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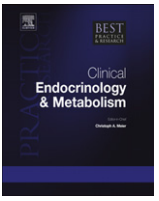

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46,XY DSD due to impaired androgen production

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LHCGR defects
Smith–Lemli–Opitz syndrome
testosterone-synthesis defects
5α-reductase type 2 deficiency

Disorders of androgen production can occur in all steps of testosterone biosynthesis and secretion carried out by the foetal Leydig cells as well as in the conversion of testosterone into dihydrotestosterone (DHT).

The differentiation of Leydig cells from mesenchymal cells is the first walk for testosterone production. In 46,XY disorders of sex development (DSDs) due to Leydig cell hypoplasia, there is a failure in intrauterine and postnatal virilisation due to the paucity of interstitial Leydig cells to secrete testosterone. Enzymatic defects which impair the normal synthesis of testosterone from cholesterol and the conversion of testosterone to its active metabolite DHT are other causes of DSD due to impaired androgen production. Mutations in the genes that codify the enzymes acting in the steps from cholesterol to DHT have been identified in affected patients.

Patients with 46,XY DSD secondary to defects in androgen production show a variable phenotype, strongly depending of the specific mutated gene. Often, these conditions are detected at birth due to the ambiguity of external genitalia but, in several patients, the extremely undervirilised genitalia postpone the diagnosis until late childhood or even adulthood. These patients should receive long-term care provided by multidisciplinary teams with experience in this clinical management.

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Q4 Prenatal disorders of sexual development (DSDs) are congenital conditions in which the development of chromosomal, gonadal or anatomical sex is a typical. They are secondary to multiple causes and this article focusses on revising the current bibliography on disorders of androgen production. For this purpose, we systematically review the main steps of testosterone biosynthesis and secretion carried out by foetal Leydig cells and the conversion of testosterone in DHT. Often, these conditions are detected at birth because of ambiguity of external genitalia but, in several patients, the extremely undervirilised genitalia postpone the diagnosis until late childhood or even adulthood. A proposed classification of 46,XY DSD due to disorders of androgen production is displayed in Table 1.

46,XY DSD due to impaired leydig cell differentiation (LHCGR defects)

The differentiation of Leydig cells from mesenchymal cells is the first step in testosterone production. Multiple genes have been involved in the specification of foetal Leydig cell lineage, such as X-linked aristaless-related homeobox gene (Arx), Desert Hedgehog (Dhh) and platelet-derived growth factor receptor alpha (Pdgfα) genes. Dhh, a Sertoli cell product, specifies the foetal Leydig cell lineage in the primordial gonad through a paracrine signalling mechanism. Postnatally, these cells are replaced in the testes by morphologically distinct adult Leydig cells. The absence of Dhh results in decreased number in foetal Leydig cell without affecting migration or proliferation of precursor cells or Sertoli cells differentiation. Steroidogenic factor 1 (Sf1) is a transcriptional regulator of hormone-biosynthesis genes, thus serving a central role in the Leydig cell. A combinatorial expression of Dhh, a paracrine signalling factor, and Sf1, a transcriptional regulator of hormone-biosynthesis genes, is required for Leydig cell development.[1–3]

In 46,XY DSD due to Leydig cell hypoplasia, there is a failure of intrauterine and pubertal virilisation due to the scarcity of interstitial Leydig cells to secrete testosterone. Leydig cells are stimulated by both hormones, chorionic gonadotrophin (CG) and luteinising hormone (LH), which act by binding and activating a common receptor (*LHCGR*) located in the cell membrane. The human *LHCGR* is a member of the G-protein-coupled super-family of receptors and, simultaneously with the receptors for thyroid-stimulating hormone (*TSHR*) and follicle-stimulating hormone (*FSHR*), belongs to the glycoprotein hormone-receptor family. The *LHCGR* has a modular architecture consisting of an ectodomain or extracellular hormone-binding domain, linked to a seven-transmembrane signal-transduction domain. This receptor has 11 exons and most of the long extracellular domain is codified by the first 10 exons and the rest of the protein by exon 11. The *LHCGR* consists of 674 amino acids and has a molecular mass of ~85–95 kDA based on the extent of glycolysation.[4] *LHCGR* is located on chromosome 2p21 in humans, close to the *FSHR* gene. Following human CG (hCG)/LH binding, the receptor undergoes a conformational change activating the G protein (Gαs) that is bound to the receptor internally.

Table 1
Q9 Classification of 46,XY DSD due to impaired androgen production.

46,XY DSD due to impaired leydig cell differentiation (*LHCGR* defects)
46,XY DSD associated with cholesterol synthesis defects
Smith-Lemli-Opitz syndrome
46,XY DSD due to testosterone synthesis defects
Enzymatic defects in adrenal and testicular steroidogenesis
StAR deficiency
P450scc deficiency
3-β-hydroxysteroid dehydrogenase type II deficiency
17α-hydroxylase and 17,20 lyase deficiency
Altered steroidogenesis due to disrupted electron transfer
P450 oxidoreductase defect
Cytochrome b5 defect
Defects in testicular steroidogenesis
Isolated 17,20-lyase deficiency
17β-hydroxysteroid dehydrogenase III deficiency
Defects in testosterone metabolism
5α-reductase type 2 deficiency

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Following the binding of *LHCGR*, the protein Gαs interchanges guanosine diphosphate (GDP) for guanosine triphosphate (GTP), releases the receptor and binds to adenylcyclase to activate cyclic adenosine monophosphate (cAMP) production. Next, cAMP activates cAMP-dependent protein kinase A. This is a tetramer of two regulatory and two catalytic subunits. cAMP binds to regulatory subunits, releasing the catalytic subunits to phosphorylate several proteins (P-proteins).[5] Some of these P-proteins migrate to the nucleus and are bound to responsive elements in the promoter zones of certain genes to modulate transcription. This process is modified by prostaglandins and other intracellular regulators.

Phenotype: In 1976, Berthezene et al.[6] described the first patients with Leydig cell hypoplasia and, subsequently, other cases have been reported.[7–10] The syndrome of Leydig cell hypoplasia has variable phenotypes.[11]

The typical phenotype of 46,XY DSD due to the complete form of Leydig cell hypoplasia is a female external genitalia leading to female sex assignment, no development of sexual characteristics at puberty, undescended testes slightly smaller than normal (Figure 1) with relatively preserved seminiferous tubules and absence of mature Leydig cells (Figure 2), presence of epididymis and vas deferens (Figure 3) and absence of uterus and fallopian tubes. It is noteworthy that well-developed epididymis and vas deferens can be found even in patients with female external genitalia. In one of our patients with Leydig cell hypoplasia, testosterone levels from the testicular vein was 108 ng dl^{-1} in comparison with peripheral levels of 24 ng dl^{-1}, indicating that small amounts of testosterone are sufficient to developed Wolffian duct derivatives (Figure 3). In contrast to the homogeneous phenotype of the complete form of Leydig cell hypoplasia, the partial form can have a broad spectrum.[12–18] Most patients have predominantly male external genitalia with micropenis and/or hypospadias. Testes are cryptorchidic or in the scrotum. While complete forms are usually not detected at birth (46,XY subjects are raised as normal girls), partial forms might be suspected because of signs of incomplete function of foetal testes. Spontaneous gynaecomastia does not occur. During puberty, partial virilisation occurs and testicular size is normal or only slightly reduced, while penile growth is significantly impaired. A milder phenotype of Leydig cell hypoplasia was recently reported in a Portuguese family that constituted

