma lead to the diagnosis of NC CAH in men (427). TARTs are rare in men with NC CAH (435–437). Therefore, routine scrotal ultrasound is not recommended in NC CAH males.

Prenatal treatment

Purpose

Since the mid-1980s, prenatal treatment with high doses of Dex has been proposed for to pregnant women with a fetus at risk for classic CAH using a treatment protocol of 20 µg/kg/day, maximum 1.5 mg/day, with the aim of preventing prenatal virilization of the external genitalia in affected girls (311, 315, 438). The treatment is effective in ameliorating virilization of the external genitalia if started by gestational week 6–7 (439); in most centers this is before a fetal diagnosis can be made (see section “Diagnostics,” “Prenatal diagnosis”). If prenatal diagnosis, most often by chorionic villus biopsy obtained in week 10–11, shows that the fetus is a girl with classic CAH, the treatment is continued until term, but otherwise stopped. The treatment is controversial due to safety concerns (440). Risk–benefit

assessments must consider that, on average, 8 pregnancies at risk for CAH must be treated for every affected female who might benefit from the treatment (441-444). Endocrine societies and others have stated that the treatment is experimental and should only be performed in centers taking part in long-term research studies of these treated pregnancies (133, 326, 373, 375, 445).

Fetal safety

There have not been randomized studies of prenatal Dex treatment, and so all discussions of adverse effects are based on animal or retrospective data. Dex is a pluripotent gene regulator and its introduction at a critical stage of embryonic development may impact much more than the developing hypothalamic–pituitary–adrenal axis. Numerous studies (Table 8) have delineated adverse outcomes affecting brain, cardiovascular, renal, reproductive, thyroid, and metabolic functions in nonhuman mammalian species exposed to glucocorticoids in utero (reviewed in (373)).

With respect to human teratogenicity, systematic review and meta-analysis found an odds ratio of 1.41 (95% CI 1.14-1.74) for cleft lip and palate in case–control series of infants whose mothers were treated with glucocorticoids in the first trimester (466). Even when exposed later in gestation, multiple doses of antenatal steroids for preterm labor increased the number of infants with birth weight <10th percentile and the risk for cerebral palsy (467). Among pregnancies at risk for CAH, prenatally Dex-treated newborns have lower, but nominally normal, birth weights than untreated controls (468); the decrease averages ~400 g (469). Other adverse events including failure to thrive, stroke-like events and midline defects have been observed in both short-term and full-term treated cases at risk for CAH (470-472).

Prenatal Dex treatment has shown inconsistent long-term effects on cognition and behavior (Table 9). One study showed no cognitive differences but increased shyness and emotionality in treated children (473). A larger follow-up study from the same group of 126 non-CAH and 48 with CAH short-term exposed children and 8 girls with CAH treated until term did not show any effects on motor, cognitive, and social development or scholastic competence using parental questionnaires (474). In a later report including 2 different age groups (5-12 and 11-24 years) using neuropsychological testing, there were no significant findings in children without CAH (478). Swedish studies of healthy non-CAH children exposed to Dex only during the first trimester have shown negative effects on cognition, especially verbal working memory (475); a sexually dimorphic effect with a more pronounced negative effect on working memory and executive function was observed

in the girls (481). A follow-up with a second neuropsychological testing in a subgroup of the cohort as adults showed less pronounced effects indicating a possibility for compensatory mechanisms over time (483).

In 2 cohorts of girls with CAH treated throughout gestation, neurocognitive outcomes were negatively affected for mental processing and spatial memory (478) and broad deficits were found in most measures of cognition (486). In contrast, a Polish study reported better cognitive results in general in 9 girls with CAH who were treated throughout pregnancy, but 8 unaffected girls who had been treated with Dex had worse results than controls (479).

Possible imprinting effects of prenatal exposure to Dex have only begun to be explored. Differences in DNA methylation in peripheral CD4+ T cells seemed to be related to sex (483). Of particular interest were methylation effects of the genes *BDNF* and *FKBP5*, relevant for the development of the central nervous system, and *NR3C1* encoding the glucocorticoid receptor. There were also associations between DNA methylation and performance on cognitive tasks.

Moreover, first trimester Dex exposure of non-CAH fetuses is associated with differences in brain morphology (487). Magnetic resonance imaging (MRI) studies in adults showed enlargement of the amygdala, increased left superior frontal gyrus, and widespread white matter changes. The pathophysiology behind the observed neuropsychological effects of early Dex exposure are largely unknown. Infants prenatally exposed to betamethasone have altered responses of the HPA axis and a higher incidence of mental and behavioral disorders (488, 489).

Negative effects on glucose and lipid metabolism in childhood and in young adulthood have been reported in individuals without CAH but exposed to Dex during the first trimester. Lower insulin secretion, followed by lower glucagon secretion was reported in a French study (484). A lower HOMA- β was reported in the Swedish cohort, significant in girls but not in boys. Plasma glucose levels were higher in the younger treated group with no sex difference. In older adolescents and young adults, total cholesterol and low-density lipoprotein cholesterol were higher in the treated individuals (485). It is unknown if this implies an increased risk of developing metabolic syndrome later in life.

In order to minimize the exposure to Dex, efforts have been made to develop diagnostic techniques using cell-free fetal DNA in maternal blood samples, but these are not yet routinely available (see section “Diagnostics,” “Prenatal diagnosis”) (323). Dose adjustments, with lower doses during the later phases of pregnancy, have been discussed (156) but such studies have not been reported.

Table 8. Animal models of prenatal glucocorticoid exposure

| Animal | Medication/Dosing ^a | Outcome | Reference |
|----------------------------------|---|---|-----------|
| Mouse | NK1R antagonists 30-300 mg/kg/day ^b | 9% cleft palate in higher dosage | (446) |
| Rat | Dex 0.1 mg/kg/day during the whole pregnancy | Lower body weight and kidney size, postnatal hypertension, albuminuria, sodium retention, and decreased glomerular filtration | (447) |
| Spiny mouse | Mini osmotic pump with Dex 125 µg/kg | Decreased the number of nephrons and altered expression of genes involved in nephron development in the spiny mouse | (448) |
| Rat | Dex | Impaired thyroid development with fewer follicular cells and C cells | (449) |
| Rat | Carbenoxolone ^c 12.5 mg/day | Lower birth weight and increased blood pressure | (450) |
| Rat | Dex 100 µg/kg/day SC in late pregnancy | Glucose intolerance, 25% increase in hepatic expression of glucocorticoid receptor | (451) |
| Rat | Dex 100 µg/kg/day SC in late pregnancy | Lower birth weight, fatty acid esterification, and triglyceride synthesis | (452) |
| Rodents | Dex 50-120-200 µg/kg/day | Impaired glucose tolerance, hyperinsulinism increased blood pressure, reduced postnatal growth at 1 year of age despite normal birth weight | (453) |
| Rat | Carbenoxolone 12.5 mg/day | Reduced birth weight | (454) |
| 11 β HSD mutant mouse | None | Mice lacking Hsd11b2 had lower birthweights and increased anxiety compared with wild type littermates | (455) |
| Sheep | Betamethasone 0.5 mg/kg during 3 days | Retardation of fetal brain development | (456) |
| Sheep | Single or repeated betamethasone injections | Reduced brain weight | (457) |
| Sheep | Repeated betamethasone injections | Reduced neuronal myelination | (458) |
| Rhesus macaque | Dex 0.5 or 5 or 10 mg/kg or repeated injections | Decreased numbers of pyramidal neurons in the hippocampal CA regions | (459) |
| Neural stem cells of newborn rat | Dex in vitro | Impairment of neuron and oligodendrocyte size and differentiation | (460) |
| Fetal guinea pig | Betamethasone 1 mg/kg for 4 days | Changes in GR DNA binding and DNA methylation in the fetal hippocampus | (461) |
| Guinea pig | Betamethasone 1 mg/kg for 4 days | Reduced locomotor activity; effect on programming HPA axis and hippocampal glucocorticoid feedback | (462) |
| Spiny mouse | 125 µg/kg Dex SC for 60 hours using mini pump | Reduction of adrenal steroidogenesis, decrease in plasma DHEA reduced adrenal expression of steroidogenic enzymes in adulthood | (463) |
| Guinea pig | Betamethasone 1 mg/kg for 4 days | Altered DNA methylation underlies both the long-term effects of glucocorticoids and of maternal stress on the fetus | (464) |

^aNote that the doses given to animals exceed the typical doses given in pregnancies at risk for CAH.

