MECHANISMS IN ENDOCRINOLOGY

Rare defects in adrenal steroidogenesis

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Abstract

Congenital adrenal hyperplasia (CAH) is a group of genetic disorders of adrenal steroidogenesis that impair cortisol synthesis, with compensatory increases in ACTH leading to hyperplastic adrenals. The term 'CAH' is generally used to mean 'steroid 21-hydroxylase deficiency' (21OHD) as 21OHD accounts for about 95% of CAH in most populations; the incidences of the rare forms of CAH vary with ethnicity and geography. These forms of CAH are easily understood on the basis of the biochemistry of steroidogenesis. Defects in the steroidogenic acute regulatory protein, StAR, disrupt all steroidogenesis and are the second-most common form of CAH in Japan and Korea; very rare defects in the cholesterol side-chain cleavage enzyme, P450_{scc}, are clinically indistinguishable from StAR defects. Defects in 3 β -hydroxysteroid dehydrogenase, which also causes disordered sexual development, were once thought to be fairly common, but genetic analyses show that steroid measurements are generally unreliable for this disorder. Defects in 17-hydroxylase/17,20-lyase ablate synthesis of sex steroids and also cause mineralocorticoid hypertension; these are common in Brazil and in China. Isolated 17,20-lyase deficiency can be caused by rare mutations in at least three different proteins. P450 oxidoreductase (POR) is a co-factor used by 21-hydroxylase, 17-hydroxylase/17,20-lyase and aromatase; various POR defects, found in different populations, affect these enzymes differently. 11-Hydroxylase deficiency is the second-most common form of CAH in European populations but the retention of aldosterone synthesis distinguishes it from 21OHD. Aldosterone synthase deficiency is a rare salt-losing disorder. Mild, 'non-classic' defects in all of these factors have been described. Both the severe and non-classic disorders can be treated if recognized.

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Introduction: Overview of steroidogenesis

The term 'congenital adrenal hyperplasia' (CAH) properly refers to any disorder of steroidogenesis in which cortisol biosynthesis is impaired and there is consequent overproduction of ACTH with resulting adrenal

hyperplasia. Approximately 95% of all patients with CAH have a disorder in adrenal steroid 21-hydroxylation, catalyzed by microsomal P450_{c21}, encoded by *CYP21A2*. The genetics, enzymology, clinical findings, hormonal

Invited Author's profile

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patterns, diagnosis and treatment of 21-hydroxylase deficiency (21OHD) have been studied and discussed in detail (1). There are also some adrenal disorders of steroidogenesis in which ACTH is not overproduced and hence there is no adrenal hyperplasia. This review will consider all disorders of adrenal steroidogenesis other than 21OHD, whether or not there is adrenal hyperplasia; these forms of CAH are generally rare, but may be common in certain genetically isolated populations (2).

The clinical and laboratory features and key therapeutic approaches in each of these disorders are summarized in Table 1. Accurate measurement of steroid hormones, often with very small volumes of blood from infants, is essential. Traditional immunoassays are

widely used for this purpose and can be quite reliable in reference laboratories with well-established standards by age and sex. Immunoassays are rapidly being supplanted by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS), which eliminates the problems of steroid cross-reactivity in immunoassays (3, 4). Some laboratories also assess urinary steroids and their metabolites by gas chromatography followed by mass spectrometry (GC/MS), but fewer samples can be processed, so that this procedure is largely used for research (5). Irrespective of the assay technology used, it is the clinician's responsibility to become familiar with whatever assay technology is used for clinical diagnosis, and its limitations.

Table 1 Clinical and laboratory findings in uncommon forms of CAH.

Enzyme (gene)	Presentation	Clinical and laboratory findings	Treatment
Lipoid CAH StAR (<i>STAR</i>)	Classic form: Salt-wasting crisis 46,XY DSD Non-classic form: Mild adrenal insufficiency without DSD	Low/absent levels of all steroids Decreased/absent response to ACTH Decreased/absent response to hCG in 46,XY DSD ↑ ACTH and renin	Glucocorticoid and mineralocorticoid replacement, salt supplementation in infancy Estrogen replacement at ≥12 year Consider gonadectomy of 46,XY
P450 _{scc} (<i>CYP11A1</i>) 3βHSD (<i>HSD3B2</i>)	As in lipoid CAH Salt-wasting crisis 46,XY and 46,XX DSD	As in lipoid CAH ↑ Δ5 steroids before and after ACTH ↑ Δ5/Δ54 serum steroids Suppression of elevated adrenal steroids after glucocorticoid administration ↑ ACTH and renin	As in lipoid CAH Glucocorticoid and mineralocorticoid replacement Salt supplementation in infancy Surgical correction of genitalia and sex steroid replacement consonant with sex of rearing
P450c17 (<i>CYP17A1</i>)	46,XY DSD Sexual infantilism Hypertension	↑ DOC, 18OH-DOC, corticosterone, 18OH-corticosterone Low 17OH steroids with poor responses to ACTH Poor response to hCG in 46,XY Suppression of elevated adrenal steroids after glucocorticoid administration ↑ ACTH and ↓ Renin Hypokalemia	Glucocorticoid administration Surgical correction of genitalia and sex steroid replacement in 46,XY DSD consistent with sex of rearing Estrogen replacement in females at ≥12 years Testosterone replacement if reared as male (rare)
17,20-Lyase deficiency syndrome (<i>CYB5</i>)	46,XY DSD	Low C19 steroids with poor responses to hCG	Estrogen replacement in females at ≥12 years Testosterone replacement in males
P450 Oxidoreductase (<i>POR</i>)	46,XX and 46,XY DSD Antley-Bixler syndrome in infants Infertility in adults	↑ ACTH, Prog, 17OHP ↓ DHEA, Andro, T Normal electrolytes	Glucocorticoid and sex steroid replacement Surgical correction of skeletal anomalies
P450c11β (<i>CYP11B1</i>)	46,XX DSD Postnatal virilization in males and females Occasional salt loss in newborns; hypertension in older children and adults	↑ 11-Deoxycortisol and DOC before and after ACTH ↑ Serum and urine androgens Suppression of elevated steroids after glucocorticoid administration ↑ ACTH and ↓ Renin Hypokalemia	Glucocorticoid administration Surgical correction of genitalia and sex steroid replacement in 46,XX DSD consistent with sex of rearing
P450c11AS (<i>CYP11B2</i>)	Failure to thrive Weakness Salt loss	Hyponatremia, hyperkalemia ↑ Corticosterone ↓ Aldosterone ↑ Renin	Mineralocorticoid replacement Salt supplementation in infancy

