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Rare forms of genetic steroidogenic defects affecting the gonads and adrenals

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Pathogenic variants have been found in all genes involved in the classic pathways of human adrenal and gonadal steroidogenesis. Depending on their function and severity, they cause characteristic disorders of corticosteroid and/or sex hormone deficiency, may result in atypical sex development at birth and/or puberty, and mostly lead to sexual dysfunction and infertility. Genetic disorders of steroidogenesis are all inherited in an autosomal recessive fashion. Loss of function mutations lead to typical phenotypes, while variants with partial activity may manifest with milder, non-classic, late-onset disorders that share similar phenotypes. Thus, these disorders of steroidogenesis are diagnosed by comprehensive phenotyping, steroid profiling and genetic testing using next generation sequencing techniques. Treatment comprises of steroid replacement therapies, but these are insufficient in many aspects. Therefore, studies are currently ongoing towards newer approaches such as lentiviral transmitted enzyme replacement therapy and reprogrammed stem cell-based gene therapy.

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Introduction

Classic congenital adrenal hyperplasia (CAH) with atypical sex development has been first reported in the Italian journal "*Il Morgagni*" by the pathologist De Crecchio more than 150 years ago [1,2]. He described the history and post-mortem findings of Giuseppe Marzo, who lived as a man, had almost normal male external genitalia, female internal reproductive organs, and massively enlarged adrenals. He died in an apparent Addison crisis at the age of 44 years. If Marzo had been born today, his life would have been much different, although medical and psychological care of DSD (disorders/differences in sex development) and CAH remain complex topics with many unsolved issues [3–6]. A genetic 46, XX persons with classic CAH presenting with severe external virilization would likely have been identified at birth, and a newborn screening program would have revealed a diagnosis of CAH. Hormonal replacement therapy for adrenal corticosteroid deficiency would have been started immediately. The sex of rearing and the gender identity would have been most likely female [7]. A feminizing genitoplasty would have been considered in the first year of life, although this is controversially discussed recently [6]. Current treatment options would have preserved Marzo's fertility and would have lowered his risk for a fatal adrenal crisis.

In the last century remarkable progress has been made in understanding the developmental biology of the human adrenals and gonads, the biochemistry of steroid hormone biosynthesis of these organs, as well as the underlying genes and regulators involved. Till this day, the advent of new technologies in both the clinical and the experimental laboratory settings have broadened the spectrum of steroid disorders to forms presenting with unexpected phenotypes. This has led to diagnostic and therapeutic opportunities for patients with a rare genetic disorder affecting the gonads and adrenals, enabling not only their survival, but also improving their quality of life. Nevertheless, further research is needed to answer open questions [8], and a cure for these genetic disorders is still missing.

Both the adrenals and the gonads originate from a common embryologic anlage, the urogenital ridge, and therefore share many genetic and steroidogenic characteristics. The adult human adrenal cortex produces steroid hormones that are crucial for life, supporting immune response, glucose homeostasis, salt balance and sexual maturation. It consists of three histologically distinct and functionally specialized zones that are controlled by the hypothalamic-pituitary-adrenal (HPA) axis and by the renin-angiotensin (RA) system. The fetal adrenal forms from mesodermal material and produces predominantly adrenal C_{19} steroids (e.g. androgens) from its fetal zone, which involutes after birth. Transition to the adult cortex occurs immediately after birth for the formation of the zona glomerulosa (zG) and fasciculata (zF) for aldosterone and cortisol production and continues through infancy until the zona reticularis (zR) for adrenal androgen production is formed and the continuous process of adrenarche starts as early as three years of age [9]. The development of this essential organ is complex and still not fully understood [10].

The gonads also originate from the mesonephros. The adult testes and ovaries produce sex steroids (androgens and estrogens) as well as sperms and oocytes, respectively, for human reproduction. In the adult testis, Leydig cells in the interstitium produce testosterone, while in tubules Sertoli cells nurture maturing germ cells. In the mature ovary, theca and granulosa cells collaborate in the biosynthesis of estrogens from cholesterol, while during a complex monthly cycle the finite pool of follicles produces a pregnable oocyte. In the fetus, a neutral gonad anlage can be detected by 6 weeks of gestation (GW6), before it starts to show sexual dimorphism in its further development. Soon after that, the genetically determined testis starts to produce testosterone, which is essential throughout fetal life and beyond for male sexual development and function [11]. By contrast, the genetically determined ovary is predominantly active from GW9 in proliferating oogonia to produce the prenatally set follicle pool for the whole female reproductive postnatal life. Although pre-granulosa cells are already found in the first trimester ovary, and many enzymes of the steroidogenic pathway seem active during ovary differentiation, the role of ovarian steroidogenesis during fetal life is largely unknown, and generally regarded as unimportant [11]. However, important for understanding the clinical manifestations of genetic disorders of gonadal and adrenal steroidogenesis at birth is the fact that adrenal and gonadal steroids are part of a bigger network forming the steroidogenic metabolome *in utero*. During fetal development, the liver and the placenta play major roles in this network, especially when relating to androgen metabolism [12]. As the normal development of the typical female and male external genitalia relies

largely on the absence or presence of testosterone and its more active form dihydrotestosterone, any disturbance in androgen production may result in apparent virilization of a 46, XX fetus or under masculinization of a 46, XY fetus. After birth and minipuberty, gonadal steroidogenesis is shut down until puberty, when activation of the hypothalamic-pituitary gonadal (HPG) axis commands the organs to resume sex steroid production for normal sexual maturation, fertility, and reproduction. But also in postnatal/adult life, steroids secreted by the adrenals and gonads are converted to active and inactive metabolites by peripheral organs such as the adipose tissue or the liver; and this complex peripheral steroid metabolism may be responsible for the formation of unusual steroid profiles in genetic disorders of steroidogenesis.

This review will summarize the current clinical, biochemical and genetic knowledge of rare genetic disorders of steroidogenesis of the adrenals and/or gonads. After providing an overview of the classic and alternate pathways of human steroid biosynthesis, specific genetic disorders of steroidogenesis are described based on the underlying genetic defects and their ensuing characteristic clinical findings. We also provide insight into specific diagnostic and therapeutic options. Finally, unsolved problems and future research needs are highlighted.

Basics of adrenal and gonadal steroidogenesis

Adrenal steroidogenesis

The adult adrenal cortex produces three distinct classes of steroid hormones, including mineralocorticoids (MCs) in the zG, glucocorticoids (GCs) in the zF and androgens in the zR. Fig. 1A provides an overview of the steroid biosynthesis pathways comprised in the human adrenal cortex. All cortical steroids are synthetized from cholesterol, which is either stored in esterified form or freely available within cells [13,14]. The key enzymes involved in steroidogenesis are either cytochrome P450 enzymes (CYPs) or hydroxysteroid dehydrogenases (HSDs). StAR facilitates the transport of 86% of the cholesterol to the inner mitochondrial membrane [13,14]. Here, cholesterol is converted to pregnenolone by the side-chain cleavage enzyme, encoded by the *CYP11A1* gene and supported by the cofactor system adrenodoxin (ADX1)/adrenodoxin reductase (ADXR). The function of all monooxygenases of the cytochrome P450 family involved in steroidogenesis critically depends on the cytochrome P450 reductase (POR), localized to the membrane of the endoplasmic reticulum [15,16]. While StAR and CYP11A1 are ubiquitously expressed within the adrenal cortex, the zonal segregation of other enzymes accounts for the compartmentalization of steroid production [17,18]. Within the zG, presence of aldosterone synthase (CYP11B2) and absence of CYP17A1 is specific and enables the production of aldosterone. Within the zF the 17α-hydroxylase activity of CYP17A1 promotes cortisol production. In the zR production of dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-S) and 11β-hydroxyandrostenedione are promoted by the lack of HSD3B2 expression, the enhanced activity of the 17,20-lyase activity of CYP17A1, and the increased expression of the sulfotransferase SULT2A1. In addition, the allosteric regulator of CYP17A1, cytochrome *b*5 is required for DHEA production [19]. Furthermore, it has been shown that bioactive androgens including 11-oxygenated and their precursors are synthesized within the adrenal cortex through the catalytic activities of the type 5 17β-hydroxysteroid dehydrogenase encoded by the *AKR1C3* gene and the 11-hydroxylase encoded by *CYP11B1* [20,21]. Among them 11-ketotestosterone and 11-ketodihydrotestosterone effectively activate the androgen receptor [22].