^bNK1R antagonists modulate the hypothalamic-pituitary-adrenal axis leading to increased corticosterone secretion.

^cCarbenoxolone is a glycyrrhetic acid derivative with a steroid-like structure. It inhibits placental Hsd11b2 activity, thereby increasing fetal exposure to maternal glucocorticoids (465).

New Medical Strategies

The treatment goals for classic CAH include both hormonal replacement and reducing adrenal androgen production. Glucocorticoid therapy is used to achieve both goals, but normalizing adrenal androgen production requires supraphysiologic doses that are higher than required to replace the cortisol deficiency, contributing to comorbidities. Modified and delayed-release HC formulations were discussed in section “Management,” “Hormonal treatment of classic CAH,” “Treatment of adults” (490). Continuous

subcutaneous delivery of HC is also suitable for mimicking physiologic cortisol secretion patterns and is useful in patients with rapid cortisol metabolism or impaired gut absorption (491), but this approach is less practical than oral drugs for widespread chronic use (Fig. 5).

Alternatively, medications that lower androgen production and/or action can be added to physiologic glucocorticoid therapy, similar to doses used to treat primary adrenal insufficiency. The combination of testolactone (an aromatase inhibitor) and flutamide (an androgen receptor

Table 9. Studies of human prenatal exposure to glucocorticoids

| Study group | | | Results | | | |
|---|--|---|--|--|--------------------|-----------|
| Dex exposed | Controls | Age at study | Questionnaire findings | Psychological tests | Laboratory and MRI | Reference |
| First trimester exposure only^a | | | | | | |
| 26 total 3 with CAH | 14 total 3 with CAH | 6 mo-5.5 yrs Mean 2.5 ± 1.3 yrs | NS overall development Dex treated higher shyness, emotionality, lower sociability (EAS), internalizing (CBCL) (parental Q) | | | (473) |
| 174 total 48 with CAH | 313 total 195 with CAH | 1-12 yrs 3 diff age groups | No developmental differences NS CBCL school scale (parental Q) | | | (474) |
| 22 total 10 F 7 M and 5 M with CAH | 35 total all healthy | 7-17 yrs Median 11 yrs | NS CBCL school scale (parental Q) Poorer scholastic competence (self-reported) | NS IQ, but Poorer working memory (WISC-III) NS learning, memory (NEPSY) | | (475) |
| Same study population | | | NS behavior (CBCL), or shyness (SPAI-CP). Higher scores sociability (EAS) (parental) more social anxiety (self-reported Q) | | | (476) |
| Same study population | | | M reported more neutral behavior KI-GRB (self-reported Q) F, NS | | | (477) |
| <i>Study 1</i> 67 total 51 non-CAH (35 F, 16 M) 8 M CAH (8 F full term Dex) | 73 total 31 F 16 M 15 CAH F 11 CAH M 13 total 2 F 1 M 4 CAH F 6 CAH M | <i>Study 1</i> 5-12 yrs <i>Study 2</i> 11-24 yrs | | <i>Study 1</i> Few significant findings K-ABC Sequential Processing positive for M alone <i>P</i> = .095 <i>Study 2</i> Non-CAH F performed significantly less well on Faces & Places M, NSerence | | (478) |
| <i>Study 2</i> 7 total (1 CAH F Full term Dex) | | | | | | |
| 9 F | 8 non-CAH 9 CAH non-DEX All F | Mean 12 yrs But CAH untreated 16 yrs | NS psychopathology (CBCL) (parental Q) | Lower scores in non- CAH Dex treated F (WAIS-R-PL, WISC-R) | | (479) |
| 34 total 16 F 18 M | 66 total 36 F 30 M | 7-17 yrs Mean age 10.5 yrs | | Poorer working memory (WISC-III) Sex difference with larger neg effects in F for executive functions and psychometric intelligence (WISC-III, WMS-III) | | (480) |

Table 9. Continued

| Study group | | | Results | | | |
|---|--|--|--|--|--|-----------|
| Dex exposed | Controls | Age at study | Questionnaire findings | Psychological tests | Laboratory and MRI | Reference |
| 34 15 F 19 M | 67 total 36 F 31 M | 7-17 yrs Mean age 10.5 yrs | CBCL, SPAI-R, and EAS (parental Q) SASC-R (self-reported Q)) | NS. Generally well adjusted. | | (481) |
| 23 Adults 12 F 11 M | Population controls 31 F 27 M | 16-24 yrs Mean age 20-21 | | No significant neuropsychological changes; no increase in anxiety, depression or autistic traits. | | (482) |
| 29 total 12 F 17 M | 37 total 18 F 19 M | Mean age 16.5-17 yrs | | Methylation in BDNF, FKBP5, and NR3C1 genes were associated with the performance on WAIS | Altered DNA methylation in peripheral CD4+ T-cells | (483) |
| 16 total 9 F 7 M | 15 total 8 F 7 M | Mean 24 yrs | | | Lower insulin secretion by 17-22%. Lower glucagon HOMA-β | (484) |
| 40 total 18 F 22 M | 75 total 35 F 40 M | Mean age 16.3 ± 6.2 Age groups Young <16 Older ≥16 | | | β-cell function in younger F Glucose level, higher in younger age group Higher cholesterol, and LDL in older age group | (485) |
| 19 total 9 F 10 M | 43 total 26 F 17 M | 16-26 yrs | | | MRI Alterations in brain structure Enlarged amygdala, surface area and volume of left sup frontal gyrus, widespread white matter changes | (481) |
| Girls with CAH, Dex-treated until term 4 | | | | 1 not able to perform neuropsychological testing Generally low IQ | | (475) |
| Study 1 8 Study 2 1 | Study 1 15 CAH girls Study 2 4 CAH F | Study 1 5-12 yrs Study 2 11-24 yrs | | Performed more poorly on K-ABC Mental Processing Composite (<i>P</i> = 0.09) Performed (marginally) less well on Hand Movements (subtest Sequential Processing) and Spatial Memory (Simultaneous Processing). | | (478) |

Table 9. Continued

| Study group | | | Results | | | |
|-------------|---------------------------------|---|--|--|--------------------|-----------|
| Dex exposed | Controls | Age at study | Questionnaire findings | Psychological tests | Laboratory and MRI | Reference |
| 9 | 8 non-CAH 9 CAH untreated | Mean 12 yrs and CAH untreated 16 yrs | NS psychopathology (CBCL) (parental Q) | Higher scores IQ in Dex treated F. Lower in non-CAH Dex treated F (WAIS-R-PL, WISC-R) | | (479) |
| 4 | 25 F CAH | | Diff in self-perceived deficits in executive function (B-DEFS-SF) | Broad deficits in most measures of cognition in Dex treated F (WAIS-IV, WMS-III) | | (486) |

HOMA- β homeostasis model assessment of beta cell function; LDL, low-density lipoprotein; MRI magnetic resonance imaging.

Abbreviations: NS, not significantly different; Dex, dexamethasone; CAH, congenital adrenal hyperplasia; CBCL, Child Behavior Checklist; EAS Temperament Survey for Children; WISC-III, Wechsler Intelligence Scales for Children; NEPSY, Developmental Neuropsychological Assessment; SPPC, Self-Perception SPAI-C-P, Social Phobia and Anxiety Inventory for Children—Parent Report; K-ABC, Kaufman Assessment Battery for Children; WAIS, Wechsler Adult Intelligence Scale; WMS, Wechsler Memory Scale; KI-GRB, The Karolinska Inventory of Gender Role Behavior; B-DEFS-SF, Barkley Deficit in Executive Functioning Scale—Short Form.