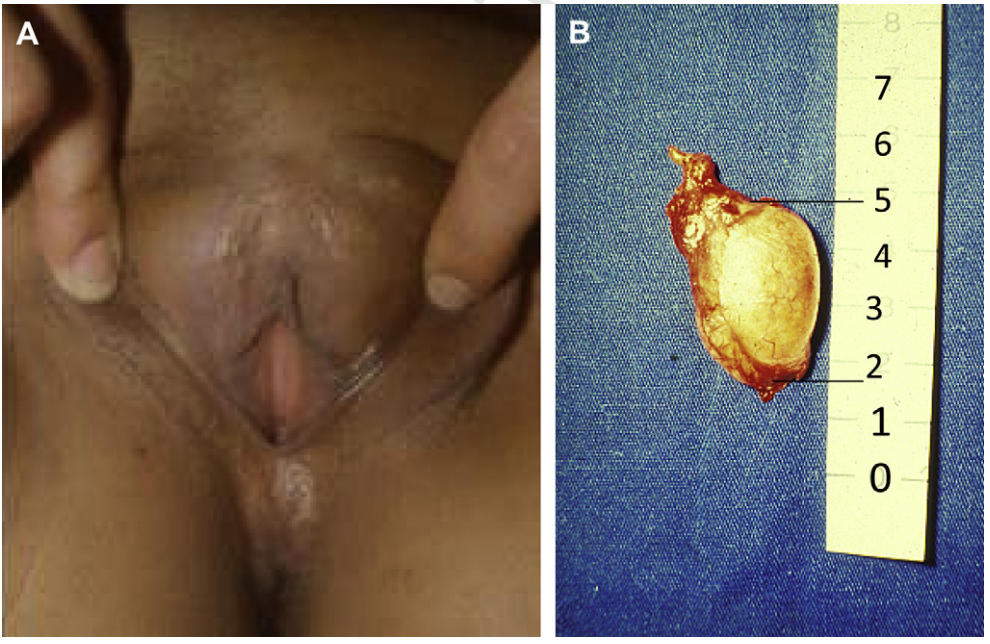

Figure 1. A: External genitalia of a 46,XY patient with a complete form of Leydig cells hypoplasia due to inactivating mutation in *LHCGR*. **B**: Right testis slightly smaller than normal.

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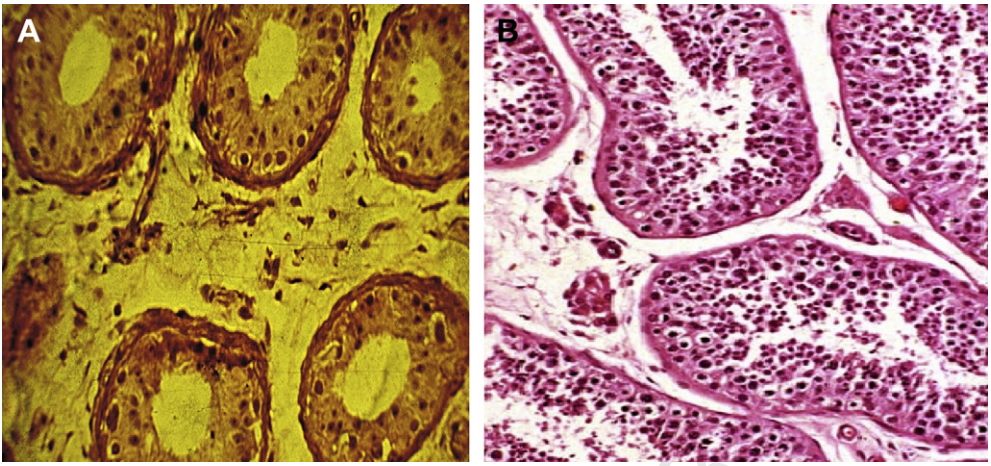

Figure 2. A Photomicrograph of testis section of a 46,XY patient with a complete form of Leydig cell hypoplasia. Seminiferous tubules contain just Sertoli cells and occasional immature germ cells and interstitium contains a sparse cell population and no mature Leydig cells, **B:** Normal testicular architecture with seminiferous tubules with Sertoli cells and germ cells. In the interstitium, the presence of mature Leydig cells.

a male patient with micropenis, hypogonadism with elevated LH levels and oligospermia and two infertile sisters.[19]

Histological analysis of the testis in both forms of Leydig cell hypoplasia did not display Leydig cells in prepubertal testes, while, in post-pubertal patients, absence or decreased numbers of Leydig cells without Reinke's crystalloids are associated with normal-appearing Sertoli cells and seminiferous tubules with spermatogenic arrest[8,9,20] (Figure 2).

Inheritance: 46,XY DSD due to Leydig cell hypoplasia presents an autosomal recessive mode of transmission. However, Leydig cell hypoplasia was found to be a genetic heterogeneous disorder since Zenteno et al. ruled out molecular defects in the *LHCGR* as the cause of Leydig cell hypoplasia in three siblings with 46,XY DSD, using segregation analysis.[21] In addition, the absence of causative mutations

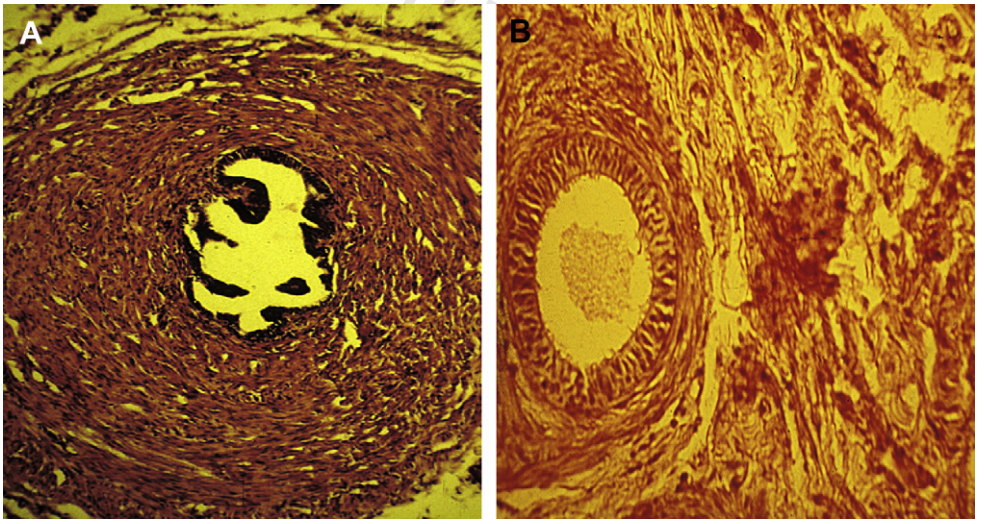

Figure 3. Presence of male ducts in a 46,XY patient with the complete form of Leydig cells hypoplasia due to inactivating mutation in *LHCGR*. **A:** Vas deferens, **B:** Epididymis duct.

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in *LHCGR*, in several patients strongly suspected to have Leydig cell hypoplasia, supported the idea that other genes must be implicated in the molecular basis of this disorder.

Biochemical diagnosis: In the complete form of Leydig cell hypoplasia, there is absence of gonadal steroid synthesis. In children, this is usually evidenced by determination of serum testosterone and its precursors following hCG stimulation test. Adrenal function is normal. In the patients with partial form of Leydig cell hypoplasia, prior to puberty the testosterone response to the hCG test is subnormal without abnormal step-up in testosterone-biosynthesis precursors.[6–9]

Following puberty, both the serum gonadotrophins are elevated but with clear predominance of LH over FSH levels; testosterone levels are intermediate between those of children and normal males.