Testicular and ovarian steroidogenesis

Not only the adrenal cortex, but also the gonads are steroid production sites. Testicular Leydig cells are the principal source of testosterone production in males (Fig. 1B). In the classic pathway, pregnenolone is converted through the delta 5 pathway by CYP17A1 to 17α-hydroxypregnenolone (17OHPreg) and DHEA. DHEA is then turned over to testosterone through androstenedione or androstenediol catalyzed by the activities of HSD3B2 and HSD17B3/5, respectively. In the delta 4 pathway pregnenolone is converted to 17α-hydroxyprogesterone (17OHP), androstendione and testosterone, but this pathway only plays a minor role in humans as 17OHP is not a preferred substrate

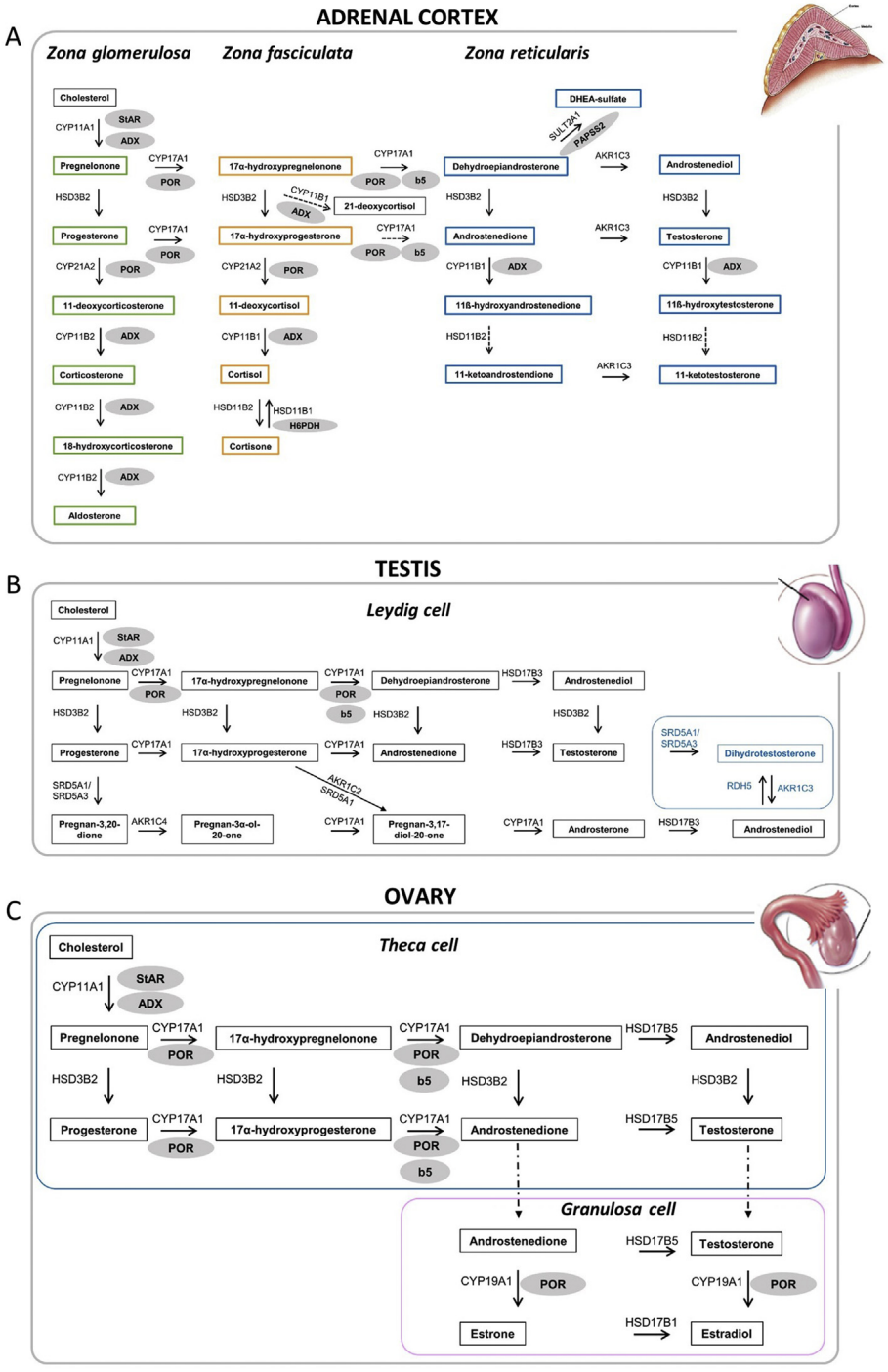

Fig. 1. – **Classic steroid biochemistry pathways of the adrenal cortex, the testis, and the ovary.** A. Adrenal steroidogenic pathways. Mineralocorticoids and their precursors are framed in green, glucocorticoids and precursor in orange, androgens, and

for the CYP17-17,20 lyase activity [23]. In addition to the classic testosterone synthesis pathway, an alternative backdoor pathway has been discovered more recently, in which 17OHP is 5α, 3α reduced (by SRD5A1 and AKR1C2/4) and then directly converted to dihydrotestosterone (DHT) without going through androstenedione or testosterone (Fig. 2).

In the ovary, theca cells respond to LH signaling by inducing the conversion of cholesterol to androgens such as androstendione and testosterone (Fig. 1C). Similarly, granulosa cells respond to FSH signaling and convert theca cell-derived androgens into estrogens [24]. Synthesis of pregnenolone and progesterone occurs in both theca and granulosa cells. But conversion of pregnenolone to 17OHPreg and DHEA via the delta 5 pathway occurs exclusively in theca cells as CYP17A1 is not expressed in granulosa cells [25]. DHEA is then further converted to androstenedione and testosterone in theca cells, before diffusing to neighboring granulosa cells where they are converted to estrogens by aromatase (CYP19A1) activity. Within the ovary, the *CYP19A1* gene is only expressed in granulosa cells, but not in theca cells. Overall ovarian steroidogenesis is devoted to produce estrogens, but small amounts of androgens produced in theca cells will also be secreted into the peripheral circulation.

Novel pathways of human androgen biosynthesis

Knowledge of human androgen biosynthesis pathways has been enhanced over the last two decades. Novel alternate pathways have been discovered (e.g. the backdoor pathway), and long forgotten androgens resurrected (e.g. 11-oxy androgens). Fig. 2 gives an overview of the biochemistry of these two 'novel' and the classic pathways of androgen synthesis.

The alternative, backdoor pathway was originally discovered in the tammar wallaby [26,27], before specific metabolites were detected in steroid profiles of humans with POR [28,29] and 21-hydroxylase deficiency [30], and first human mutations in specific genes comprised in this pathway were reported [31]. In this pathway mainly 17OHP is 5α (SRD5A1) and thence 3α (AKR1C2/4) reduced to 17-hydroxy-allo-pregnanolone, which is a perfect substrate for CYP17A1 and converted to androsterone. This metabolite is then converted to androstanediol and finally to DHT. Alternatively, the backdoor pathway may start from the delta 5 pathway, but this pathway is mainly used in rodents (Fig. 2 and Ref [27]).

The 11-oxy androgens are known since long, but that a pathway of adrenal precursors (e.g. 11β-hydroxyandrostenedione) provides an important source for active androgens in the periphery (11-ketoandrostenedione, 11-ketotestosterone), has only been recognized recently (Fig. 2 and [22]). These 11-oxy androgens seem associated with androgen excess disorders such as congenital adrenal hyperplasia (CAH), premature adrenarche and the polycystic ovary syndrome [22].