^aThe earlier studies reported results from mixed cohorts, short-term treated boys and girls without CAH, and boys with CAH, while more recent studies have assessed individuals with and without CAH, males and females, separately.

antagonist) with 8 mg/m²/day HC normalized growth and bone maturation in a 2-year randomized trial of 28 children (492). A long-term study of this combination to determine the efficacy of this regimen on improving adult height will soon be completed (NCT00001521). Abiraterone acetate is a potent CYP17A1 inhibitor used to treat prostate cancer (493). When added to HC 20 mg/day, 6 days of treatment with 100 to 250 mg/day of abiraterone acetate normalized androstenedione in 6 adult women (494) with parallel reductions in testosterone, androgen metabolites, and 11-oxo-androgens (495). Abiraterone acetate therapy can cause DOC accumulation and consequent hypertension and/or hypokalemia in patients with prostate cancer via CYP21A2-mediated 21-hydroxylation of intra-adrenal progesterone (495); however, this conversion cannot occur in patients with classic CAH (495). Abiraterone acetate is likely to be most useful in prepubertal children with classic CAH to suppress androgens and estrogens until the anticipated age of puberty, and a phase I trial testing this approach is underway (NCT02574910). Abiraterone acetate monotherapy might cause DOC accumulation in patients with NC CAH if not combined with glucocorticoid therapy or a mineralocorticoid receptor antagonist. Moreover, its use in pubertal girls would require concomitant estrogen treatment, for example with oral contraceptive pills. Third-generation antiandrogens such as enzalutamide, apalutamide, and darolutamide have not been tested in CAH patients but also might be useful treatments in women of reproductive age willing to use contraception.

Agents that reduce the ACTH-mediated drive for androgen production are possible approaches. The binding of CRH to its type 1 receptor (CRHR1) is a major input to corticotropes, raising intracellular cAMP and stimulating ACTH secretion. A single-dose, fixed-sequence study of 8 women given 300 or 600 mg of the CRHR1 antagonist NBI77860 at 22.00 (10 PM) showed significant reductions in ACTH and 17OHP over the ensuing 16 hours relative to a control period during which glucocorticoid treatment was withheld (496). The CRHR1 antagonists tildacerfont and crinicerfont were tested in 14-day continuous-dosing trials (NCT03257462 and NCT04045145, respectively), and tildacerfont therapy was extended in a 3-month trial (NCT03687242). Peer-reviewed results are unavailable as yet. Additional trials are required to further assess the long-term benefits of these treatments. Theoretically, an anti-ACTH antibody (497) or an antagonist of the melanocortin type 2 receptor (MC2R, the ACTH receptor) (498) might also reduce adrenal androgen synthesis in patients with classic CAH, but these approaches have only been tested in preclinical models (499). It should be kept in mind that most of these approaches do not eliminate the need to treat with, and monitor adequacy of, glucocorticoid replacement, albeit perhaps in lower doses.

Unilateral or bilateral adrenalectomy has been suggested as an approach to long-term management of classic CAH to limit adrenal androgens (500). A recent meta-analysis of 48 CAH cases, 34 (71%) described symptomatic improvement after bilateral adrenalectomy but with

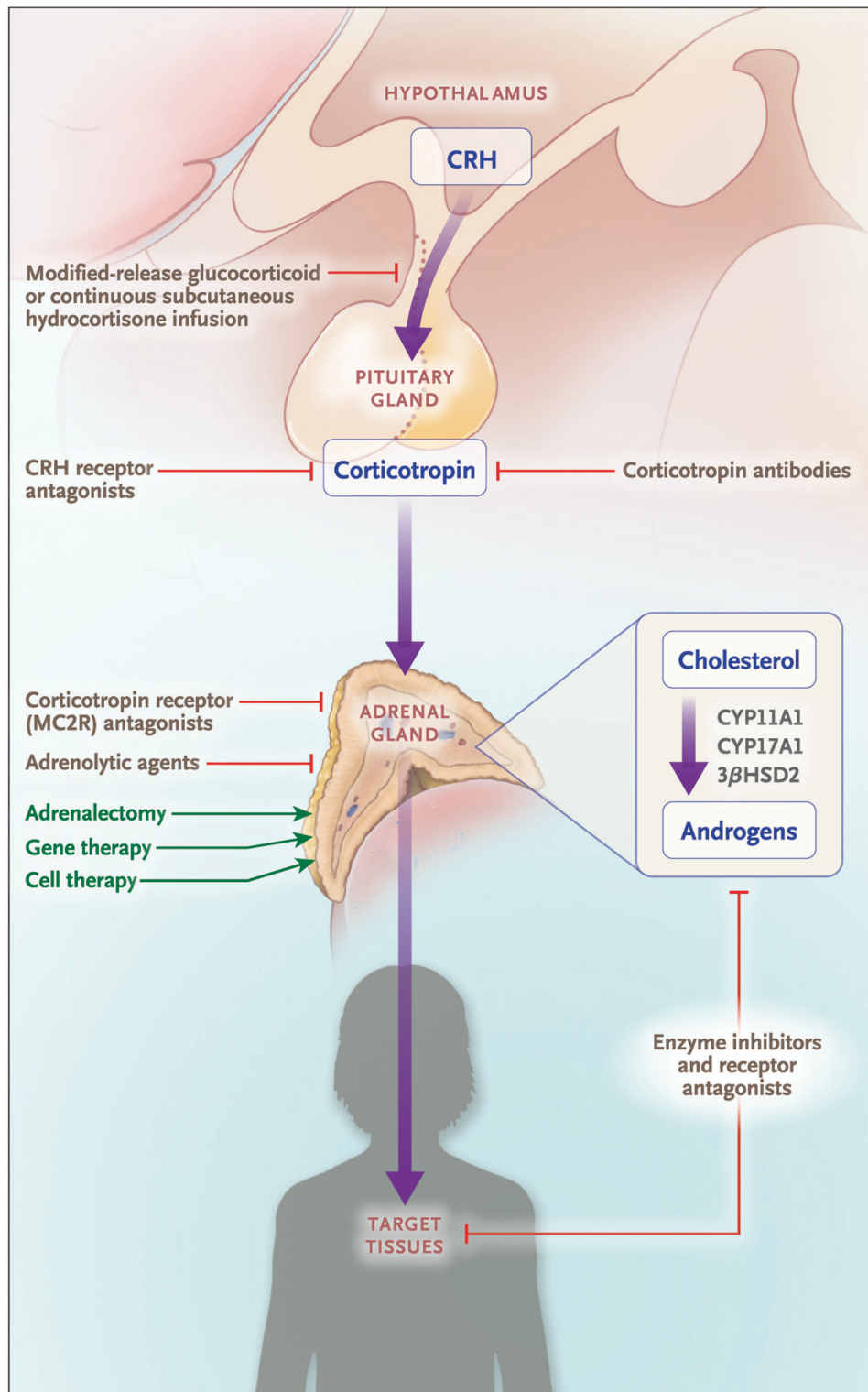


Figure 5. New therapeutic approaches target different aspects of the pathophysiology of CAH. Circadian cortisol replacement with a modified-release glucocorticoid or subcutaneous hydrocortisone infusion aim to control corticotropin-driven hyperandrogenism by replacing cortisol in a physiological manner. Other approaches to reduce androgen production without chronic supraphysiological glucocorticoid exposure include corticotropin-releasing hormone receptor-1 antagonists, adrenocorticotropic hormone (corticotropin, ACTH) antibodies, adrenocorticotropic hormone receptor (MC2R) antagonists, adrenolytic agents, adrenalectomy, and pharmacological inhibition of steroidogenic enzymes or steroid receptors in the adrenal or peripheral tissues. Since CAH owing to 21OHD is a monogenic disorder, gene therapy with cell-based and gene-editing technologies may be able to restore defective steroidogenesis. CRH denotes corticotropin-releasing hormone (sometimes referred to as corticotropin-releasing factor [CRF]). From New England Journal of Medicine, Merke DP, Auchus RJ, Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency, Volume 83, Page 1258. Copyright © (2020) Massachusetts Medical Society. Reprinted with permission.

5 cases (10%) reporting short-term and 13 cases (27%) long-term adverse outcomes, including an increased risk of adrenal crisis (501). The subsequent development of adrenal rest tumors due to elevated ACTH levels even in women has been reported (502, 503), which defeats the purpose and allows the recrudescence of androgen excess. Consequently, this approach has fallen out of favor (133). Adrenolytic therapy with mitotane has been reported in men with TARTs as an approach to restore fertility (504), but long-term outcomes have not been published. The adrenolytic drug nevanimibe was testing in a dose escalation study of 14-day treatment periods interrupted with 14-day placebo periods, up to 1000 mg twice daily (505). The median 17OHP was consistently lower in treatment periods and rose during placebo periods, consistent with a reversible effect on steroidogenesis, but only 20% met the primary endpoint (17OHP $\leq 2\times$ upper limit of normal). A study using longer treatment periods in order to achieve greater and more sustained reductions in adrenal-derived androgens was initiated (NCT03669549) but terminated after an interim analysis (<https://clinicaltrials.gov/ct2/show/NCT03669549>, accessed December 22, 2020). Thus current data do not support the approach of “medical adrenalectomy.”