Molecular defects: Several different mutations in the *LHCG receptor* gene were reported in patients with Leydig cell hypoplasia.[11,12,14,15,22–25] Inactivated mutations have been described in the three domains (extramembrane, transmembrane and intramembrane) of the protein.[26] The function is disrupted by several mechanisms, such as absence of ligand binding, interaction with G protein or transport to the cell membrane.[27] Mutations in the *LHCGR* gene have been identified in patients with complete and partial form of Leydig cell hypoplasia.[12–15] *In vitro* studies showed that cells transfected with *LHCGR* gene containing these mutations had an impaired hCG-stimulated cAMP production.[14,15] Latronico et al.[12] reported a homozygous mutation in the *LHCGR* (Ser616Tyr) in a boy with micropenis. Subsequently, mutations were identified in further patients with the partial form of Leydig cell hypoplasia.[13–15] A good correlation was observed between *in vitro* activity and clinical phenotype.[19]

Recently, the identification and characterisation of a novel, primate-specific *bona fide* exon (exon 6A) within the *LHCGR* determined a new regulatory element within the genomic organisation of this receptor and a new potential mechanism of this disorder.[28] The presence of mutations in the cryptic exon 6A were detected in three out of 16 patients with 46,DSD due to Leydig cell hypoplasia without molecular diagnosis. Functional studies revealed a dramatic increase in the expression of the mutated exon 6A transcripts, resulting in the generation of the predominantly non-functional LHCGR isoform thereby preventing its proper expression and functioning.[28]

46,XX sisters of patients with the complete form of 46,XY DSD due to Leydig cell hypoplasia, with the same homozygous mutation in the *LHCGR*, present spontaneous breast development, primary or secondary amenorrhoea, infertility and normal or enlarged cystic ovaries. The hormonal profiles of these women show elevated LH and LH/FSH ratio, measurable oestradiol levels and normal androgen levels.[12,16,29–32]

46,XX sisters of patients with the partial form of 46,XY DSD due to Leydig cell hypoplasia were recently described and include regular ovarian cycles for years, infertility and elevated or even normal LH levels.[19]

46,XY DSD due to testosterone-synthesis defects

46,XY DSD associated with cholesterol-synthesis defects

Cholesterol deficiency and abnormal increase of pre-defect sterols might be involved in the multiple anomalies reported. 46,XY DSD is one of possible phenotypes and could be present in severe defects.

Smith–Lemli–Opitz syndrome (SLOS)

This syndrome, caused by a deficiency of 7-dehydrocholesterol reductase, is the first true metabolic syndrome leading to multiple congenital malformations.[33,34] The first step of testosterone biosynthesis begins with the uptake of cholesterol from the extracellular space and/or the endogenous synthesis of cholesterol by Leydig cells. In both instances, the action of 7-dehydrosterol reductase is necessary for cholesterol synthesis from 7-dehydrocholesterol (Figure 4). SLOS is caused by an inborn error of post-squalene cholesterol biosynthesis.

Phenotype: The SLOS phenotypic spectrum is broad and variable – from early embryonic non-viability to varying levels of severity postnatally, including distinctive facial appearance, growth and mental retardation, autistic behaviour, hypotonia, failure to feed, decreased life span and variable structural anomalies of the heart, lungs, brain, gastrointestinal tract, limbs, genitalia and kidneys. Typical facial appearance is characterised by a short nose with anteverted nostrils, blepharoptosis,

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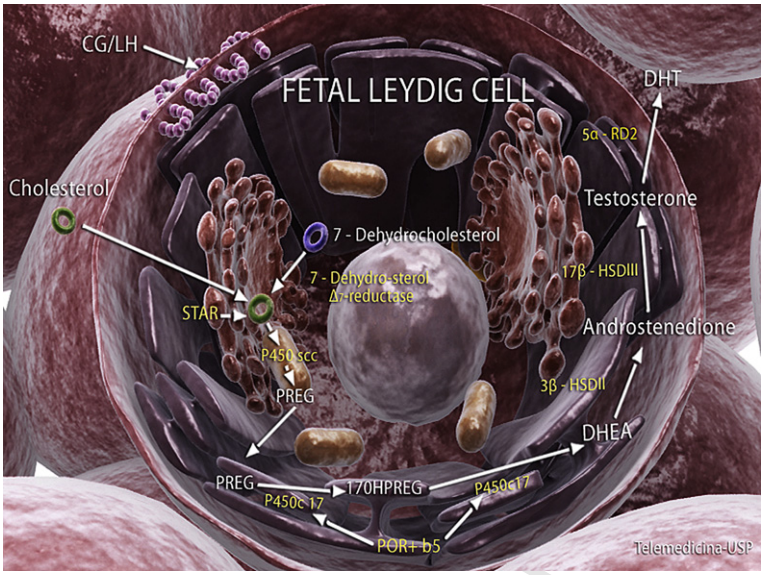

Figure 4. A cartoon of human foetal Leydig cell showing all steps involved in androgen production.

microcephaly, photosensitivity, mental retardation, syndactyly of second and third toes, hypotonia and genital ambiguity. Adrenal insufficiency maybe be present or may evolve with time. Ambiguity of the external genitalia is a frequent feature in males (71%) and ranges from hypospadias to female external genitalia despite normal 46,XY karyotype and *SRY* sequences. Müllerian derivative ducts can also be present[35,36] The mechanisms of undervirilisation of foetal external genitalia in 46,XY DSD patients is still unclear but might be due to decrease testosterone synthesis by the foetal testes secondary to lack of precursors or abnormal LH-receptor response to hCG stimulation because of abnormal plasma membrane fluidity, secondary to low cholesterol levels or excessive cholesterol precursors.[37]

However, the description of patients with SLOS who present with hyponatraemia, hyperkalaemia and decreased aldosterone-to-renin ratio suggest that the lack of substrate to produce adrenal and testicular steroids is the cause of adrenal insufficiency and genital ambiguity.[38]

Inheritance: SLOS is an autosomal recessive disorder.

Molecular defect: Loss-of-function mutations in the sterol delta-7-reductase (*DHCR7*) gene, which maps to 11q12-q13 cause SLOS.[39] It has nine exons and spans ~14 kilobases. The protein has 475 amino acids and is located in the endoplasmic reticulum of cholesterol-synthesising cells. Nine putative transmembrane segments have been identified in the amino acid sequences. The key morphogen (Sonic hedgehog and its related proteins Indian and Desert hedgehog) is affected, as this protein needs covalently attached cholesterol for regulated short- and long-range signalling processes.

Biochemical diagnosis: Low-to-undetectable levels of oestriol have been observed in the urine, amniotic fluid and serum of pregnant women carrying foetuses affected with SLOS. Affected children present with low plasma cholesterol and elevations of plasma 7-dehydrocholesterol. Considering the relative high frequency of SLOS, approximately 1:20 000–60 000 births, we suggest that at least cholesterol levels should be routinely measured in patients with 46,XY DSD.

Treatment: Treatment strategies of dietary cholesterol supplementation are focussed on supplying exogenous crystalline cholesterol by various vehicles in an attempt to increase body cholesterol levels and to secondarily decrease the levels of 7DHC/8DHC, through feedback inhibition of HMG-CoA reductase. Dietary cholesterol supplementation is recommended (e.g., two egg yolks per day), although there are no controlled studies to validate their efficacy. Some reports of isolated cases have demonstrated the beneficial impact of cholesterol supplementation (20–40 mg kg^{-1} per day) on patients' behaviour and feelings.[40,41]

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Another therapy proposed is the use of HMG-CoA reductase inhibitors, such as statins, which are recommended only in patients without residual DHCR7 enzymatic activity.[42,43] Direct cholesterol delivery to the central nervous system has been recently proposed and may allow the brain to remodel and develop normally, especially if this can be carried out as early as possible following diagnosis.[44]

46,XY DSD due to testosterone-synthesis defects

Five enzymatic defects that alter the normal synthesis of testosterone from cholesterol have been described to date (Figure 5). Three of these defects are associated with defects in cortisol synthesis leading to congenital adrenal hyperplasia. All of them present an autosomal recessive mode of inheritance. Genetic counselling is mandatory, since every additional sibling has a 25% chance of being affected with the same synthesis defect.

Defect in corticosteroid and testosterone synthesis

Adrenal hyperplasia syndromes are congenital disorders associated with hypoadrenocorticism or a mixed of hypo- and hyper-corticoadrenal steroid secretion. Synthesis of just cortisol or both gluco- and mineralocorticoids is impaired. When cortisol production is impaired, there is a compensatory increase in adrenocorticotrophic hormone (ACTH) secretion whereas impaired mineralocorticoid synthesis results in a compensatory increase in renin–angiotensin production. These compensatory mechanisms may return cortisol or aldosterone production to normal or near-normal levels, but at the expense of excessive production of other steroids causing undesirable hormonal effects.