The pathways, enzymology and disorders of human steroid biosynthesis are now well understood (Fig. 1) (6). All steroid hormone synthesis begins from cholesterol, which can derive from dietary lipids or be synthesized *de novo*; the pathways of cholesterol biosynthesis and its disorders are also well characterized (7). Cholesterol is essentially insoluble in water and in cytoplasm; hence, the intracellular transport of cholesterol involves complex mechanisms (8), disruption of which can lead to rare disorders that may include adrenal insufficiency (9); these are typically considered as genetic forms of Addison's disease rather than as forms of adrenal hyperplasia (10). There are redundancies in the intracellular cholesterol transport mechanisms; consequently, disorders in these mechanisms may not be readily apparent. The first and rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone by the mitochondrial cholesterol side-chain cleavage enzyme, cytochrome

P450scc, encoded by the *CYP11A1* gene (11). P450scc is located on the inner mitochondrial membrane, where there is very little cholesterol; hence, mitochondrial cholesterol import is rate limiting in steroidogenesis. Mitochondrial cholesterol import remains incompletely understood, but is critically dependent on the steroidogenic acute regulatory protein, StAR (encoded by the *STAR* gene), which facilitates rapid flux of cholesterol into steroidogenic mitochondria, where it is converted to pregnenolone by P450scc (9). Disorders in StAR and P450scc are clinically and hormonally indistinguishable, but disordered StAR is associated with adrenal enlargement, whereas disordered P450scc is not. Pregnenolone may be converted to 17OH-pregnenolone (17OH-Preg) and thence to DHEA by the sequential 17-hydroxylase and 17,20-lyase activities of P450c17, a microsomal enzyme encoded by *CYP17A1*. The determination of whether a steroidogenic pathway stops with a 17-hydroxy 21-carbon (C21) steroid

Figure 1

Principal pathways of steroidogenesis. The figure incorporates pathways from adrenal and gonadal steroidogenesis. StAR facilitates most cholesterol influx into steroidogenic mitochondria, but some steroidogenesis occurs in its absence. Mitochondrial P450scc removes the cholesterol side chain to yield pregnenolone, the first C21 steroid. Δ^5 -steroids are converted to the corresponding Δ^4 -steroids by 3β HSD2 in the adrenal and gonad or by 3β HSD1 in the placenta and peripheral tissues. In the zona glomerulosa, progesterone, the first Δ^4 -steroid, is 21-hydroxylated by microsomal P450c21 to yield DOC, then mitochondrial P450c11AS catalyzes 11-hydroxylase, 18-hydroxylase and 18-methyl oxidase activities, to yield aldosterone (Aldo). In the zona fasciculata, expression of microsomal P450c17 permits synthesis of 17OHP, which is converted to cortisol by mitochondrial P450c11β. In the zona reticularis and testicular Leydig cells, expression of cytochrome b5 facilitates the 17,20-lyase activity of P450c17 converting 17OH-Preg to DHEA; human P450c17 converts 17OHP to androstenedione with only ~2% of its activity to convert 17OH-Preg to DHEA, so that most human androgen synthesis proceeds via DHEA, not via androstenedione. Testicular 17βHSD3 converts DHEA to androstenediol and androstenedione to testosterone; low levels of adrenal 17βHSD5 (AKR1C3) in the zona reticularis produce small amounts of testosterone. In ovarian granulosa cells, P450aro converts androstenedione to estrone and testosterone to estradiol; in estrogenic tissues (ovary, breast fat), 17βHSD1 converts estrone to estradiol. In genital skin and in the fetal testis, 5α-reductase type 2 further activates testosterone to dihydrotestosterone. (Copyright W L Miller.)

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or undergoes subsequent 17,20-lyase activity to yield 19-carbon (C19) androgen precursors depends on the availability of reducing equivalents supplied to P450c17 by NADPH (12). Pregnenolone, 17OH-Preg and DHEA are $\Delta 5$ steroids, retaining the double bond between carbon atoms 5 and 6 in the steroid B-ring. These hormonally inactive $\Delta 5$ -steroids can be converted to the corresponding $\Delta 4$ -steroids by 3 β -hydroxysteroid dehydrogenase type 2 (3 β HSD2, encoded by *HSD3B2*) in the adrenal and gonad or by the closely related 3 β HSD1 in placenta, liver and elsewhere. The adrenal zona glomerulosa does not express P450c17, permitting progesterone to be 21-hydroxylated by microsomal P450c21. In the glomerulosa, mitochondrial P450c11AS ('AS' designates 'aldosterone synthase', encoded by *CYP11B2*) sequentially catalyzes 11-hydroxylase, 18-hydroxylase and 18-methyl oxidase activities, yielding aldosterone. Expression of P450c17 in the adrenal zona fasciculata permits synthesis of 17OH-progesterone (17OHP), which is converted to cortisol by mitochondrial P450c11 β (11 β -hydroxylase, encoded by *CYP11B1*). When facilitated by the allosteric action of cytochrome *b*₅ (*b*₅, encoded by *CYB5*), P450c17 catalyzes 17,20-lyase activity, removing two carbons from the steroid side chain thus converting C21 steroids to C19 androgen precursors. However, even with the assistance of *b*₅, human P450c17 converts 17OHP to androstenedione with only ~2% of its activity to convert 17OH-Preg to DHEA, so that most human testosterone synthesis proceeds via DHEA, and not via androstenedione; by contrast, the P450c17 of rodents, cattle and many other animals catalyzes this step efficiently. In the adrenal zona reticularis, 17 β HSD5 (encoded by *AKRIC3*) converts small amounts of androstenedione to testosterone, whereas in the testis, this reaction is catalyzed by 17 β HSD3 (encoded by *HSD17B3*) and proceeds very efficiently. P450aro (aromatase, encoded by *CYP19A1*) converts androstenedione to estrone and testosterone to estradiol in ovarian granulosa cells (and adipocytes). These tissues also express 17 β HSD1 (encoded by *HSD17B1*), which converts estrone to estradiol. In genital skin (and to a lesser extent in the testis), 5 α -reductase type 2 (5 α Red2, encoded by *SRD5A2*) further activates testosterone to dihydrotestosterone.