Growth hormone has been used to improve height in children with CAH (506-508). Growth hormone treatment for a mean duration of 5.6 years achieved nearly adult height in 34 children with CAH (12 NC-CAH patients). In some of the patients, GnRH analogue was also used to delay puberty (507). Controlled studies in larger groups of patients are lacking. Therefore, growth hormone, with or without GnRH analogue therapy, cannot be generally recommended as adjunctive therapy.

Novel Cell- and Gene-based Therapies

Potential cell-based therapies for CAH

Cellular reprogramming is the process whereby a fully differentiated, specialized cell type is forced to acquire a different phenotype that it would not reach under normal physiological conditions. Somatic cells can be induced to dedifferentiate to an embryonic stem cell (ESC)-like phenotype by forcing the expression of specific transcription factors; these cells, termed inducible pluripotent stem cells (iPSCs), are donor-specific and phenotypically highly similar to ESCs (509). An example of cell therapy used ESC- and iPSC-derived pancreatic beta cells for potential treatment for type 1 diabetes (510).

An alternative strategy for reprogramming somatic cells without an intermediate state is through lineage-conversion (also known as direct reprogramming or transdifferentiation), which entails the forced

expression of lineage-determining transcription factors (511). Various human and mouse cell types have been used for lineage conversion to an adrenocortical phenotype (512). Adrenocortical-like cells have also been established from cells derived from human skin, blood, and urine cells in humans using a combination of steroidogenic factor-1 (SF-1, NR5A1) expression (through lentiviral delivery) and activation of protein kinase A and gonadotropin-releasing hormone (GnRH) pathways (513). These reprogrammed cells displayed ultrastructural features resembling steroid-secreting cells (such as larger mitochondria with a densely packed inner mitochondrial membrane), de novo expressed steroidogenic enzymes and secreted steroid hormones in response to physiologic (such as ACTH) and pharmacologic (such as nondegradable cAMP-dependent protein kinase A activators) stimuli. They are also viable when transplanted into the mouse kidney capsule or intra-adrenally. Importantly, the hypocortisolism observed in cells reprogrammed from epithelial cells recovered from the urine of patients with CAH (due to mutations in *CYP21A2*, *STAR*, *HSD3B2*, and *CYP11A1*) was rescued by expressing the wild-type version of the defective enzyme. These studies model CAH in a dish to test personalized interventions. In the future, one could attempt to apply gene-editing on cells reprogrammed from patients to achieve normal steroidogenesis. The same approach could be employed in vivo, through delivery of gene-editing tools to the adrenal with viral vectors. Other, as yet untested, methodologies include establishment of adrenocortical-like cells from iPSCs and adrenocortical organoids capable of self-renewal.

Potential gene-based therapies for CAH

Gene therapy using adeno-associated viruses (AAVs) is an alternative option, tested in an animal model of 21OHD. The active murine gene is named *Cyp21a1*, while the duplicated pseudogene is *Cyp21a2-p*. Mice bearing a deletion of approximately 80 kilobases of chromosome 17 (514), including the *Cyp21* locus showed perinatal lethality, elevated ACTH, cortical hyperplasia with lack of proper zonation, accumulation of steroid precursors, and both glucocorticoid and mineralocorticoid deficiency (515). Intra-adrenal injection of AAVs carrying human *CYP21A2* reverted the CAH-like phenotype for 40 days. A drawback of AAVs is their induction of an inflammatory response, but adrenals of mice treated with gene therapy did not show active inflammation, possibly due to high intra-adrenal levels of glucocorticoids. (515). Restoration of adrenocortical function in *Cyp21a1*-null mice was also achieved through AAV-mediated delivery of murine *Cyp21a1* to the thigh muscles, suggesting that functional 21-hydroxylase

enzymatic activity does not have to be confined in the adrenal (516). Intravenous injections of AAVrh10-CAG-human CYP21A2-HA vector endowed with adrenocortical tropism efficiently restored near-normal adrenal function; cells in the zona fasciculata, but not in the zona glomerulosa or capsule, were efficiently transduced 2 weeks after a single AAV injection, and this was concomitant with a reduction of progesterone and ACTH levels (517). However, the restoration of proper steroidogenesis was only transient. A likely explanation of such phenomenon lies within the biology of the gland; through the use of specific transgenic mouse model (lineage tracing) we now know that the adrenal cortex undergoes a self-renewal process, and key paracrine effectors supporting a dynamic centripetal streaming of adrenocortical cells have been identified (518). Adrenocortical self-renewal relies on the differentiation of at least 2 cell populations of progenitor cells, located in capsular (expressing the transcription factor *Gli1*) and subcapsular compartments (secreting the morphogen Sonic Hedgehog, *Shh*). These 2 cell populations are able to differentiate and become fully mature steroidogenic cells forming the distinct histological and functional layers of the zona glomerulosa and zona fasciculata. If *Cyp21*--AAVs are not able to transduce adrenocortical stem/progenitor cells, as suggested from the studies cited above, newly formed steroidogenic cells will therefore be *Cyp21a1*-deficient and mice revert to a CAH phenotype. In the future, it will be important to determine AAVs serotypes that are able to efficiently transduce stem/progenitor cells in order to offer a long-term curative solution.

In considering the applicability of animal models for gene therapy of 21OHD, it must be kept in mind that mice do not express *Cyp17a1* in their adrenal glands and consequently cannot synthesize sex steroid precursors in the adrenals. Thus, mice cannot be used to model the efficacy of suppression of adrenal androgen secretion with gene therapy. Moreover, enzyme kinetics suggest that extra-adrenal expression of CYP21A2 using gene therapy is likely to produce adequate amounts of cortisol only with very high levels of precursor steroids, which means that this approach will be of limited utility in controlling adrenal androgen secretion in humans.

Psychological Risk Factors, Surveillance, and Intervention

Historically, psychological studies in CAH have emphasized the role of prenatal androgens on gender development (ie, gender identity, gender role, and sexual orientation) and other domains exhibiting sex-related variability (eg, cognitive abilities) (519-522). Most studies only concern females

with classic CAH, as only they provide opportunities to test hormonal hypotheses of gender development (523). However, this emphasis may promote the belief that atypical gender behavior or nonheterosexual attractions are causes for clinical concern to the extent that they are linked to the pathophysiology of CAH. Historically it has been assumed that men with CAH require little attention directed to their mental health because prenatal androgen exposure is typical of males.

Advances in therapeutics contribute to a disease-specific (“categorical”) approach to care (524, 525). In CAH, this approach has facilitated a fuller understanding of its genetics and pathophysiology and refinement of medical and surgical interventions (133, 526). A disease-specific approach also emphasizes the psychosexual aspects of CAH in affected females (520). Yet, a substantial body of evidence suggests that successful developmental trajectories in people with chronic medical conditions are influenced as much by the psychosocial environment, supports, and organization of healthcare delivery as by the specific nature of the person’s medical condition (524, 525). A more generic (or “noncategorical”) approach emphasizes the effects of repeated hospitalizations on the person’s psychosocial adaptation, irrespective of whether the hospitalizations were for asthma or CAH. Relatively neglected topics in CAH are those routinely addressed in more prevalent conditions, including effects on parenting and family, factors influencing adherence to the medical regimen, frequent doctor visits, impact on the person’s body- and self-image, and transition from pediatric to adult healthcare (Table 10).

Generic (or noncategorical) factors

Parental reactions to learning that their child has a serious and chronic medical condition—expressed as shock, panic, worry, and sometimes feelings of guilt—are common generic stressors (591). The mental health of patients with CAH is another example; although most studies focus on females, increased psychiatric symptomatology in both sexes mirrors observations for a wide range of chronic medical conditions (545, 592-595).