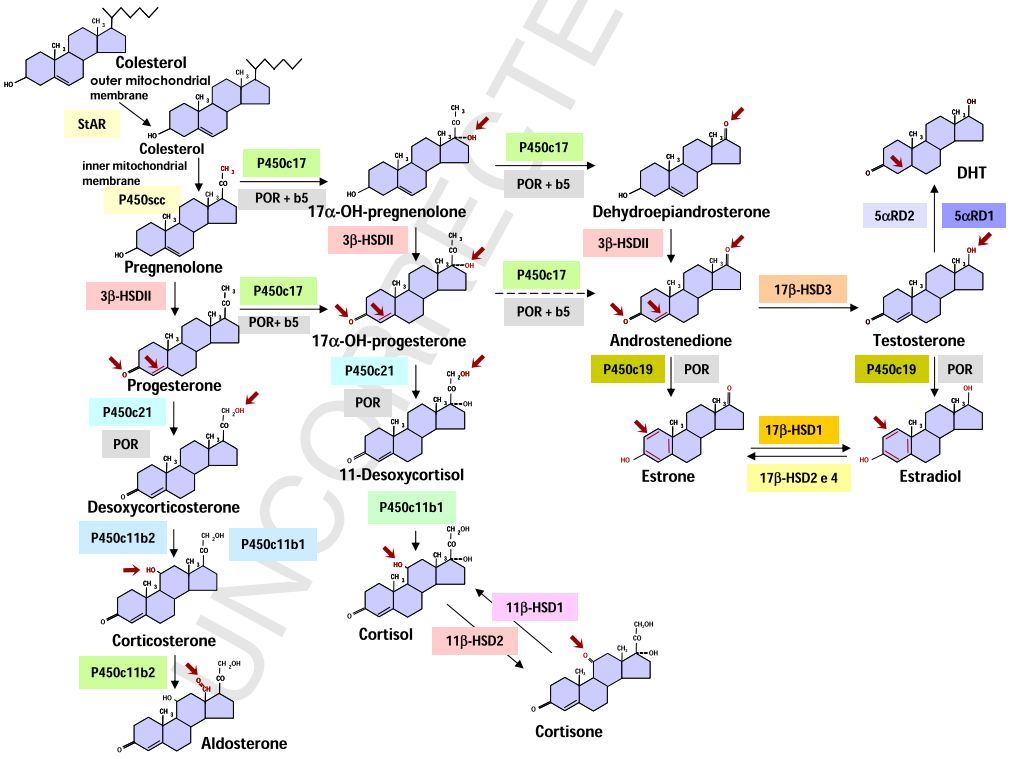

Figure 5. Adrenal and testicular steroidogenesis.

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Cholesterol side-chain-cleavage defects

The earliest step in the conversion of cholesterol to hormonal steroids is hydroxylation at carbon 20, with subsequent cleavage of the 20–22 side chain to form pregnenolone. In steroidogenic tissues, such as the adrenal cortex, testis, ovary and placenta, the initial and rate-limiting step in the pathway leading from cholesterol to steroid hormones is the cleavage of the side chain of cholesterol to yield pregnenolone. This reaction, known as cholesterol side-chain cleavage, is catalysed by a specific cytochrome P450, called P450scc or P45011A, and by the steroidogenic acute regulatory (StAR) protein, a mitochondrial phosphoprotein.[45] Cholesterol is taken up from both low-density lipoprotein (LDL) and apolipoprotein A (apoA)/high-density lipoprotein (HDL) receptors in caveolin-rich domains. Late endosomes mediate this transfer to the mitochondria via the activities of Niemann–Pick disease type C-1 (NPC-1) and possibly the StAR-like protein MLN64. Acyl-CoA:cholesterol acyltransferase converts free cholesterol derived from organelles (e.g., endosomes, endoplasmic reticulum) to the cholesterol esters that represent the predominant components of lipid droplets. StAR mRNA expression is determined by the balance between transcription and mRNA turnover, each of which is regulated by multiple factors. Promoter elements and mRNA sequence elements are subject to regulation by physiological changes, such as hormonal stimulation (which increases cAMP levels) and cholesterol depletion (which activates sterol regulatory element-binding protein (SREBP)). StAR mRNA stability is also regulated by suppression of transcription or translation. Stabilisation of an otherwise rapidly degraded mRNA is a regulatory mechanism that allows to extremely rapid and sensitive control of gene expression.[46]

Deficiency of the acute steroidogenesis regulatory protein (StAR)

It is the most severe form of congenital adrenal hyperplasia (CAH).[47] Lipoid adrenal hyperplasia is rare in Europe and America but it is thought to be the second most common form of adrenal hyperplasia in Japan. The gene for StAR is located on chromosome 8p11.2.[48] The protein has 285 amino acids and undergoes truncation when it performs its transfer function. StAR is located within the mitochondria of the adrenal and gonadal cells but has not been found in the placenta and brain.[48]

Lipoid congenital adrenal hyperplasia is caused by StAR mutations resulting in deficient steroidogenesis and 46,XY DSD.

Phenotype: Problems caused to persons with lipoid CAH can be divided into: a) mineralocorticoid deficiency, b) glucocorticoid deficiency, c) sex steroid deficiency and d) damage to gonads (and adrenals) caused by lipid accumulation. Affected subjects are, in general, phenotypic females irrespective of gonadal sex or, sometimes, have slightly virilised external genitalia with or without cryptorchidism, underdeveloped internal male organs and an enlarged adrenal cortex, engorged with cholesterol and cholesterol esters.[49] Adrenal steroidogenesis deficiency leads to salt-wasting, hyponatraemia, hyperkalaemia, hypovolaemia, acidosis and death in infancy. Adrenal sex steroid deficiency is present during pregnancy resulting in a low steroid production by the foeto-placental unit. ACTH stimulates growth of the adrenal cells and increases LDL receptors to amplify transport of cholesterol into the adrenal cells, where it accumulates because little is transferred into the mitochondria. The adrenals become markedly enlarged by the combination of ACTH-induced hyperplasia and accumulated lipid.[50] Lipid accumulation is thought to damage the cells further ("second-hit hypothesis"). Because the StAR protein is also involved in cholesterol transport into testicular and ovarian cells for sex steroid synthesis, testicular production of testosterone and ovarian production of oestrogen are also impaired. Lipid accumulation damages the Leydig cells of the testes more completely than the granulosa cells of the ovaries.

Recently, a mild form of congenital lipoid adrenal hyperplasia was described in two families. The affected children presented with late primary adrenal insufficiency at 2–4 years of age and 46,XY subjects had normal male external genital. DNA sequencing identified homozygous StAR mutations in these two families and functional studies of StAR showed that these mutants retained approximately 20% of wild-type activity.[51]

Histopathological findings of excised XY gonads included accumulation of fat in Leydig cells since 1 year of age, positive placental alkaline phosphatase and octamer-binding transcription factor (OCT4) staining indicating neoplastic potential.[52]

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Biochemical diagnosis: It is based on high ACTH, renin and gonadotrophin levels and the presence of undetectable or low levels of all glucocorticoids, mineralocorticoids and androgens. In the mild form, partial steroid production can be found.

Molecular defects: The disease was firstly attributed to P450scc deficiency, but most of the cases studied through molecular analysis showed an intact *P45011A* and its RNA.[53] Since StAR is also required for the conversion of cholesterol to pregnenolone, molecular studies were performed in *StAR* and mutations were found in most of the affected patients.[50] Congenital lipoid adrenal hyperplasia in most Palestinian cases is caused by a founder c.201_202delCT mutation, causing premature termination of the StAR protein.