StAR: the steroidogenic acute regulatory protein: classic and non-classic lipoid CAH

StAR facilitates the influx of cholesterol from the outer mitochondrial membrane (OMM) to the inner

mitochondrial membrane, where it may be converted to pregnenolone by P450scc. P450scc is the slowest steroidogenic enzyme, but the true rate-limiting step in steroidogenesis is import of cholesterol into mitochondria, facilitated by StAR acting exclusively on the OMM; the biologically active form of StAR is the extra-mitochondrial 37 kDa protein, while the intramitochondrial 30 kDa StAR is inactive because of its intramitochondrial location (8, 9, 13). Each molecule of StAR on the OMM facilitates the importation of 100–200 molecules of cholesterol, but the mechanism by which this happens remains unknown (14). The view that StAR's action requires the translocator protein (TSPO) has been disproven, at least in mice (15, 16, 17, 18), although it remains possible that it may serve some role in this process.

StAR mutations cause congenital lipoid adrenal hyperplasia (lipoid CAH) (19, 20). Lipoid CAH, the most severe steroidogenic disorder, typically presents in the first year of life with salt loss, very low serum steroids, high ACTH and plasma renin, minimal steroidal responses to long-term treatment with high doses of ACTH or human chorionic gonadotropin (hCG) and grossly enlarged adrenals laden with cholesterol and cholesterol esters (20). Because these findings indicate disruption in the conversion of cholesterol to pregnenolone, lipoid CAH was formerly misnamed '20,22-desmolase deficiency', an outmoded term for conversion of cholesterol to pregnenolone, but the *CYP11A1* gene for P450scc is not mutated in these patients (21). Rare *CYP11A1* mutations causing P450scc deficiency are clinically and hormonally indistinguishable from lipoid CAH, but are not characterized by gross adrenal enlargement (see below).

Low levels of StAR-independent steroidogenesis are seen in the absence of StAR, which suggested a two-hit model for the pathophysiology of lipoid CAH (20) (Fig. 2). The absence of StAR is the first hit, resulting in a loss of most (but not all) steroidogenesis. Diminished adrenal and testicular steroidogenesis leads to compensatory increases in ACTH and LH, which increase biosynthesis of receptors for low-density lipoproteins (LDLs), uptake of LDL cholesterol and *de novo* cholesterol synthesis. Over time, this intracellular cholesterol accumulates, causing mitochondrial and cellular damage from the accumulated cholesterol, cholesterol esters and their auto-oxidation products; this 'second hit' eventually destroys residual StAR-independent steroidogenesis (20). This two-hit model has been confirmed by clinical observations (22, 23) and by experiments in StAR-knockout mice (24, 25). In the affected 46,XY fetus, Leydig cells are destroyed early in gestation, eliminating

Figure 2

Two-hit model of lipoid CAH. (A) Normal adrenal cells derive cholesterol primarily from LDL by receptor-mediated endocytosis. LDL cholesterol is processed in lysosomes before entering the cellular pool; cholesterol can also be synthesized *de novo* from acetyl CoA. Cholesterol is stored in lipid droplets, reaches the mitochondria by both vesicular and non-vesicular means, and then travels from the OMM to the IMM by both StAR-dependent and StAR-independent mechanisms (9). (B) Early in lipoid CAH, absent StAR reduces mitochondrial cholesterol import and steroidogenesis, but some steroidogenesis persists by StAR-independent mechanisms. Decreased cortisol secretion leads to increased ACTH and increased cholesterol uptake and synthesis; cholesterol then accumulates in lipid droplets. (C) Accumulating lipid droplets damage the cell, eventually destroying all steroidogenic capacity. In the ovaries of affected females, follicular cells remain unstimulated and undamaged until they are recruited at the beginning of each cycle, when produce small amounts of estradiol, as in panel B, leading to feminization and anovulatory cycles. (From (20) with permission.)

biosynthesis of testosterone, resulting in disordered sexual development (DSD), with female-appearing external genitalia. However, the Sertoli cells continue to produce Müllerian inhibitory hormone appropriately, so that the phenotypically female 46,XY fetus lacks a cervix, uterus and fallopian tubes. In the adrenal, defective cortisol synthesis results in elevated ACTH secretion and severely impaired synthesis of DHEA, which eliminates fetoplacental estriol production. Aldosterone synthesis is severely impaired, causing salt loss, but this may be manifested at almost any time from the newborn period

to a year of age, irrespective of the *STAR* gene mutation, indicating variable destruction of the zona glomerulosa (20, 26). Genetic 46,XX females with lipoid CAH have normal genitalia at birth and appear to go through puberty, with breast development and cyclic vaginal bleeding in early adolescence (22, 23). The fetal ovary makes very little steroid and hence remains largely undamaged until it is stimulated by gonadotropins at the time of puberty, when StAR-independent steroidogenesis results in sufficient estrogen to feminize an adolescent female. Continued gonadotropin stimulation induces

cholesterol accumulation and cellular damage; although estrogen is made by StAR-independent steroidogenesis at the beginning of the menstrual cycle, progesterone synthesis late in the cycle is impaired. Gonadotropin stimulation sequentially recruits individual follicles; hence, most follicles remain undamaged so that an undamaged follicle is recruited in each cycle, resulting in monthly estrogen withdrawal bleeding and anovulatory cycles (22, 23).

Lipoid CAH is rare in most populations, but >100 patients have been reported with >40 StAR mutations (9). Lipoid CAH is the second-most common form of CAH in Korea and Japan where the heterozygous carrier rate is about 1 in 300, with the mutation p.Q258X accounting for most alleles (20, 27, 28, 29, 30). Other recurrent mutations include p.R182L, p.R182H and c.201_202delCT among Arab populations (2, 20, 26, 31) and p.L260P in Switzerland (32).

The classical patient with lipoid CAH is a phenotypically female infant with failure to thrive and salt loss in the first weeks of life, but some infants with severe StAR mutations have presented as late as 1 year of age (26). StAR mutations that retain about 20% activity, especially p.R188C, cause a mild 'non-classical' form of lipoid CAH (33, 34, 35, 36). Patients with non-classic lipoid CAH have mild adrenal insufficiency that may present at any time from childhood to adulthood, so that some of these patients have been mistaken for having familial glucocorticoid deficiency (34). Unlike classic lipoid CAH, non-classic 46,XY genetic males typically have normal-appearing external genitalia. Patients may have mildly compromised mineralocorticoid secretion, with normal serum electrolytes and elevated plasma renin, and some may have mild hypergonadotropic hypogonadism. Thus, disorders of StAR may manifest at any age.

Treatment of lipoid CAH consists of physiological replacement of glucocorticoids and mineralocorticoids, with supplementary salt in the newborn period. The glucocorticoid doses are those used in primary adrenal insufficiency and are less than those in 21OHD because it is not necessary to suppress excess adrenal androgen production. Growth should be normal. 46,XY males with female external genitalia usually undergo orchiectomy and are raised as females. Salt supplementation is typically weaned after 1 year of age, and mineralocorticoid doses are weaned thereafter, as the density of mineralocorticoid receptors in the renal epithelium increases with age, and because the diets of older children and adults tend to contain more sodium. Thus, mineralocorticoid dosing is variable and should be titrated to the blood pressure

and plasma renin. Pregnancy has been induced in 46,XX females with lipoid CAH (37, 38).

CYP11A1: the cholesterol side-chain cleavage enzyme (P450_{scc})

The first step in steroidogenesis is cleavage of the cholesterol side chain to yield pregnenolone. Three reactions are involved: 20-hydroxylation, 22-hydroxylation and scission of the 20-22 carbon bond; all are catalyzed by cytochrome P450_{scc}, encoded by *CYP11A1*. The seven human 'type I' P450 enzymes, including P450_{scc}, are found in mitochondria and mediate catalysis utilizing electrons donated by NADPH via the intermediacy of two electron transfer proteins – ferredoxin reductase and ferredoxin (Fig. 3) (39); the two isozymes of 11 β -hydroxylase (see below), and the vitamin D 1 α - and 24-hydroxylases are also type I P450 enzymes (40).

Deficient P450_{scc} activity was initially thought to cause lipoid CAH, but because P450_{scc} is needed for the placental production of progesterone, which is needed to suppress uterine contractility to permit term gestation, it was later thought that P450_{scc} deficiency would cause embryonic lethality. However, ~30 patients with P450_{scc} deficiency have been reported, including several with

Figure 3

Electron transport to mitochondrial P450 enzymes. A flavoprotein, termed ferredoxin reductase or adrenodoxin reductase (AdRed), is loosely bound to the inner mitochondrial membrane, accepts electrons (e^-) from NADPH and converts it to NADP $^+$. The electrons are passed to ferredoxin (adrenodoxin, Adx), an iron-sulfur protein in the mitochondrial matrix that functions as a freely diffusable electron shuttle mechanism that can donate electrons to any available mitochondrial cytochrome P450 such as P450_{scc}. The adrenodoxin can then recycle and receive another pair of electrons from adrenodoxin reductase. (Copyright W L Miller.)

complete loss-of-function mutations (9, 41). Fetuses with P450scc mutations probably reach term only when the maternal corpus luteum of pregnancy continues to produce progesterone into late pregnancy, but this has not been studied directly. These patients are clinically and hormonally indistinguishable from those with lipid CAH, although they typically lack the massive adrenal enlargement that is characteristic of lipid CAH; consequently, sequencing the *STAR* and *CYP11A1* genes is the only definitive way to differentiate these two disorders. A genetic isolate of P450scc deficiency has been reported in Southeastern Turkey, apparently due to a founder effect (42). A milder, 'non-classic' form of P450scc deficiency is found in patients whose P450scc mutations retain about 10–20% of WT activity (43, 44); these patients are hormonally and clinically indistinguishable from those with non-classic lipid CAH.

The discovery that complete deficiency of P450scc is compatible with term gestation in some affected fetuses suggests that mutations might be found in the genes for ferredoxin reductase or ferredoxin; these two proteins also participate in the synthesis of iron–sulfur centers utilized by some enzymes (45, 46). There are two forms of ferredoxin, FDX1 and FDX2: FDX1 interacts with mitochondrial P450s; FDX2 participates in synthesis of iron–sulfur clusters, but not in steroidogenesis (47, 48). FDX1 is especially abundant in the adrenal cortex; the corresponding *Fdx1* gene has been disrupted in zebrafish, inhibiting cortisol synthesis and causing developmental arrest (49), but no human or mouse mutations have been reported. For ferredoxin reductase, 15 missense (amino acid replacement) *FDXR* mutations causing neuropathic hearing loss and visual impairment have recently been reported in children from 17 families (50, 51). These findings apparently reflect the essential role of iron–sulfur proteins in neurologic function, but adrenal function was not examined in these patients, nor were the mutants examined functionally *in vitro* with P450scc or other mitochondrial P450 enzymes. Such studies would be of substantial interest.

***HSD3B2*: 3 β -hydroxysteroid dehydrogenase deficiency**

Using NAD as a co-factor, 3 β -hydroxysteroid dehydrogenase (3 β HSD) catalyzes two rapidly sequential reactions, 3 β -hydroxysteroid dehydrogenation and Δ 5– Δ 4-isomerization, of four adrenal Δ 5-steroids, pregnenolone, 17OH-Preg, DHEA and androstenediol,

yielding progesterone, 17OHP, androstenedione and testosterone, respectively. There are two forms of human 3 β HSD, encoded by two very similar genes: *HSD3B1* (encoding 3 β HSD1) is expressed in the placenta and peripheral tissues, and *HSD3B2* (encoding 3 β HSD2) is expressed in the adrenals and gonads (52, 53). These two isoforms share 93.5% sequence identity and catalyze the same reactions; however, the Michaelis–Menten constant (K_m) for 3 β HSD2 is about 5.5 μ M (54), approximately 10-fold higher than that of 3 β HSD1 (53, 55). 3 β HSD immunoreactivity has been found in the endoplasmic reticulum, cytoplasm and mitochondrial matrix (56); the mitochondrial intramembranous space appears to be the preferred location (57), favoring the conversion of pregnenolone to progesterone. Nevertheless, the K_m of 3 β HSD2 is much higher than that of P450c17 (~0.8 μ M) (58), which would favor production of 17OH-Preg and downstream cortisol and sex steroids. The subcellular distribution of 3 β HSD is an active area of research (14).

3 β HSD deficiency is a rare, severe form of CAH with glucocorticoid, mineralocorticoid and sex steroid deficiency. All reported cases of 3 β HSD deficiency are in the *HSD3B2* gene (2, 59, 60); mutations in 3 β HSD1 presumably would prevent placental biosynthesis of progesterone, resulting in a spontaneous first-trimester abortion. Affected 46,XX infants have clitoromegaly and mild virilization because some fetal adrenal DHEA is converted to testosterone via 3 β HSD1. Genetic males also synthesize some androgens by peripheral conversion of adrenal and testicular DHEA, but the concentrations are insufficient for complete male genital development, thus, DSD is seen in both sexes. Gonadectomy of 46,XY patients with 3 β HSD deficiency is controversial and may not be appropriate (61).

HSD3B1 expression in the liver and elsewhere persists in 3 β HSD2 deficiency; the low K_m of 3 β HSD1 permits it to produce some Δ 4-steroids from circulating Δ 5-precursors, thus complicating the diagnosis; some newborns with 3 β HSD2 deficiency have serum concentrations of 17OHP approaching those seen in patients with classical 21OHD (62). The principal diagnostic test in 3 β HSD deficiency is intravenous administration of ACTH with measurement of pregnenolone, 17OH-Preg and DHEA and their corresponding Δ 4 compounds. Steroidal responses to ACTH cannot reliably identify heterozygous carriers of 3 β HSD deficiency (63).

Ratios of Δ 5 to Δ 4 steroids that are elevated by 2–3 standard deviations following an ACTH test have led to many reports of 'partial 3 β HSD deficiency'. These patients are typically young females with premature adrenarche,

hirsutism, virilism and oligomenorrhea. However, the *HSD3B1* and *HSD3B2* genes in these patients are normal. In true 3β HSD deficiency, the ratios of $\Delta 5$ to $\Delta 4$ steroids exceed 8 SD above the mean (64, 65). Thus, ratios of $\Delta 5$ to $\Delta 4$ steroids are not reliable and cannot be used to diagnose 3β HSD deficiency; the diagnosis requires an ACTH test with a profound rise in $\Delta 5$ steroids (65). The basis of the mildly elevated ratios of $\Delta 5$ to $\Delta 4$ steroids in these hirsute individuals with normal 3β HSD genes is unknown and requires further study (14). Hirsutism can be ameliorated and regular menses can be restored in adult women with elevated ratios of $\Delta 5$ to $\Delta 4$ steroids by suppressing ACTH with low doses of glucocorticoids, but such treatment is contraindicated in girls who have not yet reached their final adult height.

CYP17A1: 17 α -hydroxylase deficiency

P450c17 is a type 2 (microsomal) P450 enzyme that is expressed in the adrenals and gonads and is encoded by the *CYP17A1* gene (66, 67). The 50 human microsomal P450 enzymes receive electrons from NADPH via P450 oxidoreductase (POR) (39); other microsomal P450s include P450c21, P450aro and most of the enzymes involved in drug metabolism and leukotriene synthesis (40). P450c17 catalyzes steroid 17 α -hydroxylation, steroid 16-hydroxylation and the 17,20-lyase activity that converts C21, 17-hydroxysteroids to C19 precursors of estrogens and androgens; thus, P450c17 is essential for both cortisol synthesis and reproduction. Human P450c17 catalyzes the 17-hydroxylation of pregnenolone and progesterone with equal efficiency, but efficiently catalyzes the cleavage of C21 steroids to C19 steroids only with 17OH-Preg. Thus, most human sex steroid synthesis proceeds via the pathway pregnenolone $\rightarrow$ 17OH-Preg $\rightarrow$ DHEA (58, 68); human P450c17 converts 17OHP to androstenedione with only ~2% of the activity to convert 17OH-Preg to DHEA, even though cattle and rodents catalyze this reaction easily (58). Human 17,20-lyase activity with 17OH-Preg is stimulated 10-fold by b5, acting as an allosteric factor rather than as an electron donor (58). Consistent with its essential role in 17,20-lyase activity, b5 is abundantly expressed in the adrenal zona reticularis but not in the zona fasciculata (69, 70). The 17,20-lyase activity of human P450c17 can also be augmented by its serine/threonine phosphorylation (71, 72), catalyzed by p38 α (MAPK14) (73). The 17,20-lyase activity of P450c17 is also essential in the alternative 'backdoor' pathway of androgen synthesis, in which 17OHP is 3 α - and 5 α -reduced,

cleaved to a C19 steroid by 17,20-lyase activity and then 17 β -reduced to androstenediol, which is then 3 α -oxidized to yield the most potent androgen, dihydrotestosterone, without going through the conventional intermediates (DHEA, androstenedione, testosterone) (6, 74) (Fig. 4). This pathway participates in the virilization seen in 21OHD (75).

Deficient P450c17 activity impairs cortisol and sex steroid synthesis, with compensatorily elevated ACTH and gonadotropins. Affected patients typically overproduce corticosterone (which compensates for the cortisol deficiency) and 11-deoxycorticosterone (DOC) (which often causes mineralocorticoid hypertension with sodium retention, hypokalemia, suppressed plasma renin and variably suppressed aldosterone); glucocorticoid replacement therapy typically normalizes these findings. Absent 17,20-lyase activity prevents sex steroid synthesis; consequently, 46,XX patients lacking all activity are phenotypically normal in childhood but do not undergo adrenarche or feminize at the time of puberty, while 46,XY patients have female or undervirilized external genitalia. Among patients with mutations that retain partial activity, females may have modest breast development and both sexes may present with DSD (76). The most common clinical presentation is a sexually infantile adolescent phenotypic female (either 46,XX or 46,XY) with mineralocorticoid hypertension. The diagnosis is established by finding elevated levels of DOC, corticosterone, 18OH-corticosterone and 18OH-DOC that are hyperresponsive to ACTH, and low concentrations of 17-hydroxylated steroids, which respond poorly to ACTH. Treatment consists of glucocorticoid replacement therapy to suppress the mineralocorticoid hypertension, and age-appropriate sex steroid replacement concordant with the sex of rearing.

Many *CYP17A1* mutations have been reported, often with a distinct ethno-geographic distribution (76, 77). The first-described mutation was a 4-base insertion among Dutch Mennonites and their Canadian descendants (78); a deletion of amino acids 487-489, first reported in a Thai patient of Chinese descent (79), has been found throughout Southeast Asia and China (80), the p.Y329Kfs frameshift mutation is common in Northern China and Korea (77, 80, 81, 82, 83); and in Brazil, where 17OHD appears to be the second most common form of CAH, the mutations p.R362C and p.W406R track with persons of Portuguese and Spanish descent, respectively (84). Most reported *CYP17A1* mutations ablate all activity, while some others partially affect both activities, usually about equally. Two studies have associated *CYP17A1*

Figure 4

The backdoor pathway of androgen synthesis. The backdoor pathway is similar to the general pathway shown in Fig. 1, except that 17OHP is sequentially 5 α - and 3 α -reduced, first by 5 α Red1 to 5 α -Pregnan-17 α -ol-3,20-dione, and then 3 α -reduced, probably by AKR1C2, to yield 17OH-allopregnanolone. P450c17 can catalyze 17,20 lyase activity using 17OH-allopregnanolone as the substrate without the allosteric action of b₅; this reaction yields androsterone, which then can be acted on by testicular 17 β HSD3 or adrenal 17 β HSD5 (AKR1C3) to yield androstanediol. Androstanediol may then be 3 α -oxidized, possibly by 17 β HSD6 (also known as retinol dehydrogenase, RoDH) or possibly by AKR1C4. The identities of the enzymes catalyzing the 3 α HSD reactions remain under investigation. (Copyright W L Miller.)

polymorphisms with hypertension (85, 86), suggesting that some individuals with low-renin hypertension that is responsive to mineralocorticoid-antagonists may have mild, unrecognized P450c17 deficiencies.

CYB5, CYP17A1 and others: 17,20 lyase deficiency

The 17,20-lyase activity of P450c17 converts C21 steroids to C19 precursors of sex steroids. Human P450c17, unlike that of most non-primate mammals, does not catalyze the conversion of 17OHP to androstenedione; hence, most human sex steroids are made via the pathway Preg $\rightarrow$ 17OH-Preg $\rightarrow$ DHEA $\rightarrow$ androstenedione (58, 68). Early reports of isolated 17,20-lyase deficiency in some patients incorrectly suggested that 17-hydroxylase and 17,20-lyase were separate enzymes, but it is now clear that P450c17 catalyzes both activities. The patients originally reported to have 17,20-lyase deficiency instead have mutations in two enzymes (AKR1C2 and AKR1C4) in the alternative, 'backdoor' pathway of androgen synthesis (87). It is now apparent that 17,20-lyase deficiency can be caused by mutations in several different genes (88). First, 17,20-lyase deficiency may be caused by specific mutations in P450c17; these are most commonly found

in the 'redox partner-binding site' of the enzyme, which interacts with and receives electrons from POR (89, 90), but mutations causing 17,20-lyase deficiency have also been found in the catalytic active site (91, 92). Second, b₅ deficiency causes 17,20-lyase deficiency. Cytochrome b₅ is a small hemoprotein that principally participates in the reduction of methemoglobin, but also acts as an allosteric factor to promote the interaction of P450c17 with POR, thus increasing 17,20-lyase activity about 10-fold (58). In the adrenal, b₅ expression is confined to the zona reticularis and coincides with the onset of adrenarche (69, 93). Cytochrome b₅ deficiency causes adrenal and testicular androgen deficiency, with associated methemoglobinemia (94, 95, 96). Third, rare mutations in the electron-donating domain of POR that interacts with P450c17 may also cause 17,20-lyase deficiency (97). Thus, 17,20-lyase deficiency is caused by mutations affecting the efficiency of electron transfer to P450c17; mutations in p38 α or other kinases that might participate in the phosphorylation of P450c17 have not (yet) been described, but might also cause 17,20-lyase deficiency (14). To date, all reported patients with 17,20-lyase deficiency have been 46,XY; this probably represents ascertainment from the DSD phenotype rather than genetic incompatibility with 46,XX, as both karyotypes can be seen in complete 17 α -hydroxylase deficiency.

POR: P450 oxidoreductase deficiency

P450c17, P450c21, P450aro and all other microsomal P450s must receive electrons from POR to mediate catalysis (40). As illustrated with 17,20-lyase deficiency, electron flow from POR is an important regulatory step. POR is a membrane-bound, butterfly-shaped protein that has a flavin adenine dinucleotide (FAD) moiety in one wing and an flavin mononucleotide (FMN) moiety in the other wing; the FAD receives two electrons from NADPH, transfers them to the FMN, which then transfers them to the P450 (39, 98) (Fig. 5).

Although POR-knockout mice die *in utero*, deficient POR activity causes an unusual form of CAH with DSD in both sexes (99, 100). Measurements of serum and urinary steroids show that POR deficiency is characterized by partially deficient P450c17 activity, with or without associated deficient activity of P450c21 and P450aro (99, 100, 101, 102). The spectrum of endocrine findings ranges from newborn males with androgen deficiency

(impaired P450c17 activity) and virilized newborn females (secondary to P450c21 deficiency) to men with infertility and women with polycystic ovary syndrome (103). POR-deficient patients usually have normal electrolytes and mineralocorticoid function, nearly-normal cortisol levels that respond poorly to ACTH, high concentrations of 17OHP that respond variably to ACTH and low levels of C19 precursors of sex steroids. Abnormal elevations in some of the steroids (and their metabolites) from the 'backdoor' pathway may assist in the diagnosis of POR deficiency (104). Some patients with POR deficiency are detected by newborn screening of 17OHP for 21OHD (99, 105). Because the 17,20-lyase activity of P450c17 is especially sensitive to perturbations in electron transport, defective fetal testicular steroidogenesis leads to incompletely developed external genitalia in affected males. The phenotypic outcome in genetic females strongly depends on the causative POR mutation (106). For example, POR p.A287P, the predominant mutation in Europe (99, 102), disrupts activity of P450c17 but not P450c21 (107), and the POR mutation p.R457H, which is common in Japan (100, 101), but not POR p.A287P, disrupts placental P450aro activity (108). Defective placental P450aro activity permits fetal C19 steroids to enter and virilize the mother; hence, pregnant women carrying a fetus with POR p.R457H (but not POR p.A287P) often experience virilization during pregnancy (99, 100, 101, 108), as evidenced by the low estriol values seen in women carrying a fetus with some POR mutations. In addition, the alternative 'backdoor pathway' of androgen production also contributes to the prenatal virilization of affected females (101). As noted, the incidence and phenotype of POR deficiency varies with ethnicity; furthermore, the POR gene is highly polymorphic: the sequence variant p.A503V, which mildly affects many P450 activities (106), is common among people of Chinese ancestry but rare among people of African ancestry, and the incidences of SNPs vary similarly (109).

Newborns with POR deficiency often have an associated skeletal defect known as Antley-Bixler syndrome (ABS), characterized by craniosynostosis, brachycephaly, radio-ulnar or radio-humeral synostosis, bowed femora, arachnodactyly, midface hypoplasia, proptosis and choanal stenosis. ABS has two causes: when seen in association with disordered steroidogenesis and DSD in either sex, the cause is autosomal recessive POR deficiency (99, 100, 103); when seen without a lesion in steroidogenesis, ABS is caused by dominant, gain-of-function mutations in fibroblast growth factor receptor 2 (100). POR deficiency impairs CYP26B1 activity, causing

Figure 5

Electron transport to microsomal P450 enzymes. P450 oxidoreductase (POR), bound to the endoplasmic reticulum, receives electrons (e^-) from NADPH and donates them to the FAD moiety of POR, eliciting a conformational change that brings the FAD and FMN moieties close together. The electrons pass from the FAD to the FMN, eliciting another conformational change that returns the protein to its original orientation. The FMN domain of POR then 'docks' with the redox partner binding site of the P450 by charge-charge interactions, and the electrons reach the P450 heme group to mediate catalysis. The substrate-binding site of the P450 lies on the side of heme ring containing the iron atom (Fe) opposite from the redox partner binding site. The interaction of human P450c17 and POR is facilitated by the allosteric action of cytochrome b_5 , and by serine phosphorylation of P450c17. (Copyright W L Miller.)

retinoic acid accumulation at embryonic sites that normally form skeletal joints and sutures, leading to their premature fusion and the ABS skeletal phenotype (110). POR-associated defects in cholesterol synthesis affecting signaling by *hedgehog* proteins may also play a role (6, 103).

POR is required by the principal hepatic drug-metabolizing enzymes (98, 106, 111). Mice with liver-specific POR knockouts metabolize drugs poorly and accumulate hepatic lipids, but similar problems have not been described in patients with POR deficiency. Numerous *in vitro* studies of drug-metabolizing enzymes show major impairment by POR mutations (98, 106, 111), but effects in POR-deficient patients are rare (112). Treatment of POR deficiency requires a multidisciplinary team of endocrinologists, geneticists, orthopedic and cranio-facial surgeons and family support; sex hormone replacement therapy should be started at pubertal age in both sexes. Some patients may require low-dose glucocorticoid replacement therapy, especially during severe illness; this should be determined individually by assessing the cortisol response to ACTH.