Specific disorders of human adrenal and gonadal steroidogenesis

Essential overlap exists in steroidogenesis of the adrenals and gonads. This concerns especially the initial steps of the biochemical pathways and involved genes (Fig. 1). Genetic defects in these genes therefore manifest with signs and symptoms of disrupted adrenal and gonadal steroidogenesis. By contrast, no overlap is seen between the two organs in their overarching regulatory feedback systems; e.g. the HPA and RA axes control the adrenal cortex, while the HPG axis controls the testes and ovaries. In addition, tissue specific expression of genes is responsible for the characteristic steroid profiles of specific steroidogenic cells and tissues. For understanding steroid disorders, it is important to keep in mind that both the adrenals and the gonads show remarkable spatio-temporal, structural and

precursors in blue. Cofactor proteins are marked in grey. Dashed arrows indicate minor adrenal pathways; conversion takes place mostly in peripheral tissues. B. Steroidogenic pathways in Leydig cells. The conversion of testosterone to dihydrotestosterone is catalyzed in e.g. genital skin and the prostate and is therefore shown in blue. C. Steroidogenic pathways in ovarian theca and granulosa cells. Abbreviations: StAR: streoidogenic acute regulatory protein; ADX: adrenodoxin; CYP11A1: cytochrome P450 cholesterol side-chain cleavage; CYP17A1: 17α-hydroxylase/17,20 lyase; POR: Cytochrome P450 reductase; b5: cytochrome *b*5; HSD3B2:3β-hydroxysteroid dehydrogenase type 2; CYP21A2: 21-hydoxylase; CYP11B1: 11β-hydroxylase; AKR1C2: Aldo-keto reductase family 1 member C2; AKR1C3: 17β-hydroxysteroid dehydrogenase type 5; AKR1C4: Aldo-keto- reductase family 1 member C4 (3alpha-Hydroxysteroid 3-Dehydrogenase); HSD11B2: 11β-hydroxysteroid dehydrogenase type 2; DHEAS: dehydroepiantrosterone sulfate; SULT2A1: steroid sulfotransferase; PAPSS2: PAPS-synthase 2; SRD5A1/2/3: Steroid 5 alpha-reductase 1/2/3; HSD17B3/5: 17β-hydroxysteroid dehydrogenase type 3/5; CYP19A1:cytochrome P450 family 19 subfamily A member 1 (aromatase); HSD17B1: hydroxysteroid 17β-dehydrogenase 1.

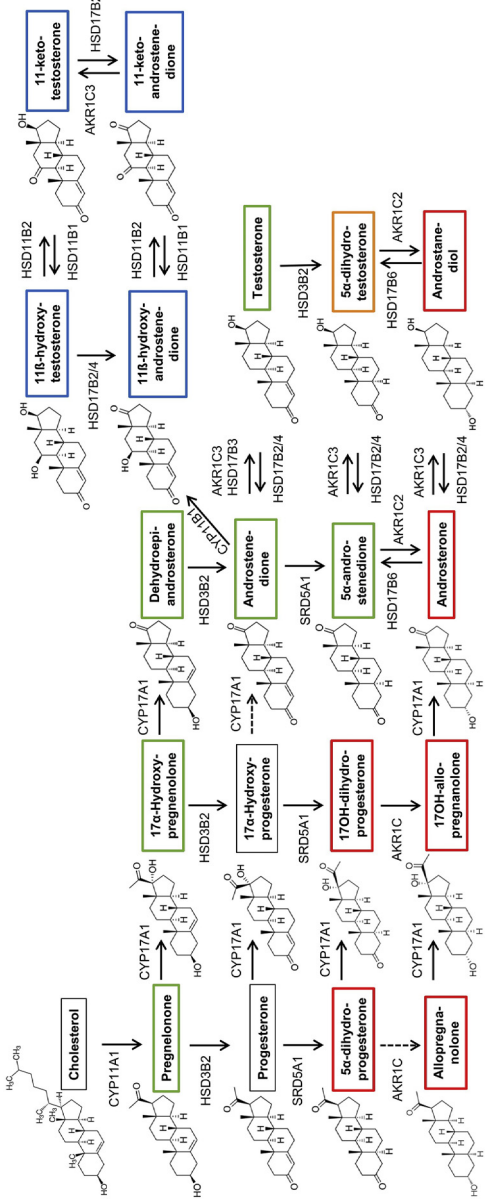

Fig. 2. The three major pathways of human androgen biosynthesis. Green framed: classic pathway; red framed: alternative pathway; blue framed: 11-oxygenated pathway. CYP11A1: cytochrome P450 cholesterol side-chain cleavage; CYP17A1: 17α-hydroxylase/17,20 lyase; SRD5A1: steroid 5 alpha-reductase 1; HSD3B2: 3β-hydroxysteroid dehydrogenase type 2; AKR1C: Aldo-keto reductase family 1 member C; AKR1C2: Aldo-keto reductase family 1 member C2; AKR1C3: 17β-hydroxysteroid dehydrogenase type 5; HSD11B1: 11β-hydroxysteroid dehydrogenase type 1; HSD11B2: 11β-hydroxysteroid dehydrogenase type 2; HSD17B2/4: 17-β-hydroxysteroid dehydrogenase type 2/4; HSD17B6: 17-β hydroxysteroid dehydrogenase 6 (3α-oxidase). Figure adapted from [98].

Table 1
Phenotypes of genetic defects affecting steroidogenesis of the gonads and/or adrenals in 46, XX and 46, XY individuals.

Disorder	Gene/ OMIM	Adrenal Insufficiency	46, XY Gonadal Phenotype (T Deficiency)	46, XX Gonadal Phenotype (E2 Deficiency)	Fertility	Other Features
Lipoid congenital adrenal hyperplasia (LCAH)	*StAR* 201710	YES	Classic form: 46, XY DSD, gonadal insufficiency Non-classic form: normal or NK	Classic: primary or secondary ovarian insufficiency (POI) Non-classic: NK or normal	Classic: Absent in 46,XY; variable in 46,XX	
P450 side chain cleavage syndrome (CAH)	*CYP11A1* 118485	YES	Classic form: 46, XY DSD, gonadal insufficiency Non-classic form: normal	Classic: primary or secondary ovarian insufficiency (POI) Non-classic: NK or normal	Reported in 46,XX	
3β-hydroxysteroid dehydrogenase II deficiency (CAH)	*HSD3B2* 201810	YES	46, XY DSD, gonadal insufficiency Non-classic form: normal, but premature adrenarche	46, XX DSD with atypical genital development; gonadal insufficiency Non-classic form: normal, but premature adrenarche	Absent in 46,XY; reported in 46,XX	
21-hydroxylase deficiency (CAH)	*CYP21A2* 201910	YES	Classic form: normal Non-classic form: normal	46, XX DSD with atypical genital development; Non-classic form: premature adrenarche, virilization, PCO	Normal in both 46, XX and 46,XY, if treated	Cave: Testicular adrenal rest tumor (m>>f) CAH-X (when combined with Ehlers-Danlos syndrome with contiguous gene variants)
11-hydroxylase deficiency (CAH)	*CYP11B1* 202010	YES	Classic form: normal Non-classic form: normal	46, XX DSD with atypical genital development; Non-classic form: premature adrenarche, virilization, PCO	Normal in both 46, XX and 46,XY, if treated	Hypertension
Combined 17-hydroxylase, 17,20 lyase deficiency (CAH)	*CYP17A1* 202110	Rare	46, XY DSD, gonadal insufficiency	Lack of pubertal development, POI	Possible in 46, XX with assisted fertility measures	Hypertension and hypokalemic alkalosis (not seen with isolated lyase deficiency)
P450 oxidoreductase deficiency (CAH)	*POR* 124015 201750	Variable	Mild to severe 46, XY DSD, gonadal insufficiency	46, XX DSD with atypical genital development or premature adrenarche, virilisation, POI, PCO	Reported	Maternal virilization during pregnancy; Antley-Bixler skeletal malformation syndrome; changes in drug metabolism
Cytochrome *b*5 deficiency	*CYB5A* 613218	NO	46, XY DSD	NK	NK	Methemoglobinemia
17β-hydroxysteroid dehydrogenase III deficiency/17-ketosteroid reductase deficiency	*HSD17B3* 264300	NO	46, XY DSD; progressive virilisation and gynecomastia at puberty	Normal	Decreased or absent in 46,XY	
5α-reductase II deficiency	*SRD5A2* 607306	NO	46, XY DSD; progressive virilisation and	Normal	Impaired in 46,XY	