Treatment: Patients treated with appropriate mineralocorticoid- and glucocorticoid-replacement therapy survive to adulthood.[54]

Deficiency of P450scc

In the next step of steroid biosynthesis (Figure 5), intra-mitochondrial cholesterol is converted into pregnenolone by P450scc. cP450scc has cholesterol monooxygenase (side-chain-cleaving) activity. *CYP11A1* is located on chromosome 15q23-24. The protein has 521 amino acids. P450scc is located in the mitochondria of the adrenals and gonads. Type I P450 enzymes are found in mitochondria, and receive electrons from nicotinamide adenine dinucleotide phosphate (NADPH) via the intermediacy of two proteins, ferredoxin reductase (a flavoprotein) and ferredoxin (an iron/sulfur protein). Type I P450 enzymes include P450scc, the two isozymes of 11-hydroxylase (P450c11beta and P450c11AS) and several vitamin D-metabolising enzymes.[55]

Phenotype: The phenotype of *CYP11A1* mutations is similar to that observed in *StAR* loss-of-function mutations.[56] However, in contrast to congenital lipoid adrenal hyperplasia caused by *StAR* mutations, adrenal hyperplasia has not been reported in patients with P450scc deficiency.[57] The phenotypic spectrum of P450scc deficiency ranges from severe loss-of-function mutations associated with prematurity, complete underandrogenisation and severe early-onset adrenal failure, to partial deficiencies found in children born at term with mild masculinisation and later-onset adrenal failure.[58]

Biochemical diagnosis: High ACTH and renin levels and the presence of undetectable or low levels of all glucocorticoids, mineralocorticoids and androgens.

Molecular defects: It has been thought that *CYP11A* mutations are incompatible with human term gestation, because P450scc is needed for placental biosynthesis of progesterone, which is required to maintain pregnancy. However, a patient has been described with congenital lipoid adrenal hyperplasia with normal *StAR* and *SF1* genes presenting a *de novo* heterozygous inactivating mutation in *CYP11A*.[59] This patient was atypical for congenital lipoid adrenal hyperplasia, having survived for 4 years without hormonal replacement.[59] More recently, the study of infants with adrenal failure and disorder of sexual differentiation identified compound heterozygous or homozygous mutations in *CYP11A1* recognising that the disorder may be more frequent than originally thought.[56,57,60,61]

Treatment: Glucocorticoid and mineralocorticoid replacement are necessary. Androgen replacement in male patients and oestrogen and progesterone replacement in females is usually necessary.

3β-hydroxysteroid dehydrogenase type II deficiency

The following step in testosterone biosynthesis is the conversion of dehydroepiandrosterone (DHEA) in androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD) type II (Figure 5). 3β-HSD converts 3β-hydroxy Δ5 steroids to 3-keto Δ4 steroids and is essential for the biosynthesis of mineralocorticoids, glucocorticoids and sex steroids.[62] Two forms of the enzyme have been described in man: type I and type II enzymes.[63] The types I and II genes are known to be closely linked on chromosome 1p13.1. The type II gene (HSD3B2), which encodes a protein of 371 amino acids, shares 93.5% identity with the type I gene and is almost exclusively expressed in the adrenals, ovary and testis. *HSD3B1* encodes an enzyme of 372 amino acids predominantly expressed in the placenta and peripheral tissues, such as the skin, mammary gland, prostate and several other normal and tumour tissues. 3β-HSD subcellular localisation patterns are unique in that they show varying degrees of endoplasmic reticular and mitochondrial distribution. The two forms are very closely related in structure and substrate specificity, although the type I enzyme has higher substrate affinities and a fivefold greater enzymatic activity than type II.[64] The structure of each of the *HSD3B2* and *HSD3B1*

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genes consists of four exons included on a 7.8-kb fragment of chromosome 1p13.1.[19] Five related pseudogenes have also been cloned.

Phenotype: Male patients with 3β-HSD type II deficiency present with ambiguous external genitalia, characterised by micropenis, perineal hypospadias, bifid scrotum and a blind vaginal pouch that may or may not be associated with salt loss.[62] Gynaecomastia is common at pubertal stage. Most of the patients were raised as males and retained the male social sex at puberty. In one Brazilian family, two cousins with 46,XY DSD due to 3β-HSD type II deficiency were reared as females; one of them was castrated in childhood and maintained the female social sex; the other was not castrated at childhood and changed to male social sex at puberty.[65] The phenotype of affected 46,XX subjects may or may not include salt wasting and absent or minimal virilisation except for one untreated adult patient with bilateral adrenal rests which developed severe virilisation.[66] Premature pubarche, acne and pubertal hirsutism were also described in affected females.[67,68] Male subjects with 46,XY DSD due to 3β-HSD type II deficiency without salt wasting showed clinical features in common with the deficiencies of 17β-HSD 3 and 5α-reductase 2.

Biochemical diagnosis: Based on the high serum levels of Δ-5 steroids (pregnenolone, 17α-hydroxypregnenolone, DHEA and DHEA-sulphate (DHEAS) as well as 17OHPreg:17OHP ratio. Basal and post-ACTH serum 17-hydroxypregnenolone and the 17α-hydroxypregnenolone/cortisol ratio are the gold standard biochemical parameters for 3β-HSD type II deficiency diagnosis, although molecular studies for diagnostic confirmation are advisable.[67,68] Serum Δ-4 steroids are slightly increased due to the peripheral action of 3β-HSD type I enzyme but the ratio of Δ-5/Δ-4 steroids is elevated. Cortisol secretion is reduced but the response to exogenous ACTH stimulation varies from decreased (in more severe deficiency) to normal.[65,69]

Molecular defects: There are nearly 40 mutations in 3β-HSD type II gene that have already been described. Mutations that lead to the abolition of 3β-HSD type II activity lead to CAH with severe salt loss.[64,70–72] Mutations that reduce but do not abolish type II activity lead to CAH with mild or no salt loss, which, in males, is associated with 46,XY DSD due to the reduction in androgen synthesis.[69,73]

Treatment: Glucocorticoid replacement is necessary along with mineralocorticoids in salt-losing patients. In male patients, androgen replacement is usually necessary when they present low levels of testosterone. However, affected males can reach normal or almost normal levels of testosterone due to the peripheral conversion of elevated Δ-5 steroids by 3β-HSD type I enzyme and also due to testicular stimulation by the high LH levels.[65]

CYP17 (17-hydroxylase and C-17-20 lyase deficiency)

The next step in the biosynthesis is the conversion of pregnenolone into 17α-hydroxypregnenolone and further down into DHEA by P450c17 (Figure 5). *CYP17A1* gene contains eight exons over 6.4 kb of DNA and is located on chromosome 10q24.3. The protein has 509 amino acids.[74] P450c17 is a steroidogenic enzyme that has hydroxylation and lyase functions and is located in the endoplasmic reticulum of the fasciculata and reticularis zone of the adrenal cortex and in gonadal tissues.[75]

Phenotype: Deficiency of adrenal 17α-hydroxylation activity was first demonstrated by Biglieri et al.[76] The phenotype of 17α-hydroxylase deficiency in most of the male patients described is a female-like or slightly virilised external genitalia with blind vaginal pouch, cryptorchidism and high blood pressure, usually associated with hypokalaemia. In 1970, New reported the first affected patient with ambiguous genitalia, who was assigned to the male sex.[77] At puberty, patients usually present sparse axillary and pubic hair. Male internal genitalia are hypoplastic and gynaecomastia can appear at puberty. Most of the male patients were reared as female and were sought treatment due to primary amenorrhoea or lack of breast development. Female patients may also be affected and present normal development of internal and external genitalia at birth and hypergonadotrophic hypogonadism and amenorrhoea at post-pubertal age, enlarged ovaries as adults and infarction from twisting can occur.[78,79] These patients do not present signs of glucocorticoid insufficiency due to the elevated levels of corticosterone, which has a glucocorticoid effect. The phenotype is similar to 46,XX or 46,XY complete gonadal dysgenesis and the presence of systemic hypertension and absent or sparse pubic hair in post-pubertal patients suggests the diagnosis of 17α-hydroxylase deficiency.[80]