CAH-specific (or categorical) factors

Patient reactions to repeated genital examinations potentially threaten mental health and well-being (596). Apart from the effects of prenatal androgens on female reproductive anatomy, the influence of early androgen exposure on brain and gender development garners significant attention. Prenatal exposure to testosterone increases the expression of behaviors and interests more typical of males than females. The largest differences

Table 10. Selected generic and CAH-specific risk factors for mental health, psychosocial/psychosexual adaptation and well-being

| | Illustrative studies |
|---|----------------------|
| A. Generic (noncategorical) | |
| Males and females | |
| Challenges to parenting with | (527) |
| accompanying caregiver psychological distress | (528, 529) |
| negative emotional spillover effects from parent to child | (530-532) |
| perceived child vulnerability and overprotectiveness | (533, 534) |
| Burdens of clinic visits and adherence to sometimes complex and changing treatment regimens; emergency room visits and hospitalizations | (535-537) |
| Threats to body-image and self-esteem | (538, 539) |
| Higher rates of missed school and peer victimization | (540, 541) |
| Academic challenges | (542) |
| Problems of psychosocial adaptation (ie, increased psychological symptomatology in youth and adults compared with healthy comparison groups) | (540, 543-545) |
| Systemic weaknesses in the process of transitioning from pediatric to adult care | (546-548) |
| Career barriers for people with chronic illness | (549) |
| B. CAH-specific (categorical) | |
| Female-specific | |
| Early reactions to newborn with atypical genitalia (experiences in medical environment) | (547, 550) |
| Stigma (anticipated or experienced) stemming from atypical genitalia and its modulation by culture | (551-561) |
| Tension between person-first (ie, CAH as a medical condition) vs identity-first (intersex and LGBT advocacy); and related human rights perspectives | (562-564) |
| Secrecy | (560, 565-568) |
| Genital examinations and medical photography | (548, 569) |
| Gender of rearing in Prader V cases | (133, 570-572) |
| Genital surgery decision making and consequences for sexual function | (573) |
| outcomes of postponing surgery | (526) |
| outcomes of postponing surgery | (574) |
| Gender identity | (575-577) |
| Effects on social support | (578, 579) |
| Model of care | (340) |
| Males and females | |
| Terminology | (580-582) |
| Early puberty/attenuated adult height; growth hormone therapy | (33, 507) |
| Neurocognitive sequelae | (486, 583, 584) |
| prenatal dexamethasone | (372, 480, 487) |
| hyponatremic episodes | (585) |
| Fertility problems (testicular adrenal rest tumors in males; low levels of fecundity in females) | (586-590) |

between females with CAH and unaffected females are observed for childhood toy preferences and adolescent and adult hobbies and interests. The majority of girls and women with CAH experience a female binary gender identity (576), yet there is evidence that the strength of that identity may be reduced (575, 597). Although the sexual orientation of women with CAH is less likely to be exclusively heterosexual than is true for unaffected women, the majority are heterosexual (225, 598). Though prenatal androgen exposure may play a role in the development of these outcomes, its influence is much smaller than effects on gender-role behavior (521, 594, 597, 599).

Psychological assessment and interventions

In the general pediatric population, the base rate for having a psychiatric disorder at any time is about 20% (600), and is similar in European adults (601, 602), yet many with mental health problems are neither identified nor referred for specialized treatment (603, 604). Specialists treating patients with CAH should consider that many of their patients (and/or their caregivers) may be struggling with mental health problems which can impact the effectiveness of medical care provided. Consequently, regular screening of patient (and family) for risk and resilience factors are indicated along with evaluating the developmental, behavioral, emotional, social, and educational status of

the patient as part of ongoing clinical care. Pediatric assessments should also encompass self-perceptions of domain-specific competencies, body image, and experiences of gender typicality and contentedness (605). Comparable surveillance in adulthood is recommended (358). Psychosocial screening should be both general (psychiatric symptoms, coping with illness) and specific (negative body image related to challenges of endocrine management, anticipated or experienced stigma, distress over nonheterosexual interests or behaviors, avoidance of potential romantic relationships as maladaptive coping strategy, sexual dysfunction potentially related to genital surgery, and fertility concerns). Adult healthcare providers need to be comfortable in assessing these topics and refer to knowledgeable specialists who understand the psychological issues in CAH. A recommendation to connect with peer support can also be extremely useful although careful consideration of where to direct patients is warranted (578). There is specific guidance for clinicians regarding the psychological aspects of CAH that warrant evaluation and possible intervention (522).

Because optimal care in CAH involves multiple subspecialties, it is recommended that clinical services be comprehensive and integrated (340, 606, 607), but inclusion of medical psychologists in interdisciplinary healthcare teams for CAH is inconsistent. There are no mental health interventions specifically designed for CAH. Psychoeducational counseling that includes detailed discussion of CAH with the patient and caregivers should be provided in an iterative and developmentally sensitive manner. For girls with genital virilization, such counseling necessarily involves education regarding the process of sex development and the influence of excess androgens on genital growth. A recent Cochrane review of psychological interventions for parents of youth with chronic illness provides clinicians with evidence-based strategies for managing parenting challenges and enhancing psychosocial adaptation in both the parent and the child (608). Interventions to promote treatment adherence in other chronic conditions should be transferable to CAH (609). Although preparation for and assessment of readiness for transition from pediatric to adult care (610, 611) does not guarantee physical health and well-being in adulthood, reports of major morbidities in adult patients with CAH (358) warrant continued efforts to improve outcomes.

Urogenital Surgery

Decisions concerning feminizing surgery

Most girls with classic CAH are born with virilized external genitalia. Virilization may consist of fusion of the outer labia, a single opening of a common urogenital sinus,

a recessed vagina that enters into the common channel and clitoromegaly. The degree of virilization is variable, and is influenced by the severity of the enzymatic defect. To indicate the severity of virilization the Prader classification, or similar scales, may be used (Fig. 4). These anatomical variations affect the decisions regarding surgery: the timing, 1- or 2-stage surgery, the technique and the extensiveness of the procedure and risk for complications (612).

Feminizing surgery is often performed in early infancy/childhood in order to provide a female appearance of the genitalia in childhood, and to enable sexual intercourse in adult life. This complex surgery may lead to short- and long-term complications. Early surgery for girls with CAH has become controversial. Many surgeons prefer complete surgical repair at an early age because of good elasticity of the tissue, prevention of possible hydrometrocolpos and reduction of parents' distress (613). However, concerns have been raised regarding body integrity and the inability of children to provide informed consent for early surgery. Unsatisfactory outcomes regarding genital sensation and sexual function, and greater acceptance of gender nonbinary status have led some to advocate that surgery be postponed until patients can express their gender and wishes (614). However, the effects of growing up with atypical genitalia on mental health or on sexual satisfaction are unknown and may vary in different cultures. The majority of patients and their parents in an American survey endorsed early reconstructive surgery (615). Families should be informed about surgical options including avoiding or delaying surgery. There should be a shared decision-making process including the family, endocrinologist, surgeon, and mental health professionals, and the surgery should be performed by an experienced surgeon (133, 579, 616, 617).

Surgical techniques, outcomes, and complications

Feminizing genitoplasty involves clitoroplasty, opening of the vaginal introitus, and labioplasty. When the patient has a high urethra–vaginal confluence, vaginoplasty may be postponed to later in life. Surgical techniques have been adjusted for best preservation of clitoral sensitivity and least vaginal stenosis (618). However, functional results of the current techniques can only be evaluated after many years.

In tandem with the Endocrine Society's 2018 Guideline, a systematic review and meta-analysis found no data to support 1 approach over another (526). The data included 29 observational studies (1178 CAH women, mean age at the time of surgery 2.7 ± 4.7 years, mostly classic CAH). After an average follow-up of 10.3 years, the majority who underwent surgery had a female gender identity (88.7%) and were heterosexual (76.2%). Women who underwent surgery reported a lower than optimal Female Sexual Function Index Score of 25.13 out of a maximum possible

score of 36, with 26 being the threshold accepted for risk for sexual dysfunction (619). Many patients reported impairment of clitoral sensitivity (620, 621), uncomfortable vaginal penetrative intercourse, and low frequency of intercourse (21). The majority of patients (79.4%) and treating healthcare professionals (71.8%) were satisfied with the surgical outcomes. The most common clinical finding was vaginal stenosis, whereas other surgical complications, such as fistulas, urinary incontinence, and urinary tract infections, were less common (622). Data on quality of life were sparse and inconclusive. To date there are no systematic prospective studies documenting outcomes in girls and women with CAH who did not undergo urogenital reconstruction; until recently most nonoperated girls have been those who were only mildly virilized. Reoperations are usually much less extensive surgical procedures than the initial genitoplasty. Most consist of widening the vaginal introitus; clitoroplasty has also been performed.

There are no studies comparing different techniques of feminizing surgery nor studies comparing early vs late surgery (526). The Endocrine Society's Guideline (133) cites urogenital mobilization with or without neurovascular-sparing clitoroplasty as the techniques now preferred by many surgeons. No evidence-based guidelines for surgical management exist, and further long-term follow-up studies are needed.

Long-term Sequelae

Gonadal Function in Males

In men with CAH, gonadal and reproductive function are often impaired due to primary gonadal failure from TARTs and/or secondary gonadal failure due to suppressed hypothalamic-pituitary-gonadal axis as a consequence of high adrenal androgen concentrations (376, 435, 436, 623).

Testicular adrenal rest tumors

TARTs are benign testicular tumors typically found in males with classic CAH (376, 435). TARTs have histological similarities to adrenocortical cells and are believed to originate from aberrant adrenal like cells in the testes but the etiology is not yet fully understood (376). TARTs are usually bilateral (70-100% of the cases) and painless (128, 435, 436, 624-629), but discomfort can occur, especially in patients with extensive tumors (630). TARTs less than 2 cm diameter are difficult to detect by palpation (435). Both ultrasound and MRI can be used to detect/confirm TARTs with similar sensitivity down to a few millimeters, but ultrasound costs less (437, 628, 631). The reported prevalence of TARTs in CAH ranges from 14% to 86% (435, 632), with an average of 25%

in adolescents, and 46% in men (376). TARTs are found occasionally in patients with NC CAH (435, 437). They occur not only in 21OHD but also in 11 β -hydroxylase and 3 β -hydroxysteroid dehydrogenase type 2 deficiencies (633, 634).

Elevated ACTH concentrations may play an important role in the development of TARTs. Suppression of ACTH secretion by increased doses of glucocorticoid can decrease the size of TARTs in some cases and may restore fertility (635-637). However, TARTs also occur in well-controlled patients and only a few studies have found a clear association between hormonal control, and TARTs (435, 638, 639). Moreover, there seems to be no correlation between TARTs and bilateral adrenalectomy, a condition that usually leads to high ACTH concentrations (501, 640).

It is important to discriminate Leydig cell tumors from TARTs, due to the malignant potential of Leydig cell tumors, but this cannot be done by either palpation or imaging (630, 641). TARTs are usually bilateral whereas Leydig cell tumors are mostly unilateral and often produce estrogens (376). TART size may decrease in some cases after intensified glucocorticoid dosing (586, 630, 635-637). Additionally, characteristic histologic structures called Reinke crystalloids can sometimes be found in Leydig cell tumors but never in TARTs (376, 630, 631). Furthermore, Leydig cell tumors are very rare in CAH while TARTs are very common.

The central location of TARTs in the testes may result in mechanical obstruction of the seminiferous tubules with azoospermia and irreversible peritubular fibrosis (642). Moreover, the paracrine effects of steroids produced by TARTs may destroy the surrounding Sertoli or germ cells (376). Testis sparing surgery has been described in TARTs but usually does not improve gonadal function, probably owing to irreversible damage to the testis (635). Regular testicular ultrasound is recommended (every 2-5 years if TARTs are small and stable) and if an increased TART burden is found, glucocorticoid therapy should be optimized and cryopreservation of sperm offered (435).

Secondary gonadal failure

Poor hormonal control in CAH results in increased risk of hypogonadotropic hypogonadism (435, 624), because high levels of adrenal androgen precursors will be aromatized to estrogens and suppress the hypothalamic-pituitary-gonadal axis. Steroids produced by TARTs can also suppress gonadotropin secretion (436). Even though most males with CAH and secondary gonadal failure will compensate for reduced testicular testosterone production with increased adrenal testosterone, low testosterone levels are found in some patients (436, 643). Overtreatment with glucocorticoids in men with CAH may also induce gonadal

failure (587); optimizing glucocorticoid therapy will usually reverse this.

Paternity

There are few controlled studies of fertility in men with CAH. In a Finnish study of 29 young men with classic CAH a child rate of 0.07 children per adult male was reported, which was significantly lower than the 0.34 in the entire Finnish male population with a similar age distribution (644). In a similar Swedish study of 30 men with CAH the child rate was 0.9 compared with 1.8 in the entire age-matched Swedish population (435). Of 30 US men with CAH only 7% had fathered children (636), and of 22 German men with CAH, 23% had children (587). Of 65 British men with CAH, 25% had become fathers, 2 after fertility treatment, but only 37% had tried to become fathers (358). Finally, of 219 French men with classic CAH, 24% had children (11% after in vitro fertilization), and this fertility rate was lower than the national reference population (624). Men with CAH seem to be less sexually active than matched controls (366). However, of 221 Swedish men with CAH and 22 100 matched controls, only those born before neonatal screening had a reduced child rate (odds ratio 0.5, ie, half as likely to have fathered a child), suggesting that fertility may not be reduced for most men with CAH in the future. Men with NC CAH had a normal child rate and of those who, irrespective of genotype or phenotype, had succeeded in having children, the number of offspring was similar to controls (588). Men with CAH adopted children more often (odds ratio 2.9) (588).

Reproductive Function in Women

CAH affects gonadal function and fertility in women. In general, there is an association between the severity of the CAH phenotype and the level of gonadal dysfunction and fertility (623).

Pubertal development

Age of menarche is normal in well-controlled girls, with no difference between SW, SV, and NC CAH (34, 354, 370, 423, 645). However, when glucocorticoid therapy is withheld or inadequate, menarche is delayed (646). Irregular menstrual patterns in CAH are associated with other hyperandrogenic signs such as acne and hirsutism and signs of insulin resistance. This clinical picture closely resembles polycystic ovary syndrome (427). Sonographic findings of polycystic ovarian appearance have been reported in adult women with CAH (about 20-50%) and in a minority of adolescent patients (647-651). Breast development can be impaired in case of inadequate androgen control (646, 652). The European multicenter dsd-LIFE study showed that only 68% of adults

with CAH had reached Tanner stage B5 compared with 90% in women without DSD (653).

Fertility

Compared with age-matched controls, women with CAH have fewer pregnancies and children. In a Finnish study, the mean child rate was 0.34 vs 0.91 in the general Finnish female population, and was lower in SW than in non-SW women (654). In a Swedish study, the number of pregnancies was 50% lower than in age-matched controls (370); 16 of 19 women who attempted pregnancy succeeded in becoming pregnant and there was a clear relationship between more severe genotypes and fewer children. Of 106 CAH patients in the UK, 25 considered motherhood and 23 had actively attempted conception, of whom 21 achieved 34 pregnancies (589). The pregnancy rate in this subgroup was similar to that in the normal UK population (95%), and similar in the SW (88.9%) and non-SW (92.9%) subgroups. However, women with SW were less likely to seek motherhood. More recently, the dsd-LIFE study reported that only 14.7% of 221 CAH women had 1 or more children without assisted reproduction techniques (ART), and 1.9% with ART (655). In a recent Swedish epidemiological study using the national CAH registry, 272 females with CAH (aged 14 years or above) were compared with 27 200 matched controls (656). Only 25.4% of women with CAH had given birth compared with 45.8% of controls. Furthermore, mothers with CAH were older and had fewer children.

All studies have emphasized that the major cause for low child rates is that women with CAH are less likely to seek motherhood. Women with the SW phenotype show the lowest interest in motherhood. This may be caused by the effects of prenatal androgen exposure on gender role behavior, including reduced interest in infants (590, 657), the lack of a partner, dissatisfaction with genital appearance, decreased sexual satisfaction, and urogenital and sexual dysfunction as a result of corrective surgery (363). When patients attempt pregnancy, the success rate seems to have increased in the last twenty years, as a result of various factors, including increased understanding of the effect of androgen and progesterone levels, and the level of mineralocorticoid substitution (35).

Optimizing fertility in women

A large review of case reports of women with classic CAH included 159 pregnancies since 1999. In 84 pregnancies the mode of conception was reported, and 62/84 pregnancies were spontaneous (363). When pregnancy is attempted and especially when spontaneous conception fails, the first approach is to optimize glucocorticoid therapy, aiming at normal androgen and follicular-phase progesterone levels

(589, 658, 659). Second, optimizing mineralocorticoid treatment appears to improve fertility SW and SV patients (363, 589, 660), but the exact mechanism remains unknown.

If needed, and especially when the above approaches are unsuccessful, assisted reproduction techniques can be used for ovulation induction and conception (661, 662).

Most (53-68%) women with NC CAH conceive spontaneously without any treatment (647, 663). Of 190 women with NC CAH, 95 wanted pregnancy and 187 pregnancies occurred in 85 women. Of these pregnancies, 99 occurred before the diagnosis of NC CAH (96/99 spontaneous), and 88 (47%) after the diagnosis (11/88 spontaneous) (647). Therefore, in case of subfertility (or recurrent miscarriages) there is a clear indication for temporary glucocorticoid treatment in NC CAH (37, 133). Glucocorticoid treatment shortens the time to pregnancy from about 1 year to less than 6 months (664). If conception cannot be achieved with glucocorticoids, ovulation induction is usually successful. The course of pregnancy is usually uneventful; however, the miscarriage rate in women with NC CAH is substantially higher (25%) than in the general population (6%) in some (647, 663) but not all (664) studies. The miscarriage rate can be reduced to normal in women treated with low to moderate doses of HC (647), prednisolone, or prednisone (663) prior to and during pregnancy.

Pregnancy outcome

Pregnancy outcome is good in women with CAH (363). Placental aromatase activity protects the fetus from maternal androgens (100). Gestational diabetes has been described relatively frequently (370, 656). Adjustments in glucocorticoid (and fludrocortisone) dose are usually necessary, especially in the third trimester (133), similar to pregnancies in women with primary adrenal insufficiency (665, 666). In the offspring, the rate for small for gestational age seems to be increased in some (363), but not all (656) studies, and no other problems are seen at follow-up (667).

Cardiovascular and Metabolic Morbidity

Metabolic consequences

The prevalence of overweight and obesity are greater in adults with CAH in the UK (358) and Sweden (668) but similar to the general population in the US (215) and France (624, 669). Increased abdominal adiposity, with a higher proportion of proinflammatory visceral adipose tissue compared with subcutaneous adipose tissue, was present in adolescents and young adults with CAH compared with age-, sex-, and BMI-matched controls (670). Metabolic syndrome was observed in nearly 20%

of adults in the NIH's cross-sectional study cohort (215), associated with older age but not with androgens, glucocorticoid type, or dose. The Endocrine Society's systematic review of relevant literature published through early 2016 included 20 observational studies (14 longitudinal, 6 cross-sectional) with a moderate to high risk of bias (671). The average dose of glucocorticoids (in HC equivalents) was 9 to 26.5 mg/m²/day. In the meta-analysis (416 patients, 14 months-63 years old), compared with controls, individuals with CAH had increased values for the homeostatic model assessment of insulin resistance (HOMA-IR; weighted mean difference [WMD] 0.49; 95% CI 0.02-0.96); however, no differences were noted in fasting blood glucose, insulin level, and glucose or insulin level after 2-hour glucose load, or serum lipids.

Blood pressure

Some studies report normal resting (215, 624, 672) and 24-hour blood pressure profiles (673) whereas others report a slight increase in either diurnal or both diurnal and nocturnal systolic blood pressure compared with matched controls even in childhood (674, 675). There are minimal data on the prevalence of hypertension in adults with CAH (676, 677), with inconsistent results in individual studies conducted in different locales (624, 668, 678). The systematic review and meta-analysis (671) found that individuals with CAH had modestly increased systolic blood pressure (WMD 4.44 mmHg; 95% CI 3.26-5.63 mmHg) and diastolic blood pressure (WMD 2.35 mmHg; 95% CI 0.49-4.20 mmHg). The authors were unable to draw conclusions regarding the effects of several important variables such as sex, glucocorticoid type and dose, fludrocortisone dose, and genotype, and bias in the individual reports was moderate to high.

Cardiovascular consequences

Cardiovascular morbidity and mortality are difficult to assess in CAH, as few of the studied patients are older than 50 years (679). Results for carotid intima media thickness (cIMT), a surrogate marker of cardiac dysfunction, vary in existing studies (674, 675, 680), without correlation between cIMT and cumulative glucocorticoid doses or androgen levels (680). A systematic review and meta-analysis showed slight but significantly greater carotid intima thickness (WMD 0.08 mm; 95% CI 0.01-0.15 mm) (671). In adolescent and adult CAH patients, normal left ventricular morphology has been reported (674, 681), but mild diastolic dysfunction and impaired exercise performance were shown. Recently, a French group reported the complex interactions between gonadotropins and steroid hormones on the duration of ventricular repolarization. QT interval duration was shorter in women with CAH than in control

women (682). A Swedish study analyzed cardiovascular and metabolic morbidity in CAH patients, finding increases in both cardiovascular and metabolic disorders including higher frequencies of hypertension, dyslipidemia, and atrial fibrillation (668). Obesity was consistently increased in all subgroups while diabetes was increased in females, SV and NC phenotypes, and those above 40 years of age. However, the nonobese patients were similarly affected by hypertension and diabetes as the entire CAH cohort. This study also found an increased frequency of venous thromboembolic events, which should be studied further to determine if, as reported in both Cushing syndrome and glucocorticoid use, there is a higher risk of venous thromboembolism due to hypercoagulability that should prompt a lower threshold for thrombosis prophylaxis in this population.

Thus, CAH may be associated with higher cardiovascular risk (683, 684). Increased cardiovascular mortality has been reported in CAH in Sweden, second only to adrenal crisis as a cause of death (366). Data on cardiac events are sparse, and most of the literature has focused on surrogate outcomes, rather than episodes of acute myocardial infarction, heart failure, or death. Some subgroups of patients seem to be more affected by cardiovascular risk factors. Regular follow-up is needed, along with lifestyle interventions, to limit weight gain, prevent obesity, and screen for diabetes (especially gestational diabetes), and dyslipidemia. Close monitoring of glucocorticoid and mineralocorticoid doses is important. Further prospective studies on larger cohorts are necessary to clarify the mechanisms leading to metabolic and cardiovascular abnormalities, and to understand the respective roles of adrenal sex hormones, lifelong glucocorticoid and mineralocorticoid treatment (364), and the impact of genetic background, such as glucocorticoid receptor gene polymorphisms, and other loci contributing to adverse cardio-metabolic risk profiles (685).

Neurological aspects

Early hormonal alterations affect the development of mammalian neural circuits. Widespread expression of androgen and glucocorticoid receptors in the brain suggest that fetal and postnatal imbalances in androgen and glucocorticoid exposure characteristic of CAH might influence brain development and function, with the potential to impact mental health (686). Compared with controls, patients with classic CAH have higher prevalence of anxiety, depression, alcohol misuse, suicidality, and adjustment disorders (392, 545, 595) (also see section “Management,” “Psychological risk factors, surveillance, and intervention”). Males diagnosed beyond the neonatal period and women with the most severe null genotype are especially at risk for mental health issues (545, 592, 595).

Neuroimaging studies in patients with CAH have revealed alterations in brain structure and function. In a functional MRI study of 14 adolescents with classic CAH compared with age-matched controls, girls with CAH showed a similar pattern of amygdala activation to control boys, suggesting an androgen effect on amygdala function in girls with CAH (687). Glucocorticoid therapy has been implicated in the development of white matter hyperintensities which reflect reduction of white matter structural integrity (688). White matter hyperintensities are found in patients with CAH, but are an uncommon finding in healthy adults aged <45 years (689, 690). Glucocorticoid therapy in CAH has been reported to affect working memory and digit span scores; patients on higher glucocorticoid doses have worse performance (688). Memory impairment is similarly found among patients with Cushing disease and Cushing syndrome (691).

Structural differences in gray matter morphometry in the medial temporal lobe were found in a cross-sectional MRI study of 27 adolescents with CAH (692). Young people with classic CAH had smaller regional volumes in the prefrontal cortex, amygdala, and hippocampus and overall smaller brain volumes than age-matched controls. In a study of 37 young adults with CAH, alterations in gray matter structure, including the middle frontal gyrus and the parietal and superior occipital cortex were found in CAH patients compared with controls (693). These regions play a role in visuospatial working memory and patients performed worse in visuospatial working memory tasks.

All of the neuroimaging studies are hindered by small sample size (686). Decreased brain volume has been observed in patients compared with controls in multiple studies and this needs to be accounted for when evaluating individual brain regions. Moreover, sex matching is essential since human male/female differences have been found in total brain volume (694), gray matter brain volume in specific regions (695) and brain connectivity (696); sexual dimorphism of the brain has also been found during childhood (697) (reviewed in (686)). In CAH, there are multiple hormonal imbalances including in utero glucocorticoid deficiency and androgen excess, postnatal androgen excess and iatrogenic glucocorticoid excess, and epinephrine deficiency, all possibly occurring during different developmental periods and with varying potential impact on neural circuits.

Bone

Since patients with CAH are on lifelong glucocorticoid supplementation, reduced BMD and osteoporosis are potential long-term outcomes. Epidemiological and other studies have demonstrated that glucocorticoids cause

secondary osteoporosis and increase fracture risk (698, 699). Both direct and indirect effects by glucocorticoids on bone result in initial increased resorption and later decreased bone formation leading to micro-architectural distortion and fracture risk (700). Moreover, glucocorticoids may cause secondary hyperparathyroidism by decreasing intestinal calcium absorption and increasing renal calcium excretion. Despite the known negative effects of glucocorticoids on BMD, studies with patients with CAH have reported inconsistent findings. A few studies have reported normal (701-706) or even high BMD (707), but most have shown low BMD at all or at least some sites (207, 215, 358, 643, 708-719). These differences may be due to both glucocorticoid and androgen exposure, since androgens stimulate osteoblast proliferation and differentiation in both genders (720). Adrenal androgens, including DHEAS, affect bone metabolism throughout life, especially during adrenarche, with effects mainly on cortical bone (721). Thus, late diagnosis and/or poor hormonal control may improve BMD due to high androgen concentrations (643, 715). Moreover, different glucocorticoid regimens may affect BMD differently; HC seems to affect BMD less than longer acting glucocorticoids, especially Dex (367). A recent meta-analysis comparing patients with CAH and matched controls found slightly decreased BMD in patients with CAH (722). Furthermore, adult women with CAH had more fractures than matched controls (715) whereas men with CAH did not (643). Patients with classic CAH had more nontraumatic fractures than those with NC CAH (721). However, osteoporosis-related fractures typically occur after 50 years of age and very few older patients have been included in studies of BMD and fractures. BMD screening is recommended by the Endocrine Society in adults with CAH and a prolonged period of higher than average glucocorticoid dosing, or in patients who have had a nontraumatic fracture (133). Others have also suggested screening any patient upon transfer to adult care and every 2 to 5 years thereafter (679).

Adrenal tumors

Approximately 20% to 30% of adult patients with CAH have adrenal masses (723). Almost a quarter of these are benign adrenal myelolipomas, which generally occur in patients with a history of poor hormonal control, suggesting that persistent ACTH stimulation may play a role in pathogenesis (626, 723, 724). There is no evidence that adrenocortical carcinoma, a rare malignancy with poor prognosis, is more prevalent in CAH. Adrenocortical carcinomas can be distinguished from benign adrenal masses by their characteristic steroid profile as assessed with mass spectrometry-based methods (725).

CAH in Developing Countries—Challenges and Limitations

CAH management in developing countries is challenging. Newborn screening for CAH is not available in many developing countries (726, 727), delaying diagnosis and increasing mortality, particularly in boys who lack atypical genitalia (728, 729). Pediatric endocrinologists are scarce (558, 727, 730), and late referral to specialized centers may delay diagnosis and treatment. Hormonal assays for diagnosis and follow-up have limited availability and are expensive (727, 730). Needed medications may be available only on the black market (136, 554, 730). Delayed diagnosis (555, 731), emotional (728), and gender assignment problems (729, 732) negatively influence quality of life (558, 733).

There are also socioeconomic and cultural issues (730). Myths and misconceptions about ambiguous genitalia in certain communities may lead to discrimination against patients and families (554, 558). Gender reassignments in late-identified patients may be met with resistance or refusal because of social stigma and cultural pressure (727). Moreover, many developing countries also face poverty and insufficient basic medical knowledge (554, 730). These issues imply the need for better primary healthcare education. Educational materials in the local language may increase understanding of CAH among families and communities. Clinical guidelines for developing countries are needed, along with advocacy to encourage government policy to improve access to essential medications and implementation of newborn screening.

Future Directions

Basic Science

As discussed previously, there has been much recent progress in adrenal steroidogenesis as regards the alternative “backdoor” pathway to androgens and the importance of 11-oxo-androgens. Other unanswered questions in steroidogenesis remain (summarized in (42)). Areas requiring further study include more detailed understandings of how StAR imports cholesterol to the mitochondria inner membrane, and how the 17,20-lyase activity of CYP17A1 is regulated. Secretion of androgens and androgen precursors by the fetal adrenal gland is a key component of the pathophysiology of CAH, yet regulation of fetal adrenal growth and postnatal involution of the fetal zone are poorly understood, and teleologically it is unclear why primate adrenal glands normally secrete DHEA and androgens either prenatally or at adrenarche. Steroid synthesizing enzymes, including CYP21A2, are found in nonglandular tissues, but the functional significance of extraglandular steroidogenesis remains uncertain.

Clinical Management

Given the relative rarity of CAH, national and international registries are valuable in developing and testing best practices throughout the lifespan (734). Whereas it may be unrealistic to expect that every clinical site caring for CAH patients possesses a comprehensive, multidisciplinary team, networks of expert centers can ensure access to specialty care when necessary. Criteria defining a comprehensive expert level of care for CAH have been published (340, 735). Surveys show that patient satisfaction, provider training, research, and quality improvement activities vary among medical centers (736, 737); thus, there is a need for clinical benchmarks in management. Real world data including patient and family satisfaction, as well as peer observation of clinical care can help develop guidelines and decision support tools. By providing robust data on epidemiology, patients' characteristics, and current standard of care, registries have the potential to shape healthcare policy and, by engaging with patients, increase stakeholder involvement and improve the patient-centered experience (738). One example of outcomes from the I-CAH Registry has been to define acute adverse events associated with adrenal insufficiency including sick day episodes, adrenal crises, and hospitalizations among CAH patients (399). The challenge for rare disease registries is to ensure that the data represent the widest range of patients, and that the data are findable, accessible, interoperable, and reusable (FAIR) within a rigorous framework of data governance, integrated with other data sources through multiomics technology (739). With anticipated therapeutic advances over the next decade, the use of registries for measuring therapeutic effectiveness, as well as maintaining clinician and patient engagement, will become imperative (399). Given the relative rarity of CAH, national and international registries are valuable in developing and testing best practices throughout the lifespan (734). As it may be unrealistic to expect that every clinical site caring for CAH patients possesses a comprehensive, multidisciplinary team, networks of expert centers can ensure access to specialty care when necessary. Criteria defining a comprehensive expert level of care for CAH have been published (340, 735). Surveys show that patient satisfaction, provider training, research, and quality improvement activities vary among medical centers (736, 737), thus there is a need for clinical benchmarks in disease management. (399, 738). Other areas that could benefit from large-scale collaborative data collection include prenatal and neonatal diagnosis and treatment. With recent data pointing to potential serious adverse outcomes, long-term follow-up studies should closely monitor both CAH patients and unaffected siblings subjected to prenatal Dex treatment. As discussed in section "Diagnostics," "Neonatal screening," the suboptimal positive predictive value for immunoassay in many newborn

screening programs mandates further studies to determine the most cost effective strategies to improve screening sensitivity and specificity. Clinical trials for novel drug targets and potential gene therapy are in progress or planned that should provide additional treatment options. At the same time, more widespread availability of mass spectrometry-based assays for new steroid biomarkers, such as 11-oxo-androgens, may improve monitoring and titrating existing medication regimens.

Long-term management should emphasize the importance of a smooth transition from pediatric to adult medical care, with continued emphasis on risk assessment for adverse reproductive, psychosexual, cardiovascular, metabolic, and musculoskeletal outcomes. To this end, implementation of telemedicine services have lately been recognized as a valuable resource in managing patients living in remote areas or lacking access to specialty centers.

Much discourse and debate has centered on whether and when surgical intervention ought to be considered. A systematic review and meta-analysis found scant sound evidence to favor early surgery, delayed surgery, or no surgery (526). More work is needed to develop evidence-based guidelines for surgical treatment of CAH, including ideal timing of surgery, surgical technique, risk of incontinence, risk of additional surgery (such as repair of vaginal stenosis at puberty), risk of loss of sexual function, and extent of clitoral surgery. Given that the likelihood of performing randomized controlled trials in this area is minimal, long-term surveillance using commonly agreed and routinely collected clinical and patient reported outcome measures should be prioritized.

Not least among desired goals is for mental health professionals in collaboration with other specialists to develop and validate quality of life instruments specific to CAH. In summary, based on what has been learned from collective clinical and basic research, the outlook is optimistic for improved modes of CAH treatment and consequently better quality of life.

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