(*continued on next page*)

Table 1 (*continued*)

Disorder	Gene/ OMIM	Adrenal Insufficiency	46, XY Gonadal Phenotype (T Deficiency)	46, XX Gonadal Phenotype (E2 Deficiency)	Fertility	Other Features
			gynecomastia at puberty			
3α-hydroxysteroid dehydrogenase deficiency	*AKR1C2/ 4* 600450 600451	NO	46, XY DSD; gonadal insufficiency	Normal	NK	
Aromatase deficiency	*CYP19A1* 107910	NO	Normal	46, XX DSD with variable degree of virilisation at birth, gonadal insufficiency, POI	Impaired in 46,XX	Overgrowth and metabolic anomalies in males
Steroidogenic factor 1	*NR5A1/ SF1* 184757	Rare	Mild to severe 46, XY DSD; gonadal insufficiency – very variable	POI or normal	Mostly impaired in 46,XY; variable in 46,XX	

Abbr.: E2 – estradiol; NK – not known; PCO – polycystic ovaries; POI – primary ovarian insufficiency; T – testosterone.

functional changes with their development pre- and postnatally. The most remarkable events are fetal adrenal transition at birth and adrenarche in postnatal adrenal development, and testis steroidogenesis in the early male fetus as well as gonadarche with puberty in postnatal sexual development of both sexes. Some changes even occur later in life, e.g. pregnancy, menopause, adrenopause. However, different steroid disorders may affect these events variably. To date numerous pathogenic variants underlying human disorders of steroidogenesis have been reported in all genes involved in the classic steroid pathways (Fig. 1 and Table 1) [14,32]. By contrast, genetic variants in alternate pathways are still rare and their role unclear (Fig. 2).

In the following, the phenotype of specific disorders is described from a biochemical and genetic perspective.

Defects affecting initial steps of adrenal and gonadal steroidogenesis

Lipoid congenital adrenal hyperplasia (LCAH) due to steroidogenic acute regulatory protein (STAR) deficiency

Classic LCAH is caused by severe autosomal recessive mutations in the *STAR* gene inhibiting cholesterol transport into mitochondria for the biosynthesis of all adrenal and gonadal steroids [13,14,32]. Thus, complete STAR deficiency is the most severe disorder of steroid hormone biosynthesis. By contrast, milder genetic variants enabling some cholesterol import manifest with a less severe phenotype (non-classic LCAH) mimicking familial glucocorticoid deficiencies, which are characterized by (gluco)corticosteroid deficiency only [32–34].

A 46, XY fetus with severe pathogenic *STAR* variants will not be able to virilize *in utero* and will present with typical female external genitalia at birth, but without cervix, uterus, or fallopian tubes, and with normally developed Wolffian duct derivatives [35]. By contrast, the prenatal sexual development of an affected 46, XX fetus will be typically female. At puberty, the 46, XY individual will fail to produce sex steroids in the testis and therefore fail to undergo spontaneous puberty, while the 46, XX patient with StAR deficiency is usually showing spontaneous pubertal development, but will then have anovulatory menstrual cycles [36,37]. Concerning adrenal function, STAR mutations cause complete adrenal insufficiency in the first weeks to months of life in both sexes, and milder mutations may even present later with an adrenal phenotype only, even in 46, XY subjects. These phenotypic characteristics of STAR deficiency are explained by the fact that 14% of cholesterol import to the mitochondria is STAR independent. Therefore, the pathomechanism and time scale of STAR disease has been explained by a "two hit model" [35]. In the first genetic hit, loss of STAR activity lowers the supply of cholesterol for

steroid biosynthesis critically. In the second hit, compensatory increase in cholesterol uptake and *de novo* synthesis lead to intracellular cholesterol accumulation as in a storage disease, which will ultimately damage the steroidogenic cells. As the Leydig cells are highly active in testosterone synthesis very early *in utero* for male sexual differentiation, loss of STAR activity leads to very early gonadal failure and thus severe 46, XY DSD. The fetal adrenals produce predominantly DHEA which absence results in low estriol levels in the pregnant mother. It produces very little amounts of other steroids, without which the fetus survives to birth without any problems. But by the end of pregnancy transition to the adult adrenal cortex occurs for the production of MCs and GCs that are essential for life and prompt potentially deadly adrenal crisis in the first months of life when missing. By contrast, human ovarian steroidogenesis seems quiescent during fetal life, is then minimally active during the event of "minipuberty" at age 1–6 months of life, before it is fully activated with the onset of puberty explaining the late gonadal deficiency phenotype of *STAR* mutations in 46, XX females [36].

Numerous variants of the *STAR* gene have been described in patients manifesting with classic and non-classic LCAH. Overall STAR deficiency is a very rare disorder. It is most often identified in the Japanese population, where 1 in 300 carries the p.Q258X variant, while in other populations founder effects may be responsible for some clusters [32]. Accordingly, non-classic LCAH caused by *STAR* variants has been identified variably in larger cohorts of individuals investigated for rare forms of primary adrenal insufficiency in Japan (30%) and Turkey (11%), respectively [38,39].

CYP11A1 deficiency

The side chain cleavage enzyme CYP11A1 converts cholesterol to pregnenolone in the first step of steroidogenesis, which is essential for the production of all steroid hormones in steroidogenic tissues such as the adrenals, gonads, and the placenta. It remains therefore an unsolved conundrum, how pregnancy with a CYP11A1 deficient fetus is maintained, when the placenta is deficient in progesterone production that is supposed to suppress maternal uterine contractions for preventing miscarriage in the second half of pregnancy. The phenotype of persons carrying *CYP11A1* mutations is clinically undistinguishable from STAR deficiency [32,40]. Loss of CYP11A1 activity leads to severe adrenal insufficiency, 46, XY DSD and sexual infantilism in both sexes. Milder, non-classic *CYP11A1* variants retaining 10–20% of wild-type activity manifest with isolated adrenal insufficiency [41]. But unlike STAR deficiency, loss of CYP11A1 does not cause lipoid hyperplasia of the adrenals.

Since 2001, few patients with pathogenic autosomal recessive *CYP11A1* variants have been reported [41], but more recently the non-classic form has been found in a notable number of European patients with adrenal insufficiency manifesting at different ages. Genetic analysis revealed compound heterozygote inheritance of a loss-of-function CYP11A1 mutation in combination with a common splice variant (p.E314K) that is found in about 1:140 individuals of European descents [42].

HSD3B2 deficiency

The enzyme 3β-hydroxysteroid dehydrogenase type 2 encoded by the *HSD3B2* gene converts delta 5 steroids to delta 4 steroids in the adrenals and gonads (Fig. 1). To understand the clinical and biochemical characteristics of HSD3B2 deficiency, it is important to know that there is a *HSD3B1* gene coding for a type 1 enzyme with similar activities, which is expressed in the placenta, the liver and many other peripheral tissues [43].

Fetus harboring autosomal recessive *HSD3B2* variants are born with variable degrees of mild to moderate 46, XY DSD and mostly mild 46, XX DSD. This is due to insufficient androgen production in testes for complete masculinization in males, and excessive production of adrenal delta 5 androgens that are peripherally converted to active androgens virilizing external genitalia of females. Classic adrenal steroidogenesis is compromised in all steroid biosynthesis pathways by loss of HSD3B2 activity. However, the clinical spectrum is broad: With severe forms, severe salt-wasting and non-salt-wasting adrenal insufficiency and DSD might be seen at birth followed by abnormal pubertal development later. Milder forms may show a late-onset with premature pubarche in childhood or a PCOS-like phenotype with puberty [32]. It is important to know that peripheral activity of the HSD3B1 enzyme can lead to confusing findings with HSD3B2 deficiency: The adrenal and gonads of a patient

with severe HSD3B2 deficiency will secrete very large amounts of pregnenolone, 17-hydroxypregnenolone, and DHEA, which are converted by HSD3B1 in the periphery to products that serve as substrates for further steroidogenesis. This explains high levels of serum 17OHProg measured in some neonates with HSD3B2 deficiency. It also explains masculinization and gynecomastia of 46, XY and virilization of 46, XX affected persons with pubertal development.

The incidence of autosomal recessive HSD3B2 variants in our population is very rare and unknown. Many different variants have been studied revealing a fairly good genotype-phenotype correlation with respect to the adrenal salt-wasting characteristics, but no correlation with the severity of 46, XY DSD [43]. So far, no pathogenic variants have been reported in the *HSD3B1* gene.

Defects of the gate keeper from mineralocorticoids to glucocorticoids and sex steroids: CYP17A1 deficiency

CYP17A1 is the only enzyme of the steroid biosynthesis pathway with dual 17α-hydroxylase and 17,20-lyase activities (Fig. 1) [14,44]. Therefore, it causes two different forms of steroid disorders, a rare form of CAH when both enzyme activities are inhibited and an extremely rare form of isolated sex hormone deficiency when only the 17,20-lyase activity is inhibited [32,44,45].

Patient with deficient 17α-hydroxylase activity show decreased cortisol synthesis but stimulated steroidogenesis in the mineralocorticoid pathway due to the enzyme blockage and HPA axis feedback. Still, these patients have only mild glucocorticoid deficiency, as the lack of CYP17A1 results in the overproduction of corticosterone, which has glucocorticoid activity [46]. In addition, patients typically reveal low renin hypertension, sodium retention, and hypokalemic alkalosis due to overproduction of DOC. Their hypertension becomes clinically only apparent in the second decade of life. Absence of 17,20-lyase activity with *CYP17A1* variants prevents the synthesis of adrenal and gonadal sex steroids. As a result, affected 46, XY neonates present with typical female or under masculinized external genitalia (46, XY DSD), while affected 46, XX females are phenotypically normal at birth. Pubertal development is missing, incomplete or atypical in both sexes. The typical person with loss of CYP17A1 activity presents as teenage female with primary amenorrhea, hypergonadotropic hypogonadism and hypertension. Gynecomastia is seen with partial enzyme deficiency.

More than 100 mutations have been reported in the *CYP17A1* gene so far [44]. Most pathogenic variants of *CYP17A1* inhibit both enzyme activities and account for about 1% of all CAH cases. CYP17A1 deficiency seems especially common in Brazil, where two recurring mutations (p.W406R and p.R362C) suggest a founder effect [47]. Extremely rare *CYP17A1* missense mutations have been found in isolated 17,20 lyase deficiency [44]. These mutations impair the enzyme's interaction with P450 oxidoreductase (POR) and especially cytochrome *b*5 (CYB5A).

Defects of adrenal steroidogenesis leading to adrenal insufficiency and androgen excess

Congenital adrenal hyperplasia owing to CYP21A2 mutations

The commonest form of classic CAH has an incidence of about 1 in 10′000–15′000 in the Caucasian population [6,32]. It is caused by *CYP21A2* mutations leading to 21-hydroxylase deficiency inhibiting GC and MC synthesis and leading to adrenal androgen excess (Fig. 1). Loss of function mutations cause adrenal insufficiency with potentially deadly salt-wasting crisis soon after birth. Less severe mutations cause the classic, simple virilizing form of CAH, in which adrenal insufficiency is associated with variable degrees of virilization of the external genitalia in 46, XX neonates owing to excess adrenal C19 steroid exposure prenatally. Normally, the female fetus is protected from androgen excess by the complex steroidogenic activity of the fetal-placental unit, in which fetal adrenal androgens are metabolized predominantly to estriol [48]. But fetal loss of CYP21A2 activity stimulates the HPA axis and thereby excessive fetal adrenal androgen synthesis, which may no longer be balanced by the fetal-placental unit [12,49]. With 21-hydroxylase deficiency, excessive androgens are produced by the classic and the alternate pathways such as the backdoor and 11-oxy pathway (Fig. 2) [50]. Non-classic CAH is due to *CYP21A2* variants retaining more than 10% enzyme activity. While classic CAH is picked up

because of an atypical genital phenotype in genetic girls, a pathologic 17OHProg newborn screening or an adrenal crisis at birth or soon after, non-classic CAH may only be diagnosed beyond the first year of life for signs and symptoms of androgen excess in infancy, childhood, adolescence or even adulthood [51]. This so-called late-onset form of CAH may lead to growth acceleration and precocious pseudopuberty or premature adrenarche in children, as well as hirsutism, menstrual disturbances and fertility problems resembling the polycystic ovary syndrome in adolescent and adult females. As *CYP21A2* is not needed for sex steroid production in the gonads (Fig. 1B and C), pubertal development, fertility and reproduction are normal in persons with an adequately treated CAH. However, non-adherence leading to HPA axis overstimulation and adrenal androgen excess can result in gonadal malfunction either through the formation of testicular adrenal rest tumors (TART) especially in males, through interference with the HPG axis in both sexes, or by disruption of the follicular cycles in the ovaries [6,52].

In Europe and North America incidence of classic 21-hydroxylase CAH is about 1:15′000, and carriers of pathogenic variants in the *CYP21A1* gene are found in about 1:50–60. Overall, classic 21-hydroxylase deficiency accounts for more than 90% of CAH cases, and is diagnosed in 80% of all 46, XX DSD cases. Incidence of the non-classic form is about 1:1000. But, incidence varies greatly with ethnicity and geographic location with very high incidence reported in Yupic Eskimos, and lower incidence in African Americans as well as people from Japan or Taiwan [32]. The *CYP21A2* gene is located on chromosome 6 in the HLA region III in tandem with the highly homologous *CYP21A1P* pseudogene and the tenascin-X (*TNXB)* gene flanking, explaining why abnormal genetic recombinations are frequent and repetitive events, and why about 10% of CAH patients also have Ehlers-Danlos-like syndrome, a connective tissue disorder responsible for chronic arthralgia, joint subluxations, hernias, and cardiac anomalies (OMIM 606408) [53]. With 21-hydroxylase CAH, very good genotype-phenotype correlation exists in salt-wasting and non-classic forms, while simple-virilizing forms show wider variability [54]. With compound heterozygous variants, the phenotype usually relates to the less severe variant.

CAH due to CYP11B1 deficiency

The enzyme 11β-hydroxylase catalyses the last step to cortisol synthesis in the GC pathway and conversion of 11-deoxycorticosterone (DOC) to corticosterone in the MC pathway (Fig. 1A). Therefore, patients with 11-hydroxylase deficiency have a profile of cortisol deficiency (CAH) with mineralocorticoid excess explained by the ability of DOC to stimulate the mineralocorticoid receptor. Like in 21-hydroxylase deficiency, HPA axis stimulation and precursor accumulation result in hyperandrogenism.

Overall, 11-hydroxylase deficiency has a similar phenotype as 21-hydroxylase deficiency with the exception of mild to severe hypertension found in two third of patients beyond infancy [32,55,56]. Its classic form causes 46, XX DSD with severe virilization of the external genitalia, and precocious pseudopuberty in both sexes. A non-classic form has been described in very rare cases [32,55,56].

Autosomal recessive variants in *CYP11B1* are found in about 5% of CAH persons of European ancestry indicating an incidence of about 1:100′000 to 1:200′000. However, its incidence is much higher in the Middle East and North Africa likely due to consanguinity [56]. Over 100 mutations have been reported in the *CYP11B1* gene. Although *CYP11B1* is located close to the highly homologous aldosterone synthase *CYP11B2* gene on chromosome 8q21, genetic unequal crossing-over events are only described in very few cases [57].

Mixed oxidase disorder due to deficient P450 oxidoreductase (PORD)

POR is the essential electron donor to all type II microsomal P450s involved in numerous body functions, including sterol and steroid biosynthesis. Other known functions of POR include metabolism of drugs, xenobiotics, arachidonic acid and eicosanoids, synthesis and metabolism of cholesterol, bile-acids and hemoglobin, as well as retinoic acid hydroxylation [58]. However, the exact function of most of these possibly interacting P450s remains unsolved. In the classic steroid pathway POR supports the enzyme reactions of CYP17A1, CYP21A2 and CYP19A1 (Fig. 1). Different variants of *POR* mimic their (combined) deficiency to variable degrees [32,58].

In 1985 Peterson described the first patient with a typical phenotype of PORD [59]. A 6-month-old 46, XY infant with a female phenotype and ambiguous genitalia was found to have a steroid profile suggesting combined 21-hydroxylase and 17-hydroxylase/17,20-lyase deficiency. But the clinical diagnosis of PORD is challenging, as it manifests with a very broad phenotype ranging from a) asymptomatic carriers, to b) minor biochemical deficiencies leading to a PCOS-like phenotype or gonadal insufficiency in young adults, c) a phenotype of congenital adrenal hyperplasia with or without 46, XX and 46, XY DSD only, or d) to a severe congenital malformation syndrome mimicking the Antley-Bixler skeletal malformation syndrome (ABS, OMIM 201750) with genital ambiguity at birth (Table 1).

During pregnancy, PORD in a fetus may by recognized by a) virilization of the mother, b) bone malformations and/or ambiguous genitalia in the fetus, and c) alterations of the steroid metabolome (e.g. low estriol) [60]. At birth, most individuals with PORD manifest with a DSD phenotype, which is seen in both 46, XX and 46, XY neonates due to disturbed intrauterine steroid hormone production resulting in androgen deficiency in 46, XY (e.g. for reduced CYP17A1 activity) and androgen excess in 46, XX (e.g. for reduced CYP21A2 and CYP19A1 activities). After birth, abnormal sex hormone biosynthesis may also result in absent or insufficient pubertal development, sexual functioning and fertility problems in both males and females. PORD usually causes mild glucocorticoid deficiency and mild mineralocorticoid excess, which may lead to arterial hypertension with aging (similar as seen in patients with *CYP17A1* mutations). Adrenal insufficiency may be diagnosed at birth through neonatal screening established for CAH due to mutations in the *CYP21A2* gene using 17-hydroxyprogesterone as marker steroid. Diagnostic ACTH testing may reveal partial, stress-related adrenal insufficiency in about 40%, and severe cortisol deficiency in another 40% of persons with PORD [61]. Minor skeletal anomalies are observed in 2/3 of PORD patients, while a severe ABS phenotype occurs very infrequently [61]. ABS is characterized by craniosynostosis and radiohumeral synostosis but can be associated with a wide range of other skeletal malformations, which may also be seen with genetic mutations in the *FGFR2* (OMIM 176943) or *CYP26B1* (OMIM 605207) genes. In fact, the skeletal phenotype of PORD probably results from diminished activity of CYP26B1, the POR-dependent microsomal enzyme that degrades retinoic acid [32].

Since identification of first autosomal recessive *POR* variants in 2004 [28,62], numerous variants have been reported in over 120 individuals with variable phenotypes. The exact incidence of PORD is unknown, but seems to vary among ethnic groups. Two mutations are especially common, the p.A287P in European patients, and the p.R457H in Japanese patients. Genotype-phenotype correlation is difficult as different POR variants interact variable with different P450 partners [58].

Isolated sex steroid biosynthesis defects

Aromatase deficiency

P450 aromatase (encoded by *CYP19A1*) is required for the synthesis of estrogens (C18 steroids) from androgen precursors (C19 steroid) (Fig. 1C) [63]. It is expressed in several tissues including the ovaries, testes, placenta, brain, breast, adipose tissue, and bone osteoblasts.

Aromatase deficiency manifests during fetal life in both sexes: Mothers carrying an affected fetus suffer from progressive virilization during pregnancy due to their inability to aromatize androgens derived from fetal adrenals in the placenta (e.g. low estriol). As a consequence elevated androgens *in utero* lead to 46, XX DSD at birth manifesting as mild to severe virilization of the external genitalia. During infancy and childhood there are mostly no symptoms of aromatase deficiency in boys, while girls may manifest with abdominal symptoms of ovarian cysts due to dysregulated HPG axis [64]. At puberty, lack of estrogens results in hypergonadotropic hypogonadism in girls with failure or incomplete spontaneous pubertal development and primary amenorrhea. Variable degree of androgen excess leads to acne and hirsutism in women. Bone age is typically delayed because estrogens are crucial for epiphyseal maturation and closure in both sexes, and decreased bone mineral density can be observed later in life [65]. A negative impact on glucose homeostasis and lipid profile has also been described in both adult males and females [66–68].

Aromatase deficiency is a very rare autosomal recessive disorder caused by *CYP19A1* variants or specific variants in *POR* (see above). In the about 50 reported cases so far a wide spectrum of CYP19A1 variants has been identified [69]. Genotype-phenotype correlation seems doubtful.

The syndrome of isolated 17,20-lyase deficiency (ILD) due to variants in CYP17A1, CYB5 and POR

Activity of 17,20 lyase is essential for androgen production of the classic and the backdoor pathway (Figs. 1 and 2). CYP17-lyase activity requires POR and CYB5 for its full functionality. Therefore, ILD may result from specific variants of *CYP17A1*, *POR* or cytochrome *b*5 [44,45,70,71].

46, XY persons with ILD are usually recognized at birth because of variable degrees of under masculinization, while affected 46, XX girls go usually unrecognized until puberty, when they show primary amenorrhea secondary to gonadal failure. Thus pubertal development and fertility are affected in both sexes, but low renin hypertension is not an issue with ILD.

Genetic variants in human *CYP17A1*, *POR* and *CYB5* causing ILD are extremely rare. Only few cases are reported [44,45,70,71].

HSD17B3 deficiency

There are more than 14 isoforms of human 17β-hydroxysteroid dehydrogenases (HSD17Bs) with various physiological functions. Some isoforms are preferentially reductases, others oxidases [14]. Human mutations are only known for the *HSD17B3* gene, which is exclusively expressed in the testes for androgen biosynthesis in the classic and alternative pathways (Fig. 1B).

HSD17B3 deficiency is a 46,XY-limited disorder causing DSD with severe to complete under virilization of the external genitalia with a blind vaginal pouch and absent Müllerian structures. Wolff structures are present, but testes are often located inguinal [72,73]. Most patients with HSD17B3 deficiency are raised female; at puberty, they virilize and reveal gynecomastia as testicular secreted androstenedione will be convert to T and estrogens by other 17βHSD isoforms' and aromatase activities in the periphery. Thus some affected individuals raised female will then change to male social gender. Clinically, HSD17B3 deficiency is indistinguishable from other causes of 46, XY DSD, especially 5α-reductase deficiency (*SRD5A2*) or partial androgen insensitivity (*NR3C4/AR*; OMIM 313700).

Autosomal recessive *HSD17B3* variants are the most common cause of 46, XY DSD of androgen synthesis with an estimated incidence in Europe of about 1:150′000 [72,73]. However, incidence varies considerably with ethnicity and consanguinity. About 50 different *HSD17B3* mutations have been reported so far.

Androgen deficiency due to variants in genes of the backdoor pathway (AKR1C2/4)

The backdoor pathway requires reductive and oxidative 3α-hydroxysteroid dehydrogenase (3αHSD) activities for androgen production (Fig. 2). The four major human 3αHSDs are aldoketoreductases of the AKR1C family and have in principal reductive activity [14]. AKR1C3 is also known as 17βHSD5 (*HSD17B5*) and catalyzes the conversion of androstenedione to T in the adrenals and ovaries (Fig. 1A and C), and in non-steroidogenic tissues. Both, AKR1C2 and AKR1C4 are able to convert 17OH-DHP to 17OH-Allo in the backdoor pathway (Fig. 2), and are both expressed in testes and adrenals [31].

In patients with a phenotype similar to ILD manifesting with moderate to severe 46, XY DSD we have identified first combined mutations in *AKR1C2/4* [31]. Autosomal recessive *AKR1C2* variants were found in the affected individuals and suggested a male sex-limited pattern of inheritance. Linkage analysis revealed an additional splicing mutation in *AKR1C4* in all affected persons. Another unrelated 46, XY DSD patient with female external genitalia and intraabdominal testes had a complex chromosomal rearrangement in the *AKR1C* locus consisting of an unequal crossing over between the *AKR1C2* and the *AKR1C1* genes, and an additional missense mutation in the *AKR1C2* gene [31]. These multigenic defects found in genes of the backdoor pathway of androgen biosynthesis in 46, XY DSD patients indicate that the backdoor pathway plays a crucial role for human fetal male sex development. However, to date we do not understand the interplay between the different androgen biosynthesis pathways pre- and postnatally in health and disease states [12,27,74,75].

So far *AKR1C2/4* variants associated with a severe 46, XY DSD phenotype have only been reported in two families [31].

5α-reductase deficiency due to variants in SRD5A2

There are two functionally active 5α-reductases (5α-Red) in humans. Both convert T to more potent DHT (Figs. 1C and 2). The type I enzyme (SRD5A1) is expressed in peripheral tissues such as the skin, while the type II enzyme (SRD5A2) is expressed in male reproductive tissues [76]. Inactivating mutations in the *SRD5A2* gene cause 5α-reductase deficiency.

Loss of function variants of *SRD5A2* usually manifest at birth with female typical external genitalia, as the virilization of the external genitalia depends largely on DHT. Apart from the severe under-/non-virilization of the external genitalia, affected patients with severe SRD5A2 deficiency show a rather normal male sex determination and differentiation during fetal development. Less severely affected individuals may present with hypospadias or isolated micropenis. At puberty, progressive virilization and gynecomastia occur spontaneously due to intact peripheral activity of 5α-Red type I. This may prompt a change in gender role to male in individuals raised as female in 16–70% [77]. However, only 30% of affected individuals are diagnosed at birth, and 70% are assigned female according to their external genital phenotype [77]. Unlike in other forms of severe 46, XY under virilization, it is currently advised to base sex assignment at birth with 5α-reductase deficiency on the molecular diagnosis (if available) rather than on external genital phenotype [77].

Worldwide close to 130 *SRD5A2* variants (mostly homozygous missense mutations) have been reported in under virilized 46, XY individuals [77]. Weak genotype-phenotype correlation has been suggested. By contrast, human mutations in *SRD5A1* have not been described so far.

Steroid disorders caused by steroidogenic factor 1, the master regulator of steroidogenesis

Steroidogenic factor 1 (SF1/NR5A1) was originally identified as an essential transcription factor for genes involved in human steroid biosynthesis including *StAR, CYP11A1, CYP21A2,* and *CYP17A1* [78]. The knockout mouse revealed a phenotype of complete sex reversal and adrenal insufficiency in males [79].

Same phenotype of 46, XY DSD and cortisol deficiency was found in a first patient with a heterozygote *NR5A1* mutation [80]. Meanwhile numerous patients have been described [81–83], most of them present with an isolated 46, XY DSD phenotype only, but with a wide spectrum ranging from mild hypospadias to complete sex reversal. By contrast, adrenal insufficiency with NR5A1 deficiency is very rare. Affected females characteristically present with primary ovarian insufficiency or remain asymptomatic [84]. Testis steroidogenesis is mostly disturbed with *NR5A1/SF1* mutations and T production low [81–83]. However, as SF1 is critically involved in early sex development, not only steroidogenesis of the gonads may be disturbed, but determination of the gonad in both 46, XY and 46, XX may show severe abnormalities that may lead to dysgenetic or streak gonads in worst case [85,86]. Müllerian structures are variably persistent reflecting variable AMH levels with SF1 variants. SF1 deficiency may also cause gonadotropin deficiency and asplenia [82,83]. Overall, a characteristic clinical or biochemical profile of NR5A1/SF1 deficiency does not exist, therefore the diagnosis can only be made by molecular analysis.

NR5A1 variants are a frequent finding in 46, XY DSD with close to 200 variants reported so far [82,83]. Most patients harbor heterozygous variants, and these may manifest variably even within families. Thus, genotype-phenotype correlation seems non-existent, and an oligogenic origin of disease may explain the extremely variable phenotype [82,83,87].

All steroid hormones are produced from cholesterol. Therefore, **defects affecting cholesterol biosynthesis or trafficking** may lead to adrenal insufficiency and atypical sexual development. However, most of these cause complex metabolic disorders, in which the genital and adrenal phenotype is often mild and plays minor roles. Examples of cholesterol trafficking disorders are Wolman disease (OMIM 278000) and Niemann-Pick type C disease (OMIM 257220). Smith-Lemli-Opitz syndrome (OMIM 270400) is the commonest genetic disorder of cholesterol biosynthesis that affects the last step in *de novo* cholesterol production due to deficient activity of 7-dehydrocholesterol reductase. These disorders are not further discussed in this article.

Diagnostic options

Clinical investigations (phenotype)

The workup of every patient presenting with a DSD with or without adrenal insufficiency should start with a comprehensive history (including family history) and an extensive physical exam. Although the phenotype of specific steroid disorders of the gonads and adrenals is quite characteristic, considerable overlap exists in clinical signs and symptoms (see above) that a specific diagnosis may only be made by at least additional biochemical investigations (steroid profile) and nowadays also a precise molecular genetic analysis for confirmation.

Biochemistry

Biochemistry of steroid biosynthesis by the human gonads and adrenals seemed solved in the past century. However, around 2000 novel pathways were (re-)discovered and found to be of importance in health and disease. This was largely paved by methodical advancement in steroid profiling using gas or liquid chromatographic, mass spectrometric techniques [88,89]. In contrast to antibody based immunological methods that measure single specific steroids, these methods allow targeted or non-targeted steroid profiling of multiple steroids in one test with highest specificity and sensitivity. Thus, steroid profiling of most steroid disorders reveals a characteristic signature of the underlying disorder that can lead to the exact diagnosis. Table 2 summarizes the characteristic changes identified in the plasma steroid profiles of the discussed disorders of gonadal and adrenal steroidogenesis. Urine steroid profiling might be an alternative method to characterize steroid disorders. However, as most steroids secreted from their organ of origin undergo complex metabolic changes before excretion in the urine, reading a urine steroid profile is even more difficult than interpreting a plasma profile [88,89]. Highly specialized laboratories performing routine urine steroid analyses therefore also offer a service for data interpretation. However, to get the best out of such complex lab analyses, it is important to provide clinical data (phenotype) that interpretation can be directed accordingly.

Genetics

All genetic disorders of gonadal and adrenal steroidogenesis are inherited in an autosomal recessive mode. Only *NR5A1/SF1* variants are mostly found in heterozygote state.

Genetic testing to reach a diagnosis at the molecular level is currently recommended for all individuals with a DSD [3,90,91]. It provides essential information for reasonable gender assignment, evaluation of gonadal and adrenal function, risk of gonadal cancer, infertility and other long-term consequences. Even in cases where clinical and biochemical studies point to a specific genetic cause, a genetic testing will give diagnostic safety and provide further insight into genotype-phenotype characteristics forming the basis for personalized medicine. This may for instance influence treatment decisions with *CYP21A2* variants, for which good genotype-phenotype correlation exists [32]. Also, the specific gene causing an isolated lyase or androgen deficiency syndrome may only be identified by genetic testing.

Before massive parallel sequencing methods were invented, genetic testing of steroid disorders was performed by candidate gene analysis and Sanger sequencing informed by the phenotypic and biochemical assessments. This method may still be used for selected cases with a clear diagnosis, e.g. in an over virilized 46, XX newborn with high 17-hydroxyprogesterone or 21-deoxycortisol levels and suspected 21-hydroxylase CAH [5,32,90]. Otherwise, current guidelines recommend the use of targeted gene panels or whole exome sequencing (WES) as a first molecular approach for routine genetic workup. If copy number variants (CNV), deletions/duplications and complex rearrangement are suspected, additional genetic investigations such as an aCGH (array comparative genomic hybridization) or MLPA (multiplex ligation-dependent probe amplification) may be employed.

Table 2
Characteristic changes in plasma steroid profiles of patients with genetic defects affecting steroidogenesis of the adrenals and the gonads.

Disorder	Mineralo-corticoids	Gluco-corticoids	Androgens	Specific markers
Lipoid congenital adrenal hyperplasia (LCAH)	▼	▼	▼	–
P450 side chain cleavage syndrome (CAH)	▼	▼	▼	–
3β-hydroxysteroid dehydrogenase II deficiency (CAH)	▼	▼	▼	DHEAS ▲ Pregnenolone ▲ 17α-hydroxypregnelonone ▲
21-hydroxylase deficiency (CAH)	▼	▼	▲	17α-hydroxyprogesterone ▲ **21-deoxycortisol** ▲ Androstenedione ▲ 11β-hydroxyandrostenedione▲ 11-ketoandrostendione▲ Testosterone ▲ 11β-hydroxytestosterone▲ 11-ketotestosterone▲ Aldosterone▼
11-hydroxylase deficiency (CAH)	▼ (Aldosterone) ▲(DOC)	▼	▲	11-deoxycorticosterone (DOC) ▲ 11-deoxycortisol ▲ Testosterone ▲ Aldosterone ▼
Combined 17-hydroxylase, 17,20 lyase deficiency (CAH)	▲	(▼)	▼	Progesterone ▲ 11-deoxycorticosterone (DOC) ▲ Corticosterone ▲ Testosterone ▼ Cortisol ▼ 17α-hydroxyprogesterone ▼
P450 oxidoreductase deficiency (CAH)	±	(▼)	▼	17α-hydroxyprogesterone ▲ Progesterone ▲
Cytochrome *b*5 deficiency	±	±	▼	17α-hydroxyprogesterone ▲ DHEA ▼ Androstenedione ▼
17β-hydroxysteroid dehydrogenase III deficiency/ 17-ketosteroid reductase deficiency	±	±	▲Androstene-dione ▼(Testosterone)	Androstenedione ▲ Testosterone ▼
5α-reductase II deficiency	±	±	▲ (Testosterone) ▼ (DHT)	▲ Testosterone ▼ Dihydrotestosterone
3α-hydroxysteroid dehydrogenase deficiency	±	±	NK	
Aromatase deficiency	±	±	**(▲)**	- (▼Estradiol)
Steroidogenic factor 1	±	(▼)	(▼)	–

Abbr.: DOC, 11-deoxycorticosterone; DHT, Dihydrotestosterone; NK, not known.

Importantly, any gene variation identified in genetic testing of a patient with a steroid disorder has to be assessed for its disease-causing effect according to international guidelines [92]. As for any other genetic disorders, the evidence framework for discriminating pathogenic from benign variants relies on population data, computational and predictive data, functional data as well as genetic characteristics (e.g. segregation).

Therapeutic options, fertility and long-term health issues

Replacement therapy

All essential steroid hormones of the human adrenal cortex (e.g. aldosterone and cortisol) and the gonads (estrogens, progestogens, and testosterone) are available as drugs for replacement therapies. Depending on the specific defect of a steroid disorder, they are supplemented in physiologic doses as needed [5,32]. However, most of these therapies bear major challenges, largely because they are not able to mimic the physiologic patterns and flexibility (e.g. diurnal rhythm and stress response for cortisol). Thus, adverse effects of overtreatment or undertreatment are always an issue when caring for patients under hormonal replacement therapies, and the search for better treatment opportunities is ongoing.

In the research setting, enzyme replacement therapy using adenoviral gene carrier vectors has been successful in transiently restoring 21-hydroxylase activity in CYP21A2 deficient mice [93], and may be used in humans in the near future. On the other hand, permanent correction of pathogenic variants would be desired. Gene therapy directed at patients' own stem cells could theoretically cure steroid disorders. First studies aiming at cell-based therapies have shown promising results, when patient derived mesenchymal cells were reprogrammed to induced steroidogenic cells with functional activity [94]. Using gene-editing technology in addition, this may be an option for future disease cure.

Fertility

Sexual development, function and fertility are issues in patients with all forms of steroid disorders that affect sex hormone biosynthesis (Table 1). Only persons with a CAH that inhibits MC and GC production isolated, and is well treated, may not have problems. These are persons with CYP21A2 and CYP11B1 deficiencies. But even with these CAH forms androgen excess in women and TART in men may affect fertility severely [5,52,95].

Nevertheless, with today's assisted reproductive technologies pregnancies have already been achieved in few women with CYP17A1, POR, STAR, CYP11A1 and CYP19A1 deficiencies, when using controlled ovary hyperstimulation and individualized estrogen and progesterone replacement therapy [96]. Fertility in men with any form of androgen biosynthesis defect is often (severely) compromised. However, if the affected testis is able to produce only few viable sperms, assisted reproductive techniques may be able to preserve fertility options. These have been quite successful for HSD17B3 and 5α-Red deficiencies [72,73,76]; while so far not reported for CYP17A1 deficiency [97]. But, with a steroid disorder, it is recommended to achieve kryopreservation of sperms or oocytes for later assisted fertility options early as with aging chances of success decrease.

Long-term health issues

Steroid disorders affect the human body in its development pre- and postnatally, at short-term (see above) and long-term. These adverse effects may be due to the underlying defect and/or due to necessary treatments that are not well controlled. Both adrenal and gonadal hormone deficiencies may have negative long-term consequences. In children, GC excess and deficit will lead to short adult stature. MC and GC excess cause hypertension and cardiovascular problems [5]. Similarly, GC excess may lead to obesity and low bone mineral density. In addition, sex hormone deficiency can have severe long-term consequences on overall health, including the brain, bone, cardiovascular and metabolic systems [91].

Summary

Pathogenic variants explaining disorders of gonadal and adrenal steroidogenesis have been identified in all genes involved in human steroidogenesis of the classic pathways. These genetic variants are in principle affecting enzyme catalytic reactions within the steroid biosynthesis pathways and are inherited in an autosomal recessive mode. Depending which gene of the steroid pathway is affected,

and whether a genetic variant causes a total or partial loss of enzyme activity, the phenotype can be inferred. Thus, genetic defects in initial steps of steroidogenesis will cause adrenal and gonadal insufficiency resulting in 46, XY DSD phenotypes. Genetic defects affecting genes involved in adrenal steroidogenesis only can lead to 46, XX DSD through androgen excess, while defects affecting sex steroid biosynthesis only may lead to a 46, XX or 46, XY DSD phenotype at birth, lack or insufficient development at puberty and sexual malfunction later in life. Diagnosis of steroid disorders relies on clinical and biochemical characterization and genetic testing.

Genotype-phenotype correlation of different genetic defects of steroidogenesis varies profoundly and is maybe best for 21-hydroxylase CAH (*CYP21A2* variants). It has been suggested that genetic and epigenetic modulators may play a role, but this is still subject of further studies. Incidence of the different genetic defects also varies largely with *CYP21A2* variants causing the most frequently found genetic steroid disorder worldwide, while all other defects occur even rarer. Treatment of steroid disorders relies today on hormonal replacement therapies, which enables survival of patients with severe cortisol insufficiency since the middle of the past century, and restoration of most effects of sex hormones. However, as these treatments bear several shortcomings, current hope is to find a real cure with future gene therapy options.

Practice points

- Diagnosis of disorders of adrenal and gonadal steroidogenesis is made by detailed phenotyping, steroid profiling, and genetic testing.
- Panel analysis or whole exome sequencing is generally recommended for genetic testing of steroid disorders, but candidate gene analysis may still be used for targeted *CYP21A2* analysis.
- Phenotype-genotype profiles may overlap that a similar phenotype may be caused by more than one gene (e.g., isolated lyase syndrome), and that one gene my lead to different phenotypes (e.g., classic, and non-classic lipoid CAH).
- Although steroid hormones are fortunately available for replacement therapies of steroid disorders, current treatments have several adverse effects and better options are needed.

Research agenda

- To better understand the role of the alternative steroid pathways in health and disease, especially for the synthesis of sex steroids.
- To assess the role of variants in genes comprised specifically in alternative steroid pathways. Do they cause novel steroid disorders?
- To investigate possible modulators of steroidogenesis: epigenetic factors, oligogenic networks, environmental factors etc.
- To consider enzyme replacement therapy using adenoviral gene carrier vectors for transiently restoring missing enzyme activities.
- To enforce research towards gene therapy allowing for a cure of genetic disorders of steroidogenesis.

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Declaration of competing interest

Authors declare no conflict of interest.

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