Biochemical diagnosis: 17α-hydroxylase deficiency is characterised by a five- to tenfold increase in the 17-deoxysteroids – corticosterone, deoxycorticosterone and progesterone – in basal and ACTH-stimulated

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conditions, while aldosterone, 17OH-progesterone, cortisol, androgens and oestrogens levels are decreased. Excessive production of deoxycorticosterone and corticosterone results in vascular hypertension and suppression of renin levels and inhibition of aldosterone synthesis. In addition, 17α-hydroxylase deficiency is characterised by elevated production of 18-hydroxycorticosterone and 18-hydroxy-DOC, in contrast to 11-hydroxylase and 21-hydroxylase deficiencies. Progesterone is always elevated in 17α-hydroxylase deficiency and its measurement is available in most laboratories. Basal progesterone measurement is a useful and practical screen for diagnosis of 17α-hydroxylase deficiency, particularly if the clinical presentation excludes other forms of CAH.[75] Basal progesterone measurement should reduce the misdiagnosis of 17α-hydroxylase deficiency in patients with 46,XY DSD, primary or secondary amenorrhoea associated to mineralocorticoid-excess syndrome.

Molecular defects: Several mutations in the *CYP17* gene have been identified in patients with combined 17α-hydroxylase and 17,20 lyase deficiencies.[75,78,79,81] Both P450c17 activities were abolished in four novel homozygous mutations recently described; the mutant proteins were normally expressed, suggesting that the loss of enzymatic activity is not due to defects of synthesis, stability or localisation of P450c17 proteins.[81]

Treatment: Glucocorticoid replacement is necessary for hypertension management. In the beginning of treatment, the use of spironolactone is, sometimes, necessary to control blood pressure. These patients are very sensitive to glucocorticoids and low doses of dexamethasone (0.125–0.5 mg at night) are sufficient to control blood pressure. Gonadectomy and oestrogen replacement at puberty are indicate for patients reared in the female social sex. In male patients, androgen replacement is usually necessary since they present very low levels of testosterone. In some patients, however, oestrogens might aggravate hypertension. The control of blood pressure can be initially achieved by salt restriction although mineralocorticoid antagonists might be necessary.[81]

Defects in testicular steroidogenesis

Two defects in testosterone synthesis that are not associated with adrenal insufficiency have been described: isolated 17,20-lyase deficiency (CYP17 deficiency) and 17β-HSD III deficiency (17-β-HSD 3 deficiency) (Figure 5).

CYP17 (17,20 lyase activity) deficiency

Human male sexual differentiation requires production of foetal testicular testosterone, whose biosynthesis requires steroid 17,20-lyase activity. The existence of true isolated 17,20-lyase deficiency has been questioned because 17-α-hydroxylase and 17,20-lyase activities are catalysed by a single enzyme and because combined deficiencies of both activities were found in functional studies of the mutation found in a patient thought to have had isolated 17,20-lyase deficiency.[82] Later, clear molecular evidence of the existence of isolated 17,20 desmolase deficiency was demonstrated.[79,83]

Phenotype: The patients present ambiguous genitalia with micropenis, perineal hypospadias and cryptorchidism. Gynaecomastia Tanner stage V can occur at puberty.[83]

Biochemical diagnosis: Elevated serum levels of 17-OHP and 17-OHPreg, with low levels of androstenedione, DHEA and testosterone. The hCG stimulation test results in a slight stimulation in androstenedione and testosterone secretion with an accumulation of 17-OHP and 17-OHPreg.

Molecular defects: These comprise mutations that alter the electrostatic charge distribution in the redox-partner binding site, so that the electron transfer for the 17,20-lyase reaction is selectively lost.[83] The *CYP17* gene of two unrelated Brazilian 46,XY DSD patients with clinical and hormonal findings indicative of isolated 17,20-lyase deficiency, since they produced cortisol normally, carrier homozygous mutations in *CYP17*.[83] When expressed in COS-1 cells, the mutants retained 17α-hydroxylase activity and had minimal 17,20-lyase activity.[83] In addition, *POR* mutations can be misdiagnosis as isolated 17,20-lyase deficiency.[84]

46,XY DSD due to 17β-HSD 3 deficiency

Final biosynthetic step in foetal Leydig cell is the conversion of androstendione to testosterone, activated by type III 17β-HSD (Figure 5). This disorder consists of a defect in the last phase of

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steroidogenesis, when androstenedione is converted into testosterone and oestrone into oestradiol. This disorder was described by Saez and his colleagues[85] and it is the most common disorder of androgen synthesis, reported in several parts of the world.[86]

There are five steroid 17β-HSD enzymes which catalyse this reaction[87] and 46,XY DSD results from mutations in the gene encoding the 17β-HSD3 isoenzyme.[87,88] The *HSD17B3* gene contains 11 exons and is located on chromosome 9q22. The protein has 310 amino acids. At least 14 isozymes have been described. They can be predominantly reductive (types 1, 3, 5 and 7) or oxidative (types 2, 4 and 8). Many are involved in the oestrogen balance in peripheral tissues. Type 1 is expressed in the ovary. Type 3 catalyses the reduction of androstenedione to testosterone, and it is almost exclusively expressed into the testis.[87,88]

Phenotype: Patients present female-like or ambiguous genitalia at birth, with the presence of a blind vaginal pouch, intra-abdominal or inguinal testes and epididymides, *vasa deferentia*, seminal vesicles and ejaculatory ducts. Most affected males are raised as females[89,90], but some have less severe defects in virilisation and are raised as males.[87] Virilisation in subjects with 17β-HSD3 deficiency occurs at the time of expected puberty (Figure 6). This late virilisation is usually a consequence of the presence of testosterone in the circulation as a result of the conversion of androstenedione to testosterone by some other 17β-HSD isoenzyme (presumably 17β-HSD5) in extra-gonadal tissue and, occasionally, of the secretion of testosterone by the testes when levels of LH are elevated in subjects with some residual 17β-HSD3 function.[87] However, the discrepancy between the failure of intrauterine masculinisation and the virilisation that occurs at the time of expected puberty is poorly understood. A limited capacity to convert androstenedione into testosterone in the foetal extragonadal tissues may explain the impairment of virilisation of the external genitalia in the newborn. Bilateral orchiectomy resulted in a clear reduction of androstenedione levels, indicating that the principal origin of this androgen is the testis.[87,90] 46,XY DSD phenotype is sufficiently variable in 17β-HSD3 deficiency to cause problems in accurate diagnosis, particularly in distinguishing it from partial androgen insensitivity syndrome.[89,91]

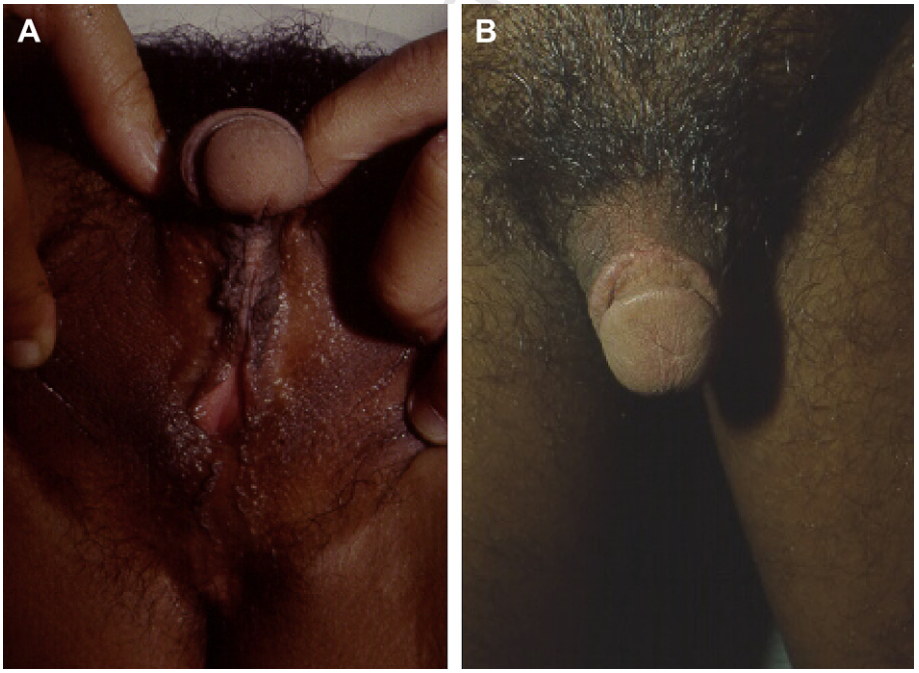

Figure 6. Adult 46,XY patient with 17β-HSD3 deficiency before and after masculinising genitoplasty.