CYP11B1: 11-hydroxylase deficiency

The final steps in the synthesis of cortisol and aldosterone are catalyzed by P450c11 β and P450c11AS, respectively, encoded by the tandemly duplicated *CYP11B1* and *CYP11B2* genes (113, 114). Like P450scc discussed earlier, these are mitochondrial P450 enzymes that require electron transport from NADPH via ferredoxin reductase and ferredoxin (39). P450c11 β is abundantly expressed in the zona fasciculata in response to ACTH and catalyzes 11-hydroxylase activity, converting 11-deoxycortisol to cortisol. P450c11AS is expressed at low levels in the zona glomerulosa in response to angiotensin II and potassium and catalyzes the 11-hydroxylation, 18-hydroxylation and 18-methyl oxidase activities needed to convert DOC to aldosterone. Both P450c11 β and P450c11AS can convert DOC to corticosterone; P450c11 β also has weak 18-hydroxylase activity, but only P450c11AS can synthesize aldosterone from 18OH-corticosterone (115). *CYP11B1* mutations causing 11-hydroxylase deficiency (11OHD) disrupt synthesis of cortisol but not aldosterone; consequent ACTH excess causes fetal adrenal androgen secretion, causing virilization of affected females. A rare, non-classic form of 11OHD has also been described (116, 117). *CYP11B2* mutations cause forms of aldosterone

deficiency in which cortisol production is unaffected (see below).

11OHD impairs cortisol secretion, causing CAH with virilization of affected females. In the zona fasciculata, defective cortisol synthesis results in accumulation of 11-deoxycortisol (Reichstein's compound S), which is hyperresponsive to ACTH administration and is the key steroid used for diagnosis; defective corticosterone synthesis also results in overproduction of DOC, potentially leading to mineralocorticoid-based hypertension. Although DOC is a less potent mineralocorticoid than aldosterone, the high levels produced in 11OHD can cause salt retention and hypertension in older children and adults (114). Nevertheless, because newborns are relatively resistant to mineralocorticoids, they may have mild, transient salt loss (118, 119). Newborns may also have elevated concentrations of 17OHP, so that 11OHD may be detected in newborn screening for 21OHD (120).

It is widely stated that 11OHD accounts for about 5% of CAH in persons of European ancestry, a higher percentage in Middle Eastern populations (121, 122) and about 13.5% in Turkey (123). No prospective study has established its general incidence, but over 100 *CYP11B1* mutations have been reported (116, 124), with some genetically isolated groups having high incidences (e.g. *CYP11B1* p.R448H among Sephardic Jews of Moroccan ancestry and p.Q356X and p.G379V among Tunisian patients). In Turkey, 13 different *CYP11B1* mutations were found among 28 patients from 25 families (125). A rare, mild, non-classic form of 11OHD has been reported manifesting as hirsutism, virilism and menstrual irregularities in otherwise asymptomatic women or sexual precocity in men with *CYP11B1* mutations that retained partial activity (126, 127). Treatment of 11OHD is glucocorticoid replacement with doses similar to those used in 21OHD, but mineralocorticoid replacement is not needed.

CYP11B2: aldosterone synthase deficiency and glucocorticoid-remediable hyperaldosteronism

P450c11AS disorders cause aldosterone synthase deficiency, also known as 'corticosterone methyl oxidase' (CMO) deficiency, clinically described as two forms (114, 128). 'CMOI deficiency' results from absent P450c11AS activity; the disrupted 18-hydroxylase and 18 methyl oxidase activities impair biosynthesis of

18OH-corticosterone and aldosterone, but synthesis of corticosterone and cortisol by P450c11 β remains intact. Thus, there is suppressed aldosterone, increased ratio of corticosterone to 18OH-corticosterone and elevated plasma renin activity. *CYP11B2* mutations include frameshift mutations, premature stop codons and the missense mutation p.R384P. Infants may experience salt loss secondary to absent aldosterone biosynthesis as the secretion of DOC may be insufficient to meet the newborn's mineralocorticoid requirements. Plasma renin activity is markedly elevated in affected children but may be normal in affected adults. These patients may survive and reach adulthood without therapy, as mineralocorticoid sensitivity and sodium intake increase with age (129). 'CMOII deficiency' results from *CYP11B2* mutations that retain partial activity so that serum 18OH-corticosterone is high and aldosterone is low (114, 128). CMOII deficiency is common among Iranian Jews: affected individuals are homozygous for the two mutations p.R181W and p.V386A, whereas individuals homozygous for only one of these mutations are clinically unaffected (130). However, the clinical findings in 'CMOI' and 'CMOII' overlap (131, 132), similarly to the various forms of 21OHD.

Glucocorticoid-remediable hypertension is the opposite of aldosterone synthase deficiency, as P450c11AS activity is increased. Genetic recombination between the *CYP11B1* and *CYP11B2* genes creates a third, hybrid *CYP11B* gene in which the ACTH-regulated upstream portions of *CYP11B1* are fused in frame to the *CYP11B2*-coding regions, resulting in the ACTH-induced expression of a chimeric protein with aldosterone synthase activity, causing a fairly common, glucocorticoid-suppressible form of hyperaldosteronism (133, 134).

Conclusions

Some rare disorders of steroidogenesis (3 β HSD deficiency, 11OHD) have been known since the 1950s, while others have been described recently (deficiencies of P450scc and POR). The discovery of the genes encoding steroid-transforming enzymes has permitted identification of the genetic causes of known diseases and the prediction of new disorders. Knowledge of the steroidogenic enzymes and pathways of steroidogenesis permit one to predict the abnormal steroid metabolomes seen in these disorders. The availability of accurate, inexpensive LC-MS/MS steroid assays and DNA sequencing now permit the correct diagnosis and treatment of these disorders; these

improved diagnostic tactics are revealing that some of these disorders are not so rare as once thought.

Declaration of interest

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