

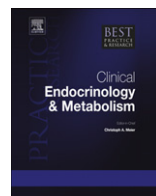


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46,XX DSD: the masculinised female

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The 46,XX disorders of sex development (DSDs) cause virilisation or masculinisation of the female foetus. The final common pathway of all 46,XX DSDs is excess dihydrotestosterone (DHT) or potent foreign androgen in the genital tissue during the critical period of sexual differentiation. Whereas the foetal testis is source of androgen in the male, it is the foetal adrenal that produces the DHT precursors in the female. By understanding the principles of human steroid biosynthesis, the pathogenesis of each disorder may be logically deduced, and treatment strategies are rationally constructed. In practice, however, therapies for many of these diseases are fraught with complications and caveats, and current approaches leave much room for improvement. This review discusses these diseases, their pathogenesis and approaches to therapy. We emphasise areas where improved treatments are sorely needed.

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Principles of foetal virilisation

Virilisation of the human foetus occurs independent of chromosomal sex when the external genitalia are exposed to androgens during certain critical times of development. Although the dominant androgen in the adult male is testosterone (T), it is dihydrotestosterone (DHT, 5 α -androstane-17 β -ol-3-one) that drives labioscrotal fusion and phallic growth in normal and pathologic conditions.¹ Labioscrotal fusion can only occur when DHT is present during the critical window between 8 and 12 weeks of gestational age. By contrast, phallic growth occurs throughout gestation but primarily manifests in the third trimester. The 46,XX disorders of sex development (DSDs) are therefore diseases

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of excessive exposure to DHT, and the clinical manifestations depend on both the amount of DHT in the circulation and the gestational age when the exposure occurs.

Whereas the foetal testis is the source of DHT in the developing male, it is the foetal adrenal that is the proximal source of 19-carbon steroids in 46,XX DSDs. The capacity of the adrenal gland to synthesise 19-carbon steroids, primarily dehydroepiandrosterone and its sulphate (DHEA[S]), is a curious property found mostly in large mammals and shared by human beings.² The need or physiologic functions supported by adrenal 19-carbon steroid synthesis remain obscure, although this process has received considerable study. Adrenal DHEAS synthesis increases exponentially throughout the first 2–3 decades of life³, leading to the phenomenon of adrenarche, which is the appearance of axillary and pubic hair in boys and girls about 8–10 years of age, independent of gonads and puberty.⁴ Circulating DHEAS concentrations become measurable in commercial assays at the same time, but recent studies have shown that adrenarche does not suddenly occur at this time but rather gradually and exponentially progresses from birth.⁵ In addition, adrenal DHEAS contributes to at least half the T production in the normal adult female. Nevertheless, it is this enigmatic capacity of the human foetal adrenal to produce 19-carbon steroids that allows 46,XX DSDs to occur.

Adrenal steroidogenesis

Normal adrenal steroidogenesis

The human adrenal cortex is divided into three zones with each having an associated major product (Fig. 1A). The zona glomerulosa, which comprises just a few cell layers underneath the adrenal capsule, synthesises aldosterone, primarily under the regulation of the renin/angiotensin system and serum potassium concentration. The zona fasciculata produces cortisol as the major glucocorticoid primarily under the stimulation of adreno corticotropin (ACTH), rather than corticosterone as in rodents and other small animals. The reason that the human adrenal makes cortisol is the presence of a third steroid hydroxylase in the human zona glomerulosa, which is 17-hydroxylase/17,20-lyase (P450c17, CYP17A1). CYP17A1 is also expressed in the inner zona reticularis, where DHEAS is the major product. The 17,20-lyase activity of CYP17A1 is manifest primarily in the zona reticularis and not in the zona fasciculata for several reasons⁴, primarily the lack of 3 β -hydroxysteroid dehydrogenase/ $\Delta^{5/4}$ -isomerase type 2 (3 β HSD2, HSD3B2)⁶, which limits drain of pregnenolone and other Δ^5 -steroids to other Δ^4 -products, and the abundance of cytochrome *b*₅ (b5)⁷, which selectively activates the 17,20-lyase activity.⁸ The enzymatic machinery in these cortical zones is selectively tailored to efficiently generate the target steroid product and to limit the accumulation of precursors and the generation of significant by-products.⁹ In enzyme-deficiency states, however, steroid flux is altered, and clinical manifestations often result.

The human adrenal gland makes little androstenedione and T compared to the massive production of DHEAS. Androstenedione synthesis is limited because the zona reticularis, where DHEA is synthesised, contains little HSD3B2 but abundant DHEA-sulphotransferase (SULT2A1).¹⁰ Consequently, nascent DHEA is mostly sulphated, and DHEAS must have its sulphate removed before it can be converted to androstenedione in the periphery, which is generally an inefficient process. In addition, 17-hydroxyprogesterone (17OHP) is a poor substrate for the 17,20-lyase reaction, with a catalytic efficiency nearly 100 times lower than 17-hydroxypregnenolone with human CYP17A1.⁸ Finally, the human adrenal does not express 17 β HSD3, the enzyme primarily responsible for conversion of AD to T in the foetal and adult testis.¹¹ Alternative enzymes of the AKR1C family, including AKR1C3 (17 β HSD5), are present and probably account for the small amounts of T production directly from the adrenal gland.¹²

The alternate or 'backdoor' pathway to DHT

In foetal and adult life, the testes synthesise primarily T, which is metabolised to DHT by the 5 α -reductase type 2 (SRD5A2), found in prostate and genital skin. In other species and in several small animals prior to puberty, the major testicular 19-carbon steroid product is 5 α -androstane-3 α , 17 β -diol (androstenediol or Adiol), rather than testosterone, which is likewise metabolised to DHT in target tissues.¹³ In this alternate or backdoor pathway to DHT, 5 α -reductase is present in the steroidogenic tissue and an oxidative 3 α -HSD, such as the RODH-like 3 α -HSD (17 β HSD6)¹⁴, completes the synthesis

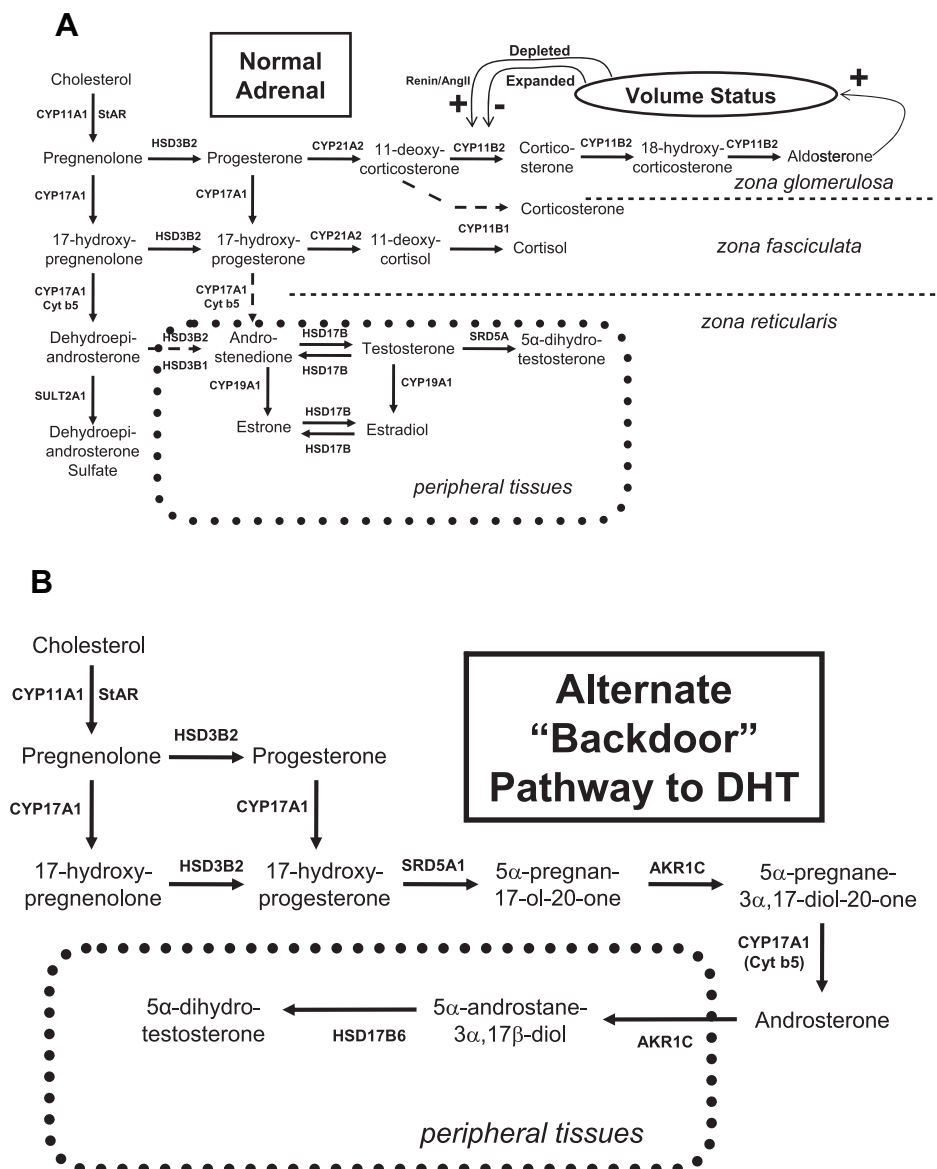


Fig. 1. Human steroid biosynthesis. Panel A, Normal steroidogenic pathways in the adrenal gland, with each zone specified, minor routes indicated by dashed arrows, and routes to androgens and estrogens in the periphery isolated by dotted box. A small amount of DHEA is converted in the adrenal by HSD3B2, and larger amounts derive from peripheral metabolism, mediated by HSD3B1. Panel B, the alternate or “backdoor” pathway to DHT, involving intra-adrenal SRD5A1.

of DHT (Fig. 1B). Accumulating evidence suggests that, in some pathologic conditions, the adrenal gland synthesises small amounts of 5α -reduced 19-carbon steroids from 21-carbon precursors, such as 17OHP. In fact, 5α - and 3α -reduced 17OHP (5α -pregnane- 3α , 17α -diol-20-one or ‘Pdiol’) is a better substrate for the 17,20-lyase activity of CYP17A1 than 17OHP or 17-hydroxypregnenolone.¹⁵ Furthermore, the product is androsterone, which is a potent androgen that is already 5α -reduced and readily metabolised to Adiol and then to DHT by several enzymes.

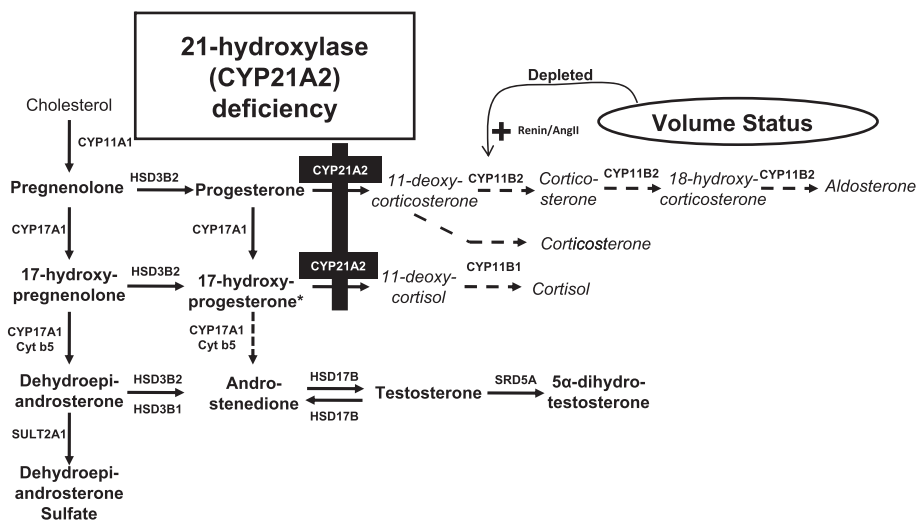


Fig. 2. Altered steroid biosynthesis in 21-hydroxylase deficiency. The block of cortisol and aldosterone synthesis shunts precursors to 19-carbon steroids. Steroids that are produced in excess are in bold, with diagnostic steroid being 17-hydroxyprogesterone (asterisk). Steroids that are deficient are in italics, with minor pathways shown with dashed arrows.

Diseases causing 46,XX DSD

The virilising congenital adrenal hyperplasias

The congenital adrenal hyperplasias (CAHs) are disorders of impaired cortisol biosynthesis. Low cortisol relieves feedback inhibition and thus increases ACTH secretion, which leads to hyperplasia of the adrenals and to disordered steroidogenesis. The clinical manifestations of these diseases result from: (1) the diversion of cortisol precursors to steroids normally produced in much lower amounts; (2) the biological activities of these cortisol precursors, which accumulate prior to the block; and (3) the deficiency of cortisol as well as often other steroids normally produced by the adrenal and sometimes also the gonads. In 17-hydroxylase deficiency (17OHD), for example, 11-deoxycorticosterone accumulates prior to the block and causes hypertension and hypokalaemia, while androgen and oestrogen production is also impaired, causing sexual infantilism, pubertal failure and 46,XY DSD. In the masculinising form of CAH, cortisol precursors are shunted to androgen synthesis, resulting in 46,XX DSD. The specific manifestations in each CAH patient varies with the severity of the enzymatic deficiency, the other steroid pathways that are disrupted, the ethnic background and additional genetic and environmental factors, which modulate the severity of the disease, many of which are not known.

21-hydroxylase deficiency (21OHD)

Far and away, the most common cause of CAH worldwide is 21OHD.¹⁶ Because of its frequency, roughly 1:15 000 live births¹⁷, 21OHD is also the most heavily studied form of CAH, although many mysteries in the development and evolution of this condition remain. The classic form of 21OHD is the severe enzymatic deficiency state, and this condition causes 46,XX DSD. A milder form of 21OHD presents in adolescence and early adulthood as androgen excess in women, and this state is called non-classic 21OHD. By definition, non-classic 21OHD is not a 46,XX DSD since newborn girls are not masculinised, and discussion here will be limited.

Physiology. Deficiency in the cytochrome P450c21 enzyme (CYP21A2) limits steroidogenesis to the reactions catalysed by cytochrome P450c17 (CYP17A1) and to a small extent cytochrome P450c11β (CYP11B1) (Fig. 2). Low cortisol allows ACTH to rise, and none of the 21-deoxysteroid precursors can

substitute for cortisol as a glucocorticoid. In addition, endogenous mineralocorticoids are all 21-hydroxylated steroids, so flux to aldosterone is also thwarted by the absence of 11-deoxycorticosterone, despite the presence of the downstream enzyme aldosterone synthase (P450c11AS, CYP11B2). With both glucocorticoid and mineralocorticoid deficiency, affected newborns are prone to volume depletion secondary to renal sodium wasting. As in other forms of adrenal insufficiency, hyperkalaemia, hyponatraemia, hypoglycaemia and hypotensive crisis may occur, if unrecognised and untreated.

ACTH and renin rise, driving conversion of cholesterol to pregnenolone in a vain compensatory response to cortisol deficiency and hypovolaemia. Without P450c21 activity, steroidogenesis is diverted to the only possible routes, which use HSD3B2 and P450c17 activities. As a result, adrenal-derived 19-carbon steroids, primarily DHEA and DHEAS, rise dramatically, and 17OHP accumulates due to its poor efficiency as a substrate for P450c17 (Fig. 2). If 5 α -reductase activity is also present, some of this 17OHP might be metabolised to androsterone via the alternate pathway (Fig. 1B). During early gestation, this androgen excess virilises the external genitalia of 46,XX fetuses, causing DSD. Progesterone also accumulates, and this steroid can have direct actions on target organs or be metabolised to other steroids in peripheral tissues.

Post-natally, the disordered adrenal steroidogenesis persists, although certain factors change and dictate changes to treatment approaches. For example, the neonatal kidney is resistant to mineralocorticoid action; the sodium content of the diet rises after infancy; and linear growth ceases in adolescence. These issues are discussed in the treatment section below.

Genetics. The high prevalence of 21OHD is a consequence of the molecular organisation of the 21-hydroxylase gene (*CYP21A2*), which is located on chromosome 6p21.1 in the highly recombinogenic HLA locus adjacent to the genes for the fourth component of complement.^{18,19} Within this locus, the *CYP21A1* pseudogene lies within a duplicated region of about 30 kilobases. Most mutations causing 21OHD derive from gene conversion events, where some or all of *CYP21A1* is transferred to *CYP21A2*, which impairs transcription of the gene or encodes an inactive enzyme. Because the two genes are extremely similar but differ primarily in nine discreet regions, a limited number of 'mutations' are found in most affected patients (Table 2). Nevertheless, the conversion events can be highly complex, including multiple copies of chimaeric genes, partial deletions and back-conversion of *CYP21A2* sequences onto the *CYP21A1* pseudogene.²⁰ Consequently, genotyping for 21OHD appears straightforward conceptually but is fraught with difficulties and ambiguities, particularly when parental DNA is not available for comparison. In addition, some sporadic mutations occur independent of gene conversions.

The severity of disease correlates generally but imperfectly with the impairment of enzyme activity for the encoded proteins and is best for the most severe and mildest mutations²¹ (Table 2). To have classic 21OHD, no copies of a gene encoding a P450c21 species possessing >5% of wild-type activity can be present. Patients with non-classic 21OHD have at least one copy of a gene with at least 15% of residual activity. Heterozygous relatives containing one copy each of a null allele and a wild-type allele cannot be reliably distinguished from unaffected individuals using basal or stimulated serum steroid concentrations. Patients with genotypes yielding 5–15% of wild-type P450c21 activity are extremely uncommon but do exist, and the entire spectrum of activity from 0% to 100% of 'normal' activity can be found. A given mutation usually affords a consistent phenotype, but the common mutation in intron 2, which impairs mRNA splicing, is associated with a variable severity, even within a kindred. Most cases of non-classical 21OHD have one or two copies of the V281L allele.¹⁶

Presentation and diagnosis. Classic CAH was historically dichotomised into 'salt-wasting' and 'simple virilising' phenotypes based on the severity of the disease. The most severely affected cases cannot synthesise sufficient aldosterone $\sim 1 \text{ nmol l}^{-1}$ (40 ng dl^{-1}) to maintain sodium balance and thus demonstrate salt wasting, which becomes clinically evident in the first week of life after catabolism of maternally derived steroids. In newborns with simple virilising 21OHD, aldosterone synthesis is partially preserved and empirically sufficient to prevent salt-wasting crises, but this amount of 21-hydroxylase activity is insufficient for normal production of cortisol, which circulates at concentrations 1000-fold higher than aldosterone. In both cases, androgen excess is present at birth in 46,XX

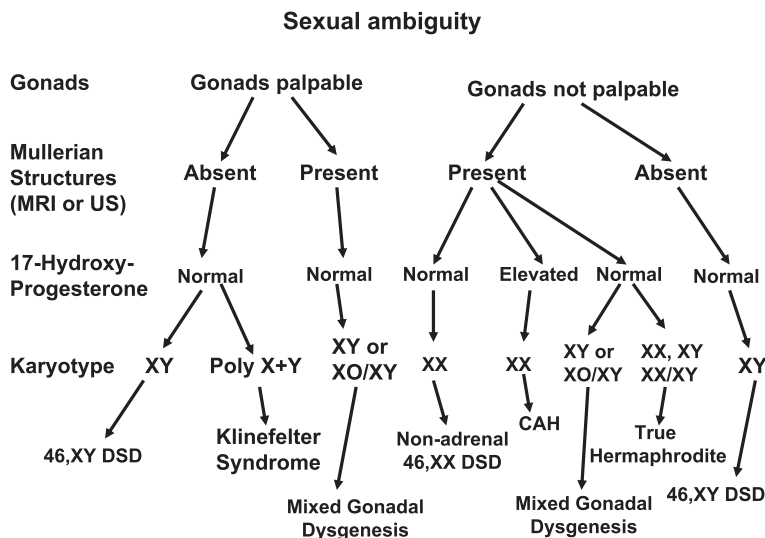


Fig. 3. Algorithm for investigating genital ambiguity and 46,XX DSD. The central role of the 17OHP in the assessment of the newborn with non-palpable gonads and lack of Y chromosome is highlighted. Note that high 17OHP alone does not diagnose 21OHD, as 17OHP is also elevated (but not as high) in other forms of CAH causing 46,XX DSD.

newborns, causing DSD with variable degrees of clitoromegaly and labioscrotal fusion. Whereas the girls are recognised due to genital ambiguity, boys with 21OHD are often undiagnosed at birth. Those severely affected typically suffer salt-wasting crises in the first weeks of life, but boys with the simple virilising genotypes are found later due to advanced somatic growth and evidence of androgen excess, including androgen-dependent hair growth, acne, penile enlargement and voice deepening.

The diagnosis of 21OHD in the newborn illustrates the general approach to the child with ambiguous genitalia and 46,XX DSD (Fig. 3). These children will have non-palpable gonads and should be investigated with imaging for Müllerian structures, while a karyotype is obtained or at least fluorescence *in situ* hybridisation (FISH) to determine if Y-chromosome DNA is present. The 17OHP determination then forms the key branch point in assembling the differential diagnosis. In classic CAH, a basal 17OHP is generally sufficient to establish the diagnosis, but a cosyntropin stimulation test remains the gold standard test. In addition to 17OHP and cortisol, additional steroids such as 11-deoxycortisol and 17-hydroxypregnenolone or DHEA are helpful to distinguish 21OHD from other conditions with high 17OHP, including 11-hydroxylase deficiency, 3 β -hydroxysteroid dehydrogenase deficiency and P450-oxidoreductase deficiency (Table 1). A stimulated 17OHP above 300 nmol l⁻¹ (10 000 ng dl⁻¹) establishes the diagnosis of 21OHD.¹⁶ Although the clinical experience and normative data for 17OHP is extensive, the most specific marker of 21OHD is actually 21-deoxycortisol^{22,23}; however, this steroid is not measured by most commercial laboratories.

Non-classic 21OHD presents in childhood or later with premature pubarche, acne, hirsutism, irregular menses and infertility. Males are rarely ascertained unless undergoing testing as part of kindred with suspected 21OHD or when undergoing genetic counselling with a partner affected by 21OHD. Some women are asymptomatic, but most menstruating women with non-classic 21OHD are clinically hyperandrogenemic and very similar to those with idiopathic polycystic ovary syndrome (PCOS).²⁴ The family history and stimulated 17OHP are the best parameters to distinguish between the two diagnoses.²⁴ Non-classical 21OHD is more common than classical 21OHD, with an incidence of about 1:300 in general Caucasian population and as much as 1:27 in the Ashkenazi Jews.¹⁹ The non-classical 21OHD patients produce normal amounts of aldosterone and cortisol, and affected females do not have ambiguous genitalia. Treatment of non-classic 21OHD is generally limited to females for symptomatic relief of hyperandrogenism and irregular menses, and details are not covered here. Some patients, particularly those heterozygous for one null allele and one with the P30L mutation,

Table 1

46,XX DSD conditions.

Disease	Cause	Diagnostic serum steroids
21-Hydroxylase deficiency	CYP21A2 mutations	17-hydroxyprogesterone, 21-deoxycortisol
11-Hydroxylase deficiency	CYP11B1 mutations	11-deoxycortisol, 11-deoxycorticosterone
3 β HSD deficiency	HSD3B2 mutations	17-hydroxypregnenolone, DHEA, DHEAS
P450-oxidoreductase deficiency	POR mutations	Progesterone, 17-hydroxyprogesterone corticosterone, DHEA, DHEAS
Aromatase deficiency	CYP19A1 mutations	Estriol
Luteoma of pregnancy	Maternal tumor	Testosterone, DHT
Androgen administration	Foreign androgens	Varies

demonstrate very high androgen and 17OHP production difficult to distinguish from classic 21OHD²¹, and these patients may require more aggressive treatment and stress dosing of glucocorticoids during surgery and illness. This comment emphasises that a broad spectrum in the degree of enzyme deficiency is observed in 21OHD, and the physician should not assume that all non-classic 21OHD is mild disease but rather can be only slightly less severe than classic disease.

Because of the dire consequences resulting from failure to diagnose 21OHD in the newborn, neonatal screening has been introduced in many countries. In the United States, all newborns are now screened for 21OHD through state programmes at 24–48 hours of age. The immunofluorescence assay used can over-estimate the 17OHP concentration, so the vast majority of abnormal screens are false-positives. The paediatrician is informed during the first week of life, and formal endocrine assessment is arranged. In Texas, which began newborn screening in 1989, the mean age at diagnosis for boys with 21OHD is now 12 days, 2 weeks younger than the mean age for nearby states prior to institution of screening.

Therapy and management issues. This section focusses on management of the newborn as a form of 46,XX DSD; however, salient features of management throughout life are briefly addressed. Many of the concepts described for 21OHD will also apply to the other disorders and are discussed in less detail subsequently.

The newborn. A newborn with a DSD constitutes a medical emergency, particularly a 46,XX DSD with 21OHD. Hospitalisation is required to perform the necessary testing in an expedient manner and to monitor for fluid and electrolyte imbalance. Treatment consists of fluid and electrolyte replacement, glucocorticoid replacement and mineralocorticoid replacement. Initial steroid therapy is given in doses higher than maintenance for several reasons. The adrenals are hyperplastic, and glucocorticoid therapy is intended not only to suppress ACTH secretion but also to allow the adrenals to regress to normal size. Hydrocortisone is generally preferred because the short half-life limits the growth suppression observed with more potent synthetic glucocorticoids. Hydrocortisone is administered as a suspension

Table 2

Common mutations in 21-hydroxylase deficiency.

Mutation	Location	Alteration	Phenotype
Partial or Complete Deletion	Variable	Deletion	SW
P30L	Exon 1	Missense	NC
656A/C-G	Intron 2	Aberrant splicing	SW, SV
G110 Δ 8nt	Exon 3	Deletion 8 bases	SW
I172N	Exon 4	Missense	SV
I236N + V237E + M239K	Exon 6	Missense x3	SW
V281L	Exon 7	Missense	NC
Q318X	Exon 8	Nonsense	SW
R339H	Exon 8	Missense	NC
R356W	Exon 8	Missense	SW, SV
P453S	Exon 10	Missense	NC
R483P	Exon 10	Missense	SW

SW, salt wasting; SV, simple virilizing; NC, nonclassical.

(branded product withdrawn from the market in the United States due to inconsistency), crushed tablets mixed into formula or via parenteral routes as the hemisuccinate salt. About 20 mg m^{-2} per day in divided doses is used initially, such as 1.5 mg given every 8 h. As the child grows, the same dose will gradually reduce exposure to 10 mg m^{-2} per day, which is a more typical maintenance dose. Periodic monitoring and dosage adjustment to maintain the 17OHP in the low normal range for the first several weeks of life are recommended. The dose is increased two- to four-fold for illnesses that cause volume depletion, such as high fever, diarrhoea, vomiting, haemorrhage or sepsis.

For reasons not entirely understood, the neonatal kidney is particularly resistant to the action of mineralocorticoids, and in 21OHD, the high-circulating 17OHP can antagonise the action of mineralocorticoids. Suppression of 17OHP to enhance mineralocorticoid action is another reason that glucocorticoids are generously dosed in the newborn period. Hydrocortisone at 20 mg m^{-2} per day has some mineralocorticoid activity but not enough for the newborn with 21OHD. Consequently, fludrocortisone acetate is given orally, typically $0.1\text{--}0.2 \text{ mg}$ per day. The dose is titrated by measuring plasma renin activity or mass, which should be suppressed, and electrolytes should be monitored as well to avoid potassium imbalances from over- or under-treatment. Fludrocortisone will not be effective in maintaining sodium balance without sodium supplementation, to compensate for the low salt content of formula and breast milk ($<10 \text{ meq l}^{-1}$) and the tendency for all newborns with 21OHD to lose sodium in the urine. Oral sodium chloride, $1\text{--}2 \text{ g}$ per day, is added to formula or breast milk if the child is feeding well; intravenous treatment may be necessary if the child is diagnosed many days later after becoming volume depleted and lethargic.

For the child with 21OHD and 46,XX DSD, the question of genital surgery arises in the nursery. The two components of genital masculinisation are clitoral enlargement and labioscrotal fusion, and while related, the two should be considered and treated independently. Mild clitoromegaly with little labioscrotal fusion (Prader scores I–II) usually do not need surgery, certainly not in the newborn period, as the clitoris will become relatively more normal in size as the child grows. Newborns with Prader scores III–V are more difficult to assess and manage. At a minimum, the lower urinary tract should be defined by cystoscopy and imaging studies as needed to locate the urethral opening. A common urogenital sinus, where the urethra and vagina merge into a single orifice, is associated with frequent urinary tract infections. Although a complete discussion of surgical management is beyond the scope of this article, the general principle is that surgery should be kept to a minimum in the neonatal period and mainly to correct hygiene problems rather than to improve aesthetics or prepare for sexual function in adulthood.

The child and adolescent. During childhood, the goals of treatment are to prevent adrenal crises, to optimise growth and to prevent premature initiation of puberty. To do so, the hypovolaemic drive to ACTH production is first minimised, by maintaining fludrocortisone acetate therapy, usually at the same dose of $0.1\text{--}0.2 \text{ mg}$ per day. The glucocorticoid treatment is moderated as the adrenal size falls to normal and the sodium content of the diet increases, yet the dose must be slowly increased to keep up with the child's increasing body size. Hydrocortisone is still preferred, usually in three daily doses, now giving the largest dose in the morning, such as 5 mg , 3 mg and 2 mg with the three meals. Sustained-release preparations have been studied and offer good recapitulation of the normal diurnal rhythm, but these medications are not commercially available yet. Under-treatment can cause bone age and linear growth advancement, acne, hirsutism, voice deepening and further clitoral growth. More potent synthetic steroids such as prednisone or prednisolone, methylprednisolone and dexamethasone are more convenient, requiring one or two daily doses, but these medications are more difficult to titrate and cause more glucocorticoid-related side effects. Over-treatment can cause growth suppression, obesity, loss of bone mass, dyslipidaemia, glucose intolerance, poor sleep and skin thinning with easy bruising. Nevertheless, sometimes these drugs are preferable to regain control of the disease if compliance or other factors render hydrocortisone therapy problematic, preferably for short periods of time. Throughout life, the dose of glucocorticoid is increased for concurrent illnesses. Sodium supplements are usually not necessary, but salt craving by the child suggests that the dietary sodium is inadequate and can be supplemented either with salt tablets or adding salty foods.

Exact algorithms for treatment adjustment and monitoring are difficult if not impossible to develop due to the variability of the disease. In general, plasma renin, electrolytes and blood pressure are monitored to titrate mineralocorticoid and salt dosing. Titrating glucocorticoid therapy is problematic

no matter how many steroids are measured. The ultimate metabolites of adrenal 19-carbon steroids, androstenedione and testosterone, should be kept in the age- and Tanner-stage-adjusted normal range. The 17OHP is often monitored, but 17OHP fluctuates markedly throughout the day, so the timing of blood testing after a particular dose of hydrocortisone should be consistent. If the 17OHP is normalised, the patient is probably over-treated, so levels are allowed to drift to somewhat above normal. Bone age and auxologic parameters should be checked at least twice a year to assure that growth is neither too rapid nor too slow. Many of these children advance their bone age too quickly, leading to rapid growth in childhood but short stature as adults. Bilateral laparoscopic adrenalectomy has been proposed as a definitive treatment for severe 21OHD when androgen excess cannot be controlled medically.²⁵ An argument against this approach is that a small amount of adrenal steroids and metabolites with some mineralocorticoid and/or glucocorticoid activities are made by the 21OHD adrenals in adulthood, which might defend against adrenal crisis in severe illness.²⁶ The selection criteria that should be used to identify patients who will benefit most from bilateral adrenalectomy and the long-term consequences of this practice are not currently known. Treatment regimens that incorporate androgen receptor antagonists²⁷ or inhibitors of androgen synthesis such as ketoconazole and trilostane have received little attention, possibly due to cost, side effects and drug–drug interactions. Improved methods to reduce or blockade androgens as a means to avoid over-treatment with glucocorticoids are clearly needed for the management of 21OHD.

A common complication of CAH, especially if control is poor, is precocious puberty, probably related to the action of high and fluctuating androgens and oestrogens on the pituitary and hypothalamus. Treatment involves long-acting GnRH agonists as for other children with precocious puberty. In addition, recent studies suggest that recombinant growth hormone therapy improves final height²⁸, but 21OHD is not an accepted indication for growth hormone therapy at this time.

The adult. With the transition to adulthood, the goals of treatment change somewhat. Salt-wasting and adrenal crises become very uncommon, except during severe concurrent illness. Linear growth has completed, reproduction may be desired and long-term health issues become dominant. The intensity of treatment and complexity of treatment have to be carefully balanced with long-term consequences of therapy over many years.²⁹ Mineralocorticoid replacement is generally continued, although patients with 21OHD develop elevated blood pressure over time³⁰, necessitating dose reduction or discontinuation. Androgen excess remains problematic, particularly if control has been poor in childhood. Busy working adults may not be able to comply with thrice daily dosing regimens, so simplified hydrocortisone schedules or longer-acting steroids may be necessary. After instituting all other measures to reduce androgen exposure, the minimum dose of corticosteroid should be used to control adrenal 19-carbon steroid production. Many women develop a secondary PCOS, and the ovary becomes a second source of androgens and 17OHP that persists even after adrenal suppression.³¹ Menses may not become regular even with good control of adrenal hyperfunction. If pregnancy is not desired, an oral contraceptive pill offers several advantages: raising sex hormone binding globulin, which lowers free testosterone; lowering ovarian androgen production; and encouraging monthly menses. Anti-androgen therapy is a logical approach, but use of spironolactone, a common agent used for other forms of hirsutism, is problematic due to concomitant mineralocorticoid receptor antagonism, which could exacerbate salt wasting. Pure anti-androgens, such as flutamide and bicalutamide, and 5 α -reductase inhibitors finasteride and dutasteride, have not been studied much in 21OHD.

If pregnancy is desired, women with 21OHD face multiple impediments. Problems include oligoanovulation due to high androgens from all sources, inadequate vaginal calibre for coitus and chronically high progesterone, which prevents favourable endometrial maturation and cervical mucus formation.³² Consequently, glucocorticoid treatment often must be intensified during the period prior to attempting pregnancy. In a series of 23 patients, 21 were able to bear children using either hydrocortisone or prednisolone therapy divided 2–3 times daily, with attention to the timing of the doses.³³

The best approach to achieve vaginal adequacy for intercourse and childbirth remains a major unsolved problem in the management of adult women with 21OHD. Staged procedures have been the norm, with definitive reconstruction and dilatation reserved for the age when the woman desires to begin sexual activity. Results with skin or bowel grafts are mixed and often unsatisfactory if the degree of virilisation at birth was severe.³⁴ Recent results with buccal mucosa grafts are promising³⁵, but long-

term data are lacking. Regardless of the procedures, consistent use of lubricants and dilatation are essential for sustained satisfaction.

Limited data on adults with 21OHD have not identified major consistent new health problems developing with age. When control is poor for long periods, males are prone to develop adrenal rests in the testes, and in both males and females, the hyperplastic adrenals may develop massive myelolipomas.³⁶ Some studies have found a higher prevalence of low bone density³⁷, glucose intolerance, hypertension and dyslipidaemia in adults with 21OHD than in controls.³⁰ The reasons for these results are not known, but the findings reflect the constellation of problems experienced by patients with Cushing's disease and thus may reflect a lifetime of excessive glucocorticoid exposure.

Prenatal treatment. Given the difficulties encountered with vaginal reconstruction, schemes to prevent virilisation of infants have been devised. The logic of the approach is that suppression of the hypothalamic–pituitary–adrenal axis during the window of sexual differentiation will reduce androgen production in affected females. To do so, a corticosteroid that crosses the placenta is administered to the mother starting 6–8 weeks of gestation. In practical terms, as soon as a woman who has given birth to a child with severe 21OHD becomes pregnant again, dexamethasone 0.2 mg kg⁻¹ per day (~1.5 mg in two to three divided doses) is administered.³⁸ The family should be informed of the prenatal treatment option well in advance and have the medication available to avoid delay in commencing treatment. Ideally, chorionic villous sampling is performed as early as possible at 10–12 weeks, and both the chromosomal sex and *CYP21A2* genotype for the foetus are ascertained. Treatment can be discontinued if the foetus is an unaffected female or male; women bearing affected females are treated throughout pregnancy.³⁹ The evidence from uncontrolled studies shows that prenatal dexamethasone treatment reduces virilisation of newborns.⁴⁰ Disadvantages and caveats of prenatal treatment include the considerable expense, side effects of excessive weight gain and cushingoid features experienced by the mother, and the concern that seven of eight foetuses are exposed to dexamethasone unnecessarily.¹⁶ Prenatal dexamethasone exposure is associated with a high prevalence of hypertension and reduced brain size in rodents, raising the concern of long-term consequences to children treated prenatally. Limited long-term data on treated children do not demonstrate severe problems, but some differences in cognitive function have been found.⁴¹ One potential improvement to the current approach derives from the capacity to obtain foetal cells in the maternal blood before 6 weeks of gestation. The presence of Y-chromosome material would obviate the need to commence dexamethasone, limiting treatment of half of the pregnancies bearing females. Since labioscrotal fusion, the most difficult anomaly to correct in girls with 21OHD, occurs only at 8–12 weeks of gestation, another potential modification to the approach is to reduce the dose of dexamethasone throughout the pregnancy, as moderate clitoromegaly poses less of a problem than the need for vaginal reconstruction.

11 β -hydroxylase deficiency (11OHD)

While 11OHD is often considered the second most common cause of CAH, this is true mainly in Israel where the disorder occurs in up to 1 in 5000 births⁴²; elsewhere, the disease is rare, occurring in <1 in 100 000 births. Clinically, 11OHD blends the androgen excess of 21OHD with the mineralocorticoid excess of 17OHD.

Physiology. Deficiency in the cytochrome P450c11 β enzyme (*CYP11B1*) blocks only the final step of cortisol and corticosterone synthesis, such that many steroids accumulate. In contrast to 21OHD, aldosterone synthesis is not impaired, since 11-hydroxylation in the zona glomerulosa is mediated by aldosterone synthase (e.g., P450c11AS and *CYP11B2*). Absence of *CYP11B1* in the zona fasciculata, however, results in a marked rise in the potent mineralocorticoid 11-deoxycorticosterone, which causes salt and water retention, hypertension, hypokalaemia and suppression of plasma renin activity with secondary suppression of aldosterone synthesis (Fig. 4).⁴³

As in 21OHD, cortisol deficiency leads to increased ACTH and cortisol precursor production, including not only 11-deoxycortisol and 11-deoxycorticosterone but also 17OHP and progesterone. These upstream steroids are shunted towards androgen biosynthesis, and 17OHP might also drive flux along the backdoor pathway to DHT (Fig. 1B). As a result, newborn girls are virilised as in 21OHD, causing 46,XX DSD; boys are difficult to ascertain at birth but rather present with precocious pseudopuberty in

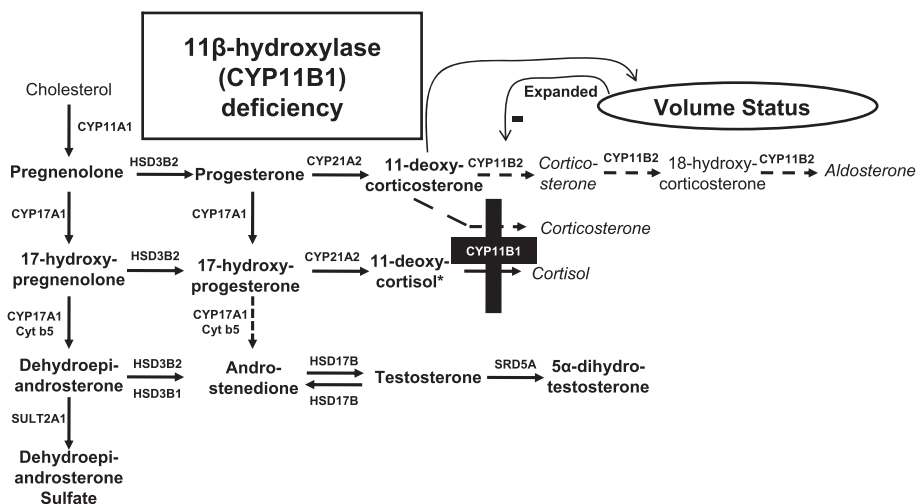


Fig. 4. Altered steroid biosynthesis in 11-hydroxylase deficiency. The block of cortisol and aldosterone synthesis shunts precursors to 19-carbon steroids, but DOC also accumulates, mitigating the volume depletion ordinarily caused by aldosterone deficiency. Steroids that are produced in excess are in bold, with diagnostic steroid being 11-deoxycortisol (asterisk). Steroids that are deficient are in italics, with minor pathways shown with dashed arrows.

childhood. A non-classical form has been suggested by analogy with 21OHD, but a genetic basis for this diagnosis is lacking. Unlike 21OHD, hypotension and hyperkalaemia are rare due to preservation of mineralocorticoid biosynthesis, but a wide range of blood pressure phenotypes are observed.

Genetics. The *CYP11B1* gene is located on chromosome 8q21–q22, within 40 kb of the homologous *CYP11B2* gene for aldosterone synthase.⁴⁴ Mutations in *CYP11B1* cause 11OHD, and most described mutations cause loss of essentially all enzyme activity.⁴⁵ Curiously, although the *CYP11B1* and *CYP11B2* genes lie in a duplicated locus analogous to the *CYP21A* genes, gene conversion events as in 21OHD rarely cause 11OHD.⁴⁶ The R448H mutation is the most common cause of 11OHD, and this founder mutation explains the high prevalence of 11OHD in the Jews of Moroccan ancestry.⁴⁷ Other missense, frameshifts and nonsense mutations have been described. Unequal recombination between the two *CYP11B* genes can produce a third, hybrid gene in which aldosterone synthase activity is expressed under the control of ACTH in the zona fasciculata, which causes the disease glucocorticoid-remediable aldosteronism.⁴⁸ In two patients with 11OHD, unequal recombination yielded a locus with a single hybrid *CYP11B* gene that caused the disease due to lack of enzyme activity or expression.^{49,50}

Presentation and diagnosis. Female newborns are identified with 46,XX DSD, including clitoral enlargement and labioscrotal fusion clinically indistinguishable from 21OHD.⁴³ Children undiagnosed at birth, particularly boys or girls with less severe deficiency, progress with rapid somatic growth and bone age advancement, premature adrenarche, acne and isosexual or contrasexual precocious pseudopuberty. Adolescent girls and women suffer from irregular menses and hirsutism or pattern baldness. Although some women with hirsutism show mildly elevated 11-deoxycortisol after cosyntropin, causative molecular defects in the *CYP11B1* gene have not been identified in these cases, such that hirsute women should only be evaluated for 11OHD also virilised.

Hypertension is common in 11OHD, with a prevalence of 60–70% and early age of onset.⁵¹ Although 11-deoxycorticosterone is the presumed mediator of the hypertension, blood pressure correlates poorly with circulating 11-deoxycorticosterone concentrations⁵² and the severity the genetic defect is not predictive of hypertension.^{43,53} In fact, salt-wasting or hypotensive crises have been described in children with 11OHD⁵², possibly because the high circulating concentrations of 17OHP and progesterone can antagonise the action of mineralocorticoids at their receptor, particularly in newborns.

Nevertheless, the presence of hypertension and/or hypokalaemia are clinical features that help to distinguish 11OHD from 21OHD and 3 β -hydroxysteroid dehydrogenase deficiency, and the androgen excess distinguishes 11OHD from 17OHD (which is not a 46,XX DSD).

Profound elevation of basal or stimulated 11-deoxycortisol and 11-deoxycorticosterone with low cortisol is diagnostic of 11OHD. The 17OHP may be quite high, usually not as high as in severe 21OHD, but 21-deoxycortisol will not be elevated (Table 1).²² Elevated urinary excretion of tetrahydro metabolites of 11-deoxycortisol and 11-deoxycorticosterone, as well as 19-carbon steroids or 17-ketosteroids, will also establish the diagnosis. Heterozygous carriers of 11OHD have biochemical profiles indistinguishable from normal controls.

Therapy. The newborn. The principles of diagnosis and management of the newborn with 46,XX DSD due to 11OHD are the same as followed for 21OHD (Fig. 3). Newborn screening is not performed for 11OHD; however, these children will have high 17OHP and will be identified in newborn screening for 21OHD. Clinical and laboratory testing is then necessary to distinguish the two diseases. Glucocorticoid replacement is mandatory, and salt supplementation is prudent until the diagnosis is established, due to the similarity in presentation of the two conditions and the potential for salt wasting in the newborn with 11OHD. Evaluation and reconstruction of the external genitalia were discussed for 21OHD.

The child and adolescent. The goals of glucocorticoid replacement therapy in children with 11OHD are similar to those for 21OHD in moderating androgen production to allow normal growth and development. Unlike 21OHD, mineralocorticoid replacement is rarely needed, and in fact, reduction of cortisol precursors often ameliorates the hypertension and hypokalaemia when present. Regimens include hydrocortisone (10–25 mg m⁻² per day), prednisone (0.1 mg kg⁻¹ per day) and dexamethasone (up to 0.5 mg per day). Stress doses of glucocorticoids are recommended during concurrent illness. Even more so than in 21OHD, treatment must be tailored to the individual, relying heavily on blood pressure, physical examination (assessment of hirsutism and acne), electrolytes, bone age and growth rate to determine adequacy of glucocorticoid replacement and avoid unnecessary over-replacement. Serum testosterone concentrations should be in the age- and gender-specific normal range, and 11-deoxycortisol concentrations should decline, although usually not to normal. Correction of mineralocorticoid excess is indicated by normal blood pressure and potassium, as well as measurable plasma renin activity and aldosterone. If androgen excess has been controlled but hypertension and hypokalaemia with suppressed plasma renin activity persist, targeted therapy with amiloride (1.25–5 mg per day) or spironolactone (12.5–25 mg per day) is very effective in normalising potassium and blood pressure. These potassium-sparing diuretics can be viewed as glucocorticoid-sparing agents and should always be considered when designing a treatment regimen. Spironolactone also antagonises the action of androgens at their receptor and thus has a unique spectrum of benefits in 11OHD, and its use is discussed in the following subsection. Vaginal reconstruction and dilation is considered when the patient is ready to become sexually active.

The adult. Adult women with 11OHD usually require treatment to control androgen excess and hypertension with potassium wasting, and spironolactone, 25–200 mg per day, treats both problems by antagonising both androgen and mineralocorticoid receptors. Since the source of both the androgens and mineralocorticoids are the adrenal zonae fasciculata and reticularis and spironolactone does not cause a further rise in ACTH, the receptor blockades are not overcome by a reflex increase in tropic hormone synthesis. Thus, a simple and logical regimen is replacement doses of hydrocortisone (15–20 mg per day divided in two doses) plus sufficient spironolactone to control any remaining androgen and mineralocorticoid excess. If pregnancy is not desired, an oral contraception pill should generally be added, to increase SHBG, regularise menses and prevent vaginal spotting, which is a common side effect of spironolactone. Calcium channel blockers and other vasodilators are a good choice if blood pressure is not controlled on such a regimen. The hypertension can be severe, and cardiomyopathy, blindness and death have been reported in cases of 11OHD with long-standing uncontrolled hypertension. Spironolactone is potentially teratogenic and contraindicated in pregnancy, so affected women attempting pregnancy require intensified glucocorticoid therapy. A successful pregnancy has been reported in one woman with 11OHD treated with dexamethasone, metformin and clomiphene citrate.⁵⁴

Prenatal treatment. The principles of prenatal treatment in 21OHD are mirrored for 11OHD, but experience is much more limited due to the relative rarity of 11OHD. The prenatal diagnosis of 11OHD is made by sequencing the *CYP11B1* gene amplified from DNA in a chorionic villus biopsy or by measuring tetrahydro-11-deoxycortisol in amniotic fluid.⁵⁵ The dose of dexamethasone ($20\ \mu\text{g kg}^{-1}$ daily in three divided doses) is the same as in 21OHD. Treatment is commenced as early as possible, and at least one report confirms that this regimen prevents virilisation of the external genitalia in at-risk pregnancies.⁵⁶

3 β -hydroxysteroid dehydrogenase/ $\Delta^5/4$ -isomerase type 2 (3 β HSD) deficiency

Bongiovanni defined the rare clinical entity of 3 β HSD deficiency 50 years ago.⁵⁷ This condition is a very severe steroid deficiency state, since production of delta-4 steroids in both the adrenal and gonads are impaired, which abrogates normal synthesis of all steroid hormones. The disease is similar to 21OHD with both glucocorticoid and mineralocorticoid deficiency, but the androgen excess, at least in the foetus and newborn, is less severe than in 21OHD. One of the rarest forms of CAH, the condition occurs in only 1 in ~1000 000 births, and no ethnic pockets with higher prevalence due to founder mutations have been described.⁵⁸ A non-classic form has been proposed, but a genetic basis for this condition has not been described⁵⁹, and use of this terminology and diagnosis is discouraged.

Physiology. Deficiency of 3 β HSD activity in the adrenals precludes normal aldosterone and cortisol synthesis, causing a rise in ACTH and the flooding of cortisol precursors along the delta-5 pathway and culminating with marked rise in DHEA and DHEAS production. Glucocorticoid and mineralocorticoid deficiency cause salt wasting, hyperkalaemia and volume depletion, as described for 21OHD. A puzzling feature of 3 β HSD deficiency is that both male and female infants are born with ambiguous genitalia. Deficiency of 3 β HSD activity in the gonads precludes normal testosterone synthesis in male foetuses, explaining under-virilization of males, but the androgen excess in females remained a paradox until the molecular genetics of the disease was clarified. As described below, human beings have two 3 β HSD genes and cognate enzymes: the type 1 enzyme⁶⁰, expressed in the placenta, skin, liver and other peripheral tissues, and the type 2 enzyme, expressed only in the adrenals and gonads.⁶¹ In the female foetus, excess DHEA is a substrate for the type 1 enzyme, yielding abundant androstenedione. Peripheral AKR-type HSDs convert androstenedione to T, and 5 α -reductase type 2 in genital skin completes the synthesis of DHT (Fig. 5).

This flux to DHT in 3 β HSD deficiency is not as robust as in 21OHD and 11OHD, and modest androgen excess causes some clitoromegaly at birth but little labioscrotal fusion.^{58,59} The reason for lesser virilisation in 3 β HSD deficiency is unlikely a lower degree of enzyme deficiency, since salt wasting can be as severe as in 21OHD. More likely, the absence of adrenal 3 β HSD activity precludes elevation of intra-adrenal 17OHP, which drives flux to DHT via the backdoor pathway, suggesting that the alternate pathway is the major source of virilising androgens in CAH (Fig. 1B).⁶² In addition, pregnenolone and 17-hydroxypregnenolone are metabolised to their delta-4 congeners progesterone and 17OHP, respectively, explaining the elevations in circulating concentrations of these steroids as well. This peripheral conversion does not rescue cortisol and aldosterone deficiency, however, because the 21-carbon steroid precursors do not efficiently return to the adrenal for subsequent hydroxylations necessary for complete these pathways. Peripheral cytochromes P450 cannot substitute for the adrenal 21- and 11-hydroxylases to make significant amounts of cortisol, but progesterone 21-hydroxylation by CYP3A4 and CYP2C9 in the liver can partially compensate for mineralocorticoid deficiency by making 11-deoxycorticosterone in older children and adults.⁶³

Genetics. The molecular basis of this disorder was defined 30 years after the disease was described, and this work revealed that two genes encoding steroid 3 β HSD enzymes are found on chromosome 1p13 in human beings. The *HSD3B2* gene is mutated in 3 β HSD deficiency, and mutations in the *HSD3B1* gene have not been described, possibly because placental 3 β HSD deficiency would preclude progesterone formation in mid-pregnancy and cause abortion. Mutations throughout the gene have been described, and the severity of the disease correlates fairly well with the degree of enzyme deficiency as assayed in model systems. Mutations that impair steroid binding, nicotinamide cofactor binding and enzyme

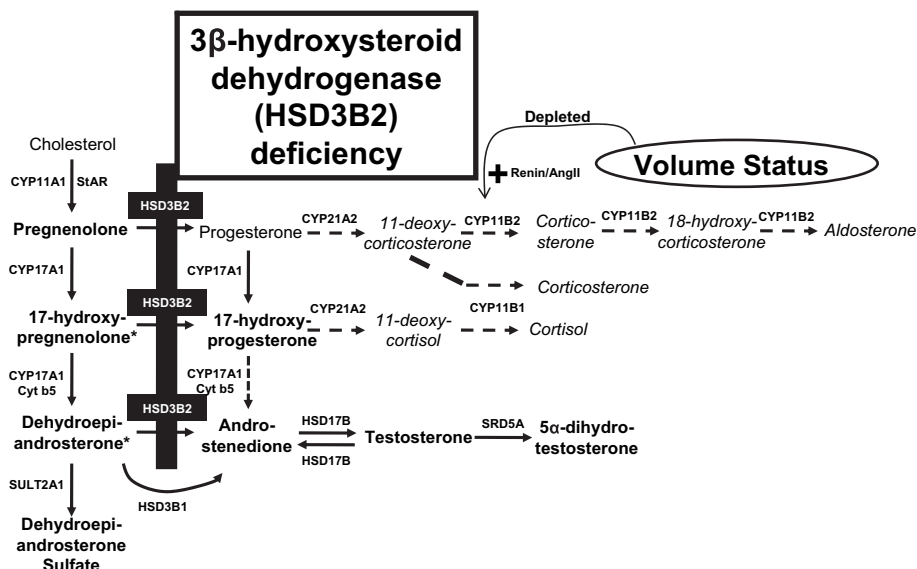


Fig. 5. Altered steroid biosynthesis in 3β -hydroxysteroid dehydrogenase deficiency. The block of cortisol and aldosterone synthesis shunts precursors to 19-carbon steroids. Peripheral conversion of Δ^5 steroids to Δ^4 steroids results in high 17OHP and androstenedione in 46,XX patients, but these precursors recirculate to the adrenal poorly and are ineffective substrates for glucocorticoid or mineralocorticoid synthesis. Note that the “backdoor” pathway to DHT is precluded since intra-adrenal Δ^4 steroids (17OHP) are essential to this pathway. Steroids that are produced in excess are in bold, with diagnostic steroids being 17-hydroxypregnenolone and DHEA (asterisks). Steroids that are deficient are in italics, with minor pathways shown with dashed arrows.

stability have all been described, and in many instances, the enzymatic defect is complex and multifactorial. Gene conversions with the *HSD3B1* gene have not been described, and founder mutations account for only small clusters of cases in remote areas. No mutations have been found in patients suspected of having ‘non-classic 3β HSD deficiency’ based on cosyntropin stimulation testing, suggesting that the basis for these laboratory abnormalities is functional rather than genetic.

Presentation and diagnosis. Unlike 21OHD and 11OHD, male newborns are more likely to be ascertained at birth due to significant 46,XY DSD. Females may go undiagnosed at birth when the clitoromegaly is not severe and labioscrotal fusion is mild or absent. Salt wasting occurs in the first week of life if undiagnosed, leading to hypotension, hyperkalaemia and failure to thrive. Children with less severe enzymatic deficiency who maintain normal fluid and electrolyte balance will develop signs of androgen excess, including body odour, axillary and pubic hair growth, rapid somatic growth and advanced bone age. As with 11OHD, high 17OHP will trigger an abnormal neonatal screen for 21OHD, and a discrepancy between the modest degree of genital virilisation compared with the high 17OHP should suggest a diagnosis other than 21OHD.

The presence of peripheral 3β HSD1 activity confounds interpretation of basal steroid profiles; therefore, the diagnosis in a newborn with 46,XX DSD is established by comprehensive steroid analysis after cosyntropin stimulation. Low cortisol and aldosterone despite elevated ACTH and plasma renin activity persists after cosyntropin stimulation. DHEAS, androstenedione and T concentrations are elevated as well. The finding of a 17-hydroxypregnenolone/17OHP ratio (precursor/product ratio across the block, Fig. 5) after cosyntropin at least six standard deviations above normal is required for diagnosis, with the 17-hydroxypregnenolone in excess of 100 nmol l^{-1} (3300 ng dl^{-1}). Elevated ratios of pregnenolone/progesterone and DHEA/androstenedione are helpful for confirmation.

Therapy. The newborn. As androgen excess is neither severe nor difficult to control in the newborn period, the major goal of therapy is to restore fluid and electrolyte balance. Glucocorticoid, mineralocorticoid

and salt supplementation are administered as in the newborn with 21OHD, although the glucocorticoid dose can be quickly reduced to about 10 mg m^{-2} per day once the child is well. Interrogation of the urinary tract and genital surgery are rarely required.

The child and adolescent. As the child grows, androgen excess becomes more problematic, probably because DHEA(S) production increases as the adrenal zona reticularis develops and the capacity for peripheral metabolism to T and DHT increases as well. Therapy with mineralocorticoid and glucocorticoid is continued to prevent unwanted hair growth, acne and advanced linear growth with skeletal maturation. Monitoring for this disease is similar to monitoring in 21OHD, with attention mainly focused on normalising T and suppressing DHEAS concentrations. The use of spironolactone is complicated by its mineralocorticoid receptor antagonism.

Unlike 21OHD and 11OHD, the simultaneous ovarian 3 β HSD deficiency prevents normal oestrogen synthesis, and secondary sexual characteristics are induced at the age of normal puberty. Ethinyl oestradiol, conjugated equine oestrogen or oestradiol is started at the lowest possible dose about age 10 and advanced very slowly over time, for example, ethinyl estradiol $2.5 \mu\text{g}$ per day, increasing the dose by $2.5 \mu\text{g}$ per day every 6 months. After 2–3 years, a progestin is added to induce cyclic menses; oral contraceptive pills with progestins low in androgenic activity (e.g., drospirenone, desogestrel and norgestimate) are ideal for this purpose.

The adult. Almost no data exist concerning optimal management of adult women with 3 β HSD2 deficiency. In general, the mineralocorticoid is titrated to lower the plasma renin activity to the normal range as tolerated by the blood pressure and potassium concentration. Hydrocortisone is the preferred glucocorticoid replacement to minimise over-replacement, and simple replacement regimens (15 mg on arising and 5 mg after lunch) are generally adequate. Oestrogen replacement with withdrawal bleeding every 1–3 months is necessary to prevent osteoporosis and endometrial hyperplasia. Adjunctive mechanical means to control hirsutism are preferred over chronic therapy with supra-physiologic doses of glucocorticoids. Vaginal reconstruction is rarely required, but dilatation and lubrication aid in achieving satisfaction with intercourse. Pregnancy in women with 3 β HSD deficiency has not been described.

Prenatal treatment. The mild virilisation experienced by newborns with 3 β HSD deficiency is readily controlled, and vaginal reconstruction is rarely required if treatment is instituted early. Consequently, prenatal treatment is not advised given the considerable concerns of maternal side effects and long-term risks to the foetus, yielding an unfavourable risk/benefit profile.

P450-oxidoreductase deficiency (PORD) or Antley–Bixler syndrome with disordered steroidogenesis

PORD was originally thought to be a combination of 21OHD and 17OHD due to the accumulation of steroids characteristic of both disorders, namely 17OHP and corticosterone, respectively.⁶⁴ The confusing mixture of biochemical and phenotypic disturbances, the wide range of phenotypes and the lack of reliable diagnostic criteria make this the most difficult form of CAH to diagnose and to comprehend. As in 3 β HSD deficiency, both males and females may have DSD, and mothers carrying an affected foetus often virilise during pregnancy as in aromatase (CYP19A1) deficiency, yet testosterone synthesis is minimal in postnatal life.⁶⁵ Children often demonstrate skeletal malformations including synostoses in a pattern described as the Antley–Bixler syndrome or other eponymic conditions. These malformations resemble disorders of cholesterol biosynthesis including Smith–Lemli–Opitz syndrome or infants born to mothers who ingest azole antifungals (fluconazole) during pregnancy. An understanding of the physiology of PORD must explain all of these bizarre and seemingly unrelated features.

Physiology. POR is the obligate electron transfer protein for all microsomal (type II) P450 enzymes⁶⁶, including the steroidogenic enzymes CYP17A1, CYP21A2 and CYP19A1; hepatic P450s that participate in drug metabolism; prostaglandin- and retinoid-metabolising P450s; and CYP51A1 (lanosterol demethylase), which is required for cholesterol biosynthesis. Mice with complete absence of Por die before birth, probably because of disordered retinoid synthesis.⁶⁷ In parallel, no human being has been described with complete POR deficiency, such that at least one allele in all patients retains partial activity. POR deficiency can thus be viewed as *multiple partial defects* in steroidogenesis (Fig. 6). The complexity of this disease derives not only from the variable degree of enzymatic deficiency but also from the capacity of different

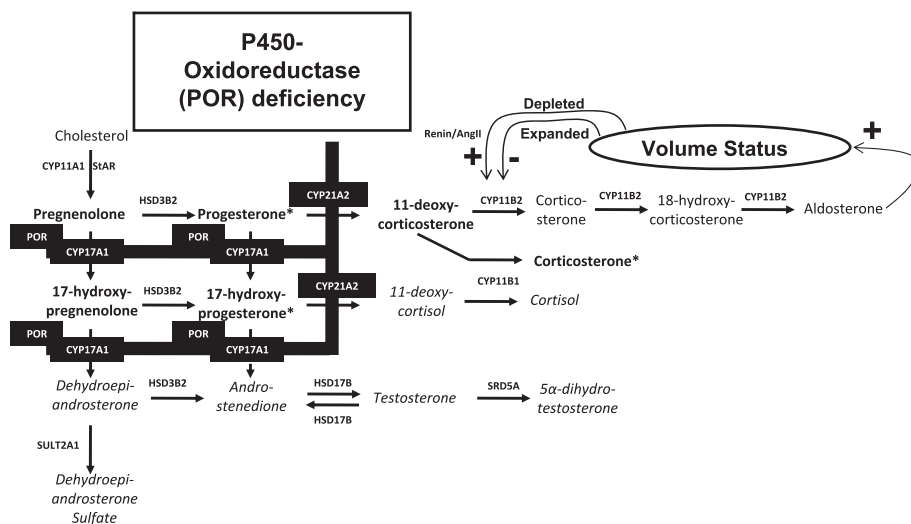


Fig. 6. Altered steroid biosynthesis in P450-oxidoreductase deficiency. The physiology is complex and variable, but both 17OHP and corticosterone accumulate, albeit not as high as other forms of CAH (21OHD and 17OHD, respectively). Progesterone, which is proximal to any block, is markedly elevated out of proportion to the moderate and variable elevation of 17OHP, in contrast to 21OHD in which 17OHP is the most elevated. Steroids that require two steps mediated by CYP17A1 and/or CYP21A2 are deficient, including cortisol and 19-carbon steroids. Steroids that are produced in excess are in bold, with diagnostic steroids being the combination of very high progesterone with moderately elevated 17OHP and corticosterone (asterisks). Steroids that are deficient are in italics, with minor pathways shown with dashed arrows. The virilization of 46,XX newborns with POR deficiency is probably the consequence of flux from 17OHP to androsterone via the alternate pathway to DHT (Fig. 1B).

mutations to affect the various enzymes differentially.⁶⁸ In general, the 17,20-lyase activity of CYP17A1 is most vulnerable to disruption, explaining the low postnatal androgen synthesis in most cases. Nevertheless, some mutations impair 21-hydroxylation or aromatase activity very little.

To understand the disordered steroidogenesis, the various consequences of POR deficiency should be conceptualised first before specific steroid changes are predicted. The four guiding principles are listed below:

- 1) Steroids requiring two activities impaired by PORD are low. For example, cortisol requires hydroxylations mediated by CYP17A1 and CYP21A2, and cortisol deficiency is essential universal except in the mildest cases. DHEAS and androgens require two reactions catalysed by CYP17A1, and androgens are always low.
- 2) Steroids proximal to all blocks are high. Progesterone is always elevated in PORD, since only CYP11A1 and 3βHSD are required.
- 3) Steroids requiring one activity impaired by PORD are normal or moderately elevated. In general, 17OHP is elevated but not as high as in 21OHD. This paradox of having a defect in 17-hydroxylase activity yet high 17OHP is understood by the very high progesterone concentrations, such that the progesterone/17OHP ratio (precursor/product ratio across the block) is indeed elevated in PORD.
- 4) The impairment of each step is variable. For mutations that affect 21-hydroxylase activity much less than 17-hydroxylation, aldosterone and 17OHP are relatively normal, whereas corticosterone will be quite high. Some mutations greatly impair aromatase activity, and others do not. Homozygosity for mutation G539R presents clinically as isolated 17,20-lyase deficiency. Presumably, the Antley–Bixler phenotype occurs only when CYP51A1 activity is obliterated, but this hypothesis has not been tested experimentally.

As with 3 β HSD deficiency, both adrenal and gonadal steroidogenesis are disrupted. The gonadal phenotype may be as mild as infertility and irregular menses in an otherwise normal adult woman or severe DSD in boys or girls. The incomplete masculinisation of boys is readily understood as a consequence of low T synthesis from the CYP17A1 impairment. By contrast, the explanations for virilisation of newborn girls and maternal virilisation are not obvious from the classical pathway (Fig. 1). Low placental CYP19A1 (aromatase) activity is common in PORD, and mutations in CYP19A1 itself cause maternal virilisation. In PORD, androgen production by the foetal adrenal is low, suggesting that aromatase deficiency is not the only mechanism. In the face of high 17OHP, however, flux to DHT might be driven along the backdoor pathway (Fig. 1B). These 5 α -reduced 19-carbon steroids such as androsterone are very potent as DHT precursors, so small amounts are sufficient to cause partial virilisation. A high ratio of 5 α /5 β -reduced 19-carbon steroids (androsterone/aetiocholanolone) in the urine of women carrying PORD fetuses⁶⁹ and in newborns with PORD⁷⁰ is evidence for elevated androsterone production directly from the foetal adrenal in PORD. The role of the alternate pathway to DHT in male genital development in PORD is not known and is likely to vary with the specific genotype.

Genetics. The molecular basis of this disorder was elucidated in 2003 and found to be incomplete loss of POR activity.^{71,72} Since POR is the essential electron donor for all type II cytochromes P450, these mutations result in severe but incomplete loss of the activities of CYP17A1, CYP21A2 and CYP19A1—which explains the disordered steroidogenesis. The skeletal malformations probably derive from impaired activity of CYP51A1 (lanosterol demethylase), which disrupts cholesterol synthesis *in utero* and thus may disturb body patterning by disrupting hedgehog transcription factor signalling and during development. The A287P is the most frequently reported mutation in Caucasians⁶⁸ while R457H is found worldwide but is particularly frequent in Japanese populations.⁷³

Presentation and diagnosis. The presentation is highly variable, but the classic presentation is a child with ambiguous genitalia (46,XX DSD or 46,XY DSD) and craniofacial abnormalities characteristic of Antley–Bixler syndrome. The absence of skeletal dysplasia does not exclude the diagnosis. Salt wasting and hypotension are rare, except in significant illness. Curiously, hypertension is rare, despite elevation in 11-deoxysteroids as in 11OHD and 17OHD. Since the disorder was only characterised with molecular genetics in the past 6 years, many of these children have been misdiagnosed or never diagnosed, particularly when the phenotype is mild. The 17OHP is mildly to moderately elevated, and most newborns will be identified in screening for 21OHD, prompting evaluation, while the virilisation is usually much less than is typical for 21OHD.

The diagnosis is established only with detailed steroid profiling after cosyntropin stimulation testing⁷³ or by analysis of urinary steroids by mass spectrometry⁷⁴, if available. The typical pattern shows high progesterone, variably elevated or normal 17OHP and corticosterone, low cortisol and 19-carbon steroids (Fig. 6). Definitive criteria for the diagnosis have not been established due to the variable impairment of multiple activities, and genetic analysis of the POR gene is often the only way to establish the diagnosis with certainty.

Therapy. The newborn. Basal cortisol production is often low or marginally normal but does not stimulate with cosyntropin, and the degree of glucocorticoid deficiency must be assessed to tailor replacement therapy. Since postnatal androgens are low, high doses of glucocorticoid to prevent further androgen excess in 46,XX DSD newborns are not necessary. Conventional replacement doses of glucocorticoids and stress dosing for illness are generally sufficient, and mineralocorticoid supplementation is rarely indicated. Orthopaedic care for the skeletal malformations may dominate the management in the child, and vaginal reconstruction is generally deferred unless virilisation is severe.

The child and adolescent. Glucocorticoid supplementation, stress dosing and supportive care treatments are continued, as there is no evidence that the deficiency ameliorates over time. While boys often experience some pubertal progression, girls generally show little maturation⁷³, possibly because an additional type II P450 (CYP19A1) is required to convert androgens to oestrogens. Consequently, oestrogen replacement is commenced at the time of expected puberty as in 3 β HSD deficiency. Large ovarian cysts may develop at puberty but regress with oestrogen replacement therapy.

The adult. Children without life-threatening skull malformations should survive into adulthood. No substantive data exist on the care of women with PORD, as children diagnosed since the characterisation of the disease have not reached adulthood. Glucocorticoid supplementation and cyclic oestrogen/progestin will be necessary and complete the endocrine management. Pregnancy is unlikely, but may be possible in mild cases with ovulation induction and assisted reproduction. Vaginal reconstruction may be necessary for adequate intercourse.

Prenatal treatment. The issue of prenatal treatment is much more complex in PORD than in 21OHD, since both boys and girls are born with DSD. In addition, depending on the mutation, adrenal 19-carbon steroids produced via the backdoor pathway might contribute significantly to DHT production in males. Thus, treatment of an affected male could make the genital ambiguity worse. In principle, dexamethasone treatment of an affected female using the same approach as in 21OHD and 11OHD should reduce genital virilisation, but data to support this approach are lacking. The impetus for treatment is less than in 21OHD, since the degree of genital ambiguity is generally mild in PORD, although some Prader IV–V cases have been described. Until a much better understanding of how each unique genotype correlates with virilisation of a female foetus, prenatal therapy is not recommended.

Materno-foetal disorders

Aromatase deficiency

The aromatase enzyme (CYP19A1) is the only cytochrome P450 enzyme with the capacity to convert androgens to oestrogens.⁷⁵ Aromatase is expressed in many tissues⁷⁶, and studies of individuals with aromatase deficiency⁷⁷ demonstrate the importance of oestrogens even in men for bone development, metabolism and immune function.^{78,79} During pregnancy, placental aromatase plays a critical role in protecting the foetus from high concentrations of androgens synthesised normally by the foetal adrenal gland and also androgens of maternal origin, yet the oestrogen production *per se* is not required for a successful term pregnancy. Aromatase deficiency is extremely rare, with fewer than 20 cases reported to date.⁸⁰ Milder cases may present in adolescence with pubertal failure and be difficult to distinguish from other forms of ovarian failure.⁸¹

Physiology. DHEA(S) and 16 α -hydroxyDHEA from the foetal circulation are normally converted by placental 3 β HSD1 to androstenedione and 16 α -hydroxyandrostenedione, which are in turn converted to estrone and 16 α -hydroxyestrone by placental aromatase. Placental 17 β HSD1 then completes the synthesis of the active oestrogens, oestradiol and oestriol, respectively. In the rare cases of the autosomal recessive aromatase deficiency, accumulation of androstenedione, testosterone and DHT lead to maternal virilisation after the second trimester of pregnancy and virilisation (46,XX DSD) of the female affected foetus if the enzymatic deficiency is very severe.⁷⁷ Because aromatase executes only the very distal steps of steroidogenesis, most other steroids are not affected. Post-natally, oestrogen production by the ovary and peripheral tissues is likewise impaired, resulting in androgen accumulation in adolescence and adulthood with failure to develop female secondary sexual characteristics.⁷⁷

Genetics. Over 15 different CYP19A1 mutations have been described, and these defects include missense, nonsense, insertion and deletion mutations. These defects can interfere with substrate binding, heme incorporation or protein folding. No common or founder mutations have been described. The few cases not attributable to consanguinity are compound heterozygotes.⁸⁰

Presentation and diagnosis. Affected females are born with ambiguous genitalia, similar to presentations of the more common 46,XX DSD caused by CAH, but these newborns have normal 17OHP and 11-deoxycortisol concentrations. Glucocorticoid and mineralocorticoid production are likewise normal, and fluid and electrolyte imbalance is not observed. The diagnosis is suspected if maternal virilisation occurs during pregnancy in a woman who fails second-trimester birth defects screening due to low unconjugated serum oestriol or urinary oestriol.⁸⁰ The differential diagnosis mainly involves distinguishing aromatase deficiency from PORD, which is resolved by the presence of other blocks in adrenal steroidogenesis and cortisol precursor accumulation in PORD. Since oestrogen production by

the neonatal ovary is normally very low, biochemical testing of the child is difficult until puberty, at which time ovarian androgen are elevated and oestrogen is markedly reduced.⁷⁷ Affected girls often develop polycystic ovaries before puberty and delayed bone maturation during childhood and adolescence. The girls typically progress to have hypergonadotrophic hypogonadism with failure of breast development and primary amenorrhoea as well as hirsutism, acne and virilisation.⁸⁰ The spectrum of disease is broad, however, with some girls experiencing significant breast development despite severe androgen excess and virilisation during foetal life.⁸¹

Therapy. The newborn. The newborn needs no specific therapy, since androgen excess ceases, and no adrenal defects are present.

The child and adolescent. Treatment of aromatase deficiency almost always involves replacement of oestrogens in the affected female at the time of expected puberty. The dose is carefully titrated to optimise skeletal maturation and bone mineral density, an appropriate adolescent growth spurt and appropriate female secondary sexual maturation at puberty.⁸⁰ Cyclic menses is initiated 2–3 years later with addition of progestin. In particular, if not treated early enough, the ovaries can become severely hyperplastic with large cysts⁷⁷ and require surgical removal for pain or bleeding.

The adult. Cyclic oestrogen and progestin therapy is continued to protect against osteoporosis and endometrial hyperplasia. Vaginal reconstruction surgery is performed if necessary when the woman is ready to have sexual intercourse. Very little data exist about the course of the disease in adulthood.

Prenatal treatment. Because the source of androgens is the foetal adrenal, prenatal dexamethasone therapy might reduce virilisation of both mother and child in aromatase deficiency, but no such cases have been reported. Unlike CAH, virilisation occurs primarily in the second and third trimester when adrenal 19-carbon steroid production accelerates. Consequently, it may be possible to delay treatment until a prenatal diagnosis of an affected female foetus has been established and achieve comparable results to those obtained by beginning presumptive treatment in early gestation. Treatment of a woman carrying a male foetus with aromatase deficiency should not alter genital development of the child but should lessen virilisation of the mother.

Maternal-derived androgens

In rare instances, virilisation of the foetus derives from a maternal source of androgens. In most cases, the maternal source can be identified before delivery with work-up for the most common ovarian and adrenal sources of androgen-producing tumours. Chronic, more common conditions of androgen excess such as PCOS⁸² or CAH³² in the mother are rarely reported as the cause of foetal virilisation and illustrate the ability of the placental aromatase to protect the foetus from elevated androgens. Even with much higher androgen concentrations that cause more significant maternal virilisation, the female foetus is not always virilised.

Table 3

Sources of maternal-derived androgens.

Endogenous	Exogenous
<i>Benign</i>	<i>Synthetic androgens</i>
Luteoma of pregnancy ⁸⁴	Danazol ⁸⁸
Adrenal adenoma ^{89,90}	Progestins (medroxyprogesterone acetate) ⁹¹
Hyperreactio luteinalis	Potassium-sparing diuretics
Thecoma/fibroma	
Stromal hyperthecosis	
Brenner tumour	
Serous cystadenoma	
Mature cystic teratoma (dermoid cyst)	
<i>Malignant</i>	
Metastatic carcinomas (Krukenberg tumour) ⁹²	
Sex-cord stromal tumours—granulosa cell and Sertoli-Leydig tumours ⁹³	
Adrenal cortical carcinoma ⁹⁴	
Cystadenocarcinoma	
Hilar cell tumour	

The most common cause of maternal virilisation during pregnancy is a luteoma, a benign hyperplastic ovarian growth of luteinised stroma, granulosa or thecal cells.⁸³ About 25% of luteomas will secrete androgens and fewer than half of affected mothers will have signs of hyperandrogenism. Only about half of the girls born to symptomatic mothers will show some signs of virilisation.⁸⁴ Other reported maternal sources of androgen excess are listed in Table 3. Although these other causes of maternal virilisation should always be considered, few or no cases of foetal virilisation and 46,XX DSD have been reported with many of these potential sources of endogenous or exogenous androgens (Table 3). It is not known why conditions with similarly elevated androgen concentrations have variable affect on the presentation of foetal virilisation.^{85,86} The proportion of androgens that are 5 α -reduced may be a critical factor, as these steroids are not substrates for aromatase and cannot be converted to oestrogens by the placenta.

The degree of foetal virilisation will vary depending on the timing of maternal symptoms and the severity of androgen excess. If a work-up has not already been initiated during pregnancy for a symptomatic mother, the diagnostic work-up for the foetus is initially the same as the infant with CAH. When the mother experiences virilisation during pregnancy, it is important to measure oestrogens to identify POR and aromatase deficiencies by the low circulating concentrations of oestrone, oestradiol and oestriol.⁸⁰ Even post-partum, a negative work-up of an endogenous source of androgens in the foetus necessitates a work-up in the mother to exclude rare but malignant sources including adrenal tumours and rare malignancies (see Table 3).

Treatment for the mother will depend on the timing of the diagnosis and appearance of the ovarian mass on imaging tests. Suspected luteomas may be observed and can resolve spontaneously by 4–8 weeks postpartum. Exploratory laparotomy with frozen section during pregnancy may be indicated for ovarian or adrenal tumours with a suspicious appearance based on size, density and heterogeneity.⁸⁴ Hormonal treatments for the foetus are rarely necessary, and genital evaluation and reconstruction can be tailored based on the presentation. Mild-to-moderate clitoromegaly alone may reduce spontaneously and not require therapy.

Related disorders

Several other conditions cause androgen excess but are not strictly 46,XX DSDs, since these women are normal at birth but develop increased androgen production. These disorders include generalised glucocorticoid insensitivity; Cushing's disease; androgen-producing adrenal and ovarian tumours; apparent cortisone reductase deficiency and phosphoadenine phosphosulphate sulphotransferase type 2 deficiency.⁸⁷ These conditions are mentioned for completeness only and to clarify that these diseases do not cause 46,XX DSD.

Summary

The androgens responsible for virilisation in 46,XX DSDs are almost always derived from the foetal adrenal glands and rarely due to maternally derived or exogenous androgens. The presentation and screening laboratories can be similar amongst these disorders and require detailed evaluation to distinguish 21OHD—the most common cause—from other possible diagnoses. Clues to the diagnosis include presence or absence of maternal virilisation, the severity of the genital ambiguity, the co-existence of gonadal dysfunction and associated skeletal malformations. Advances in the pathophysiology and molecular genetics of the 46,XX DSDs during the last 2 decades also provide insight to normal and abnormal steroid biosynthetic pathways in the adrenals and gonads. Treatment strategies generally consist of glucocorticoid replacement with fluid and electrolyte balancing as necessary with vaginal reconstruction surgeries. These treatments often fail to control androgen excess adequately, bring long-term side effects, or establish satisfactory female external genitalia for intercourse and childbearing. Consequently, additional therapies are required to optimise growth and development and to mitigate consequences of chronic glucocorticoid treatment. Prenatal treatment strategies hold the promise for reducing the virilising consequences of these diseases, but enthusiasm is tempered and unknown long-term consequences of foetal exposure to potent synthetic glucocorticoids. The role of bilateral adrenalectomy, the management of the adult with 46,XX DSD and the optimal use of anti-androgens or steroidogenesis inhibitors are a few of the unsolved problems in these diseases.

Practice points

- Although 21OHD is the most common cause of 46,XX DSD, circulating 17OHP concentrations are elevated in several other forms of CAH causing 46,XX DSD
- Cosyntropin-stimulated serum 11-deoxycortisol, 11-deoxycorticosterone, 17-hydroxypregnenolone, DHEA and corticosterone can be used to distinguish the different types of CAH with elevated 17OHP.
- Adjunctive measures to ameliorate androgen excess should always be considered in the treatment of CAH to avoid long-term over-treatment with glucocorticoids.
- When maternal virilisation accompanies 46,XX DSD children, the child should be evaluated for POR deficiency and aromatase deficiency, and the mother should be evaluated for ovarian or adrenal tumours.

Research agenda

- Long-term outcomes and risk/benefit assessment for prenatal treatment and bilateral adrenalectomy in 21OHD.
- Definitive diagnostic criteria for POR deficiency.
- The relative proportions of DHT derived from the classical and backdoor pathway in various forms of 46,XX DSD.
- Optimal management strategies for adults with 46,XX DSD, including lower doses of corticosteroids and best approaches to vaginal reconstruction.

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