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Most 46,XY patients are raised as girls during childhood and change to male gender-role behaviour at puberty has been frequently described in individuals with this disorder who were reared as females[90,92–94], including members of a large consanguineous family in the Gaza strip.[95] 46,XX subjects homozygous for *HSD17B3* mutations presented with normal phenotype.[96]

Biochemical diagnosis: Laboratory diagnosis is based on elevated serum levels of androstenedione and oestrone and low levels of testosterone and oestradiol in basal conditions and following hCG stimulation resulting in elevated androstenedione/testosterone and oestrone/oestradiol ratios indicating impairment in the conversion of 17-keto into 17-hydroxysteroids. At the time of expected puberty, serum LH and testosterone levels increase in all affected 46,XY subjects and testosterone levels may be into the normal adult male range.[90]

Molecular defect: The disorder is due to homozygous or compound heterozygous mutations in the gene that encodes the 17β-HSD3 isoenzyme and several mutations have been reported.[87,97]

Treatment: Gonadectomy and oestrogen replacement at puberty are indicative of patients reared in the female social sex. In male patients, androgen replacement is necessary when they present low levels of testosterone. In the patients with mild defects, testosterone replacement is not usually necessary.

Altered steroidogenesis due to disrupted electron donor proteins

Two defects in steroid synthesis due to disrupted electron donor have been described: cytochrome P450 reductase (POR) deficiency and cytochrome b5 defect.

Cytochrome P450 reductase (POR) deficiency

POR is required for the activity of all 50 human type II P450 enzymes including POR and the steroidogenic enzymes P450c17, P450c21 and P450aro.[49]

Nevertheless, mutation of the human *POR* gene is compatible with life, causing multiple steroidogenic defects and a skeletal dysplasia called Antley–Bixler syndrome.[98] P450 oxidoreductase deficiency typically presents a steroid profile suggesting combined deficiencies of steroid 21-hydroxylase and 17α-hydroxylase/17,20-lyase activities. The clinical spectrum of P450 oxidoreductase deficiency ranges from severely affected, 46,XX children with ambiguous genitalia, adrenal insufficiency or polycystic ovary syndrome and the Antley–Bixler skeletal malformation syndrome to normal or mildly affected 46,XY individuals.[98,99] P450 oxidoreductase deficiency, with or without Antley–Bixler syndrome, is autosomal recessive, whereas Antley–Bixler syndrome without disordered steroidogenesis is caused by autosomal dominant fibroblast growth factor receptor 2 mutations.[100] A complete revision on *P450* reductase deficiency can be find in chapter X.

Methaemoglobinaemia, type IV, with 46,XY DSD due to cytochrome b5 defect

Cytochrome b5 is a heme protein associated primarily with the endoplasmic reticulum codified by *CYB5A* gene located at 18q23 locus. The reductase contains flavin adenine dinucleotide and is nearly twice the size of cytochrome b5. Enhancement of P450 reactions by b5 occurs by a direct electron transfer of both required electrons from NADH-cytochrome b5 reductase to P450, in a pathway separate and independent of NADPH-cytochrome P450 reductase. Another pathways are the transfer of the second electron to oxyferrous P450 from either cytochrome b5 reductase or cytochrome P450 reductase and allosteric stimulation of P450 without electron transfer.[101]

A single patient with type IV hereditary methaemoglobinaemia and with 46,XY DSD was described.[102] The patient exhibited female genitalia at birth and had a homozygous 16-bp deletion in the cytochrome b5 mRNA leading to a new in-frame termination codon and a truncated methaemoglobin.[103] The parents and six siblings had normal methaemoglobin levels, whereas the patient's levels varied between 12% and 19%.

The aetiology of 46,XY DSD in this patient was attributed to the cytochrome b5 defect since cytochrome b5 has been shown to participate in 17α-hydroxylation in adrenal steroidogenesis by serving as an electron donor.[103]

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46,XY DSD due to defects in testosterone metabolism

5α-Reductase type 2 deficiency

There are two steroid 5α-reductase enzymes that catalyse 5α-reductase reaction.[104–106] 46,XY DSD results from mutations in *SRD5A2* gene which encodes the steroid 5α-RD2 isoenzyme.[107–109] The 5α-RD2 isoenzyme promotes the conversion of testosterone to its 5α-reduced metabolite DHT. The *5α-RD2* gene contains five exons and four introns and is located at chromosome 2 p23.

Phenotype: Affected patients present with ambiguous external genitalia, micropenis, normal internal male genitalia, prostate hypoplasia and testes with normal differentiation with normal or reduced spermatogenesis (Figure 7). The testes are usually located in the inguinal region, suggesting that DHT influences testis migration to the scrotum.[109] Virilisation and deep voice appear at puberty, along with penile enlargement and muscle-mass development without gynaecomastia. These patients present scarce facial and body hair and absence of temporal male baldness, acne and prostate enlargement, since these features depend on DHT action. Most of the patients are reared in the female social sex due to female-like external genitalia at birth, but many patients who have not been submitted to orchiectomy in childhood undergo male social sex change at puberty.[109–113] In our experience with 30 cases of 46,XY DSD due to 5-α-RD 2 deficiency, from 18 families, all subjects were registered in the female social sex except for two cases – one who has an affected uncle and the other who was diagnosed before being registered.[112,114] Fourteen patients changed to the male gender role (Figure 7). No correlation was observed between *SRD5A2* mutation, testosterone/DHT ratio and gender-role change in these patients. In one family, the two siblings carried the same mutation but presented a different gender role.[112] Ten cases are adults now and nine of them are married. Three cases adopted children and in two cases *in vitro* fertilisation using the patient's sperm cells resulted in twin siblings in one family and in a singleton pregnancy in the other.[112,114] Fourteen patients maintained the female sexual identification. Three of them were castrated in childhood and the others, despite the virilisation signs developed at puberty, kept the female social sex and sought medical treatment to correct absence of breast development and primary amenorrhoea. None of the 10 adult female patients, now in the age range of 22–49 years, are married but eight of them have satisfactory sexual activity. The main differential diagnosis of 5α-RD2 deficiency is with 17β-HSD3 deficiency and partial androgen insensitivity syndrome although in these two disorders it is common to observe the presence of gynaecomastia.

Inheritance: The mode of inheritance for 5α-RD2 deficiency is autosomal recessive. A different mode of transmission of 5-α RD2 deficiency due to uniparental disomy was described in two unrelated patients.[115]

Biochemical diagnosis: Following hCG stimulation, affected children show lower DHT levels and elevated testosterone/DHT ratio.[88,116] Post-pubertal affected patients present normal or elevated testosterone levels, low DHT levels and elevated testosterone/DHT ratio in basal conditions. Low DHT production following exogenous testosterone administration is also capable of identifying 5α-RD2 deficiency.[112] Elevated 5β/5α urinary metabolites ratio is also an accurate method to diagnose 5α-reductase 2 even at prepubertal age and in orchiectomised adult patients.[112,117]

Molecular defects: There are more than 50 families with this disorder described in several parts of the world.[109–111,113] In a few cases of 46,XY DSD due to 5-α RD2 deficiency diagnosed by clinical and hormonal findings, no mutations were identified in *SRD5A2* gene.[107,109–111,113]

Treatment: In male patients with 5-α RD2 deficiency, higher doses of testosterone esters (250–500 mg twice a week) are used to increase DHT levels and consequently penis size and male secondary characteristics. Maximum penis enlargement is obtained following 6 months of high doses and after that the normal dosage is re-instituted.[109,112] The use of topic DHT gel is also useful to increase penis size with the advantage of not causing gynaecomastia and promoting a faster increase of penis size as it is 50 times more active than testosterone. DHT is not aromatised, allowing the use of higher doses than testosterone during prepubertal age and consequently attaining a higher degree of virilisation.

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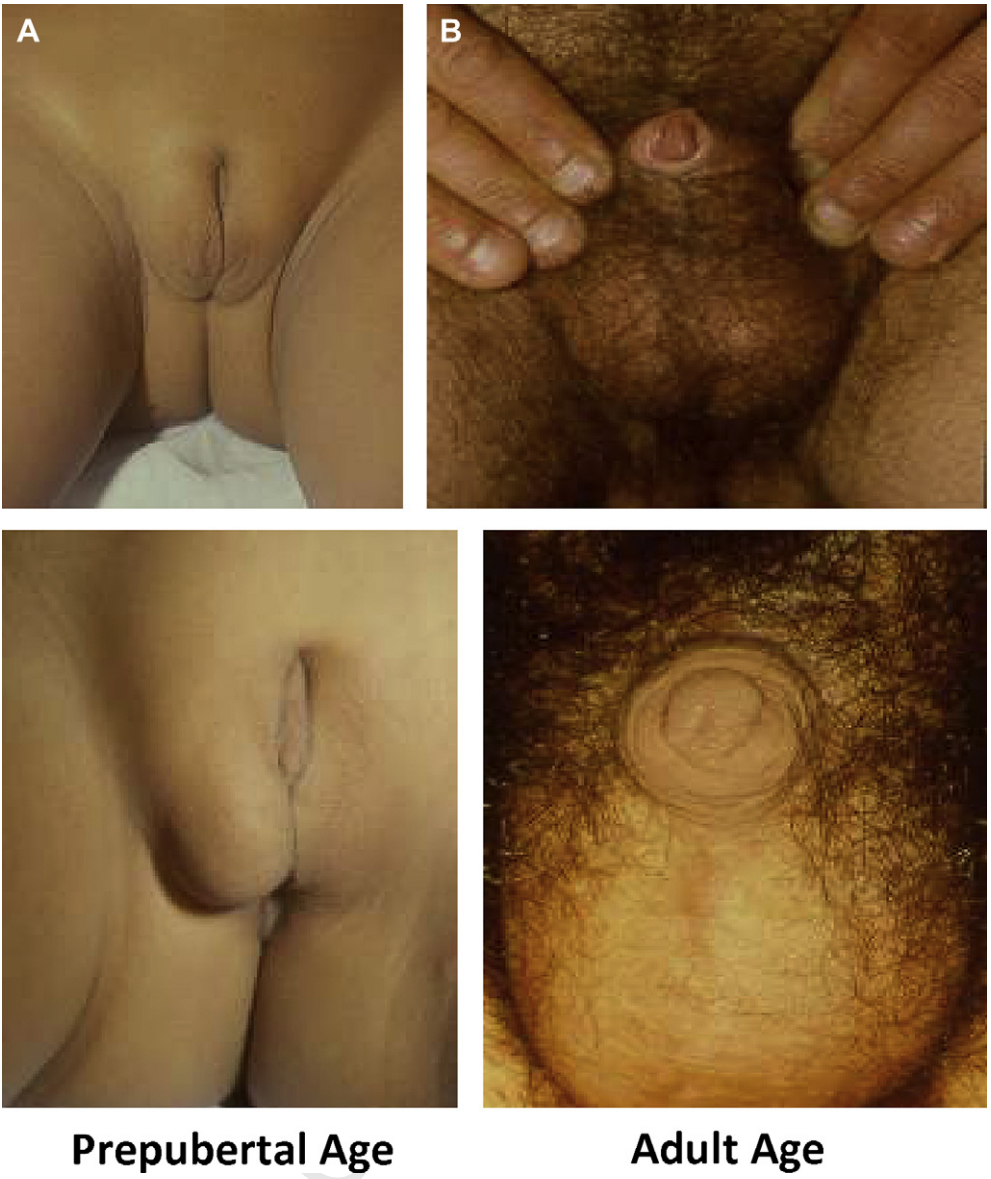

Figure 7. **A** Prepubertal 46,XY due to 5α-reductase 2 deficiency children with ambiguous genitalia, small phallus and bifid scrotum. **B:** Adult males with 46,XY DSD due to 5α-reductase 2 deficiency after masculinising genitoplasty.

Investigation of 46,XY DSD patients with androgen-production defects

In patients with androgen-production defects, post-pubertal diagnosis is made through basal steroid levels. Testosterone levels are low and steroids past the enzymatic blockage are elevated. This pattern can be confirmed with an hCG-stimulation test, which increases the accumulation of steroids past the enzymatic blockage with a slight elevation of testosterone. In prepubertal individuals, hCG-stimulation test is essential for the diagnosis, since basal levels are not altered.

There are several hCG-stimulation protocols and normative data have to be established to each of them. We established normal testosterone response 72 and 96 h following the last of four doses of hCG,

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50–100 U per kilogram of body weight, given intramuscularly every 4 days in boys with cryptorchidism but an otherwise normal external genitalia. Peak testosterone levels reached 391 ± 129 ng dl^{-1} (17.5 ± 5.7 nMol l^{-1}) and we consider a subnormal response a value <130 ng dl^{-1} (5.8 nMol l^{-1}) (corresponding to -2 SD).[118]

We also established the normal levels of DHT in prepubertal boys using the same hCG protocol. DHT peak was 29 ± 8 ng dl^{-1} (1.0 ± 0.27 nMol l^{-1}) and testosterone/DHT ratio was 14 ± 5. In adult males, basal DHT levels was 46 ± 10 ng dl^{-1} (1.6 ± 0.34 nMol l^{-1}) with a testosterone/DHT ratio of 14 ± 5; following a single 6000 IU hCG intramusculary whereas DHT peak value was 64 ± 16.5 ng dl^{-1} (2.2 ± 0.57 nMol l^{-1}) with a testosterone/DHT ratio of 21 ± 9.7.[109]

Markers of Sertoli cell function (serum AMH and inhibin B) are useful in the differential diagnosis of 46,XY DSD due to disorders of androgen production and 46,XY DSD due to abnormalities of gonadal development. In 46;XY DSD due to impaired androgen production, but not in gonadal dysgenesis, Sertoli cell function is normal. This is particularly useful in the newborn period and in prepubertal ages.

Imaging is indicated in neonatal period when genital ambiguity is identified. If apparent female genitalia with clitoral hypertrophy, posterior labial fusion, foreshortened vulva with single opening or inguinal/labial mass is present, imaging study may also be performed. A family history of DSD and later presentations as abnormal puberty or primary amenorrhoea, cyclic haematuria in a male or inguinal hernia in a female require an imaging evaluation. The ultrasonography is always the first and, often, the most valuable imaging modality in investigation of DSD patients. Ultrasound shows the presence or absence of Müllerian structures at all ages and can locate the gonads and characterise its echo texture. This exam can also identify associated malformations such as kidney abnormalities.[119] Genitography and cystourethrography can display the type of urethra, the presence of vagina, cervix and urogenital sinus. Although, the imaging features are non-specific for the cause of DSD, these diagnostic methods are important in gender assignment and, specially, to the planning of surgery. It is important, though, that the procedure be carried out by an experienced examiner.

The genetic evaluation includes karyotype, fluorescence *in situ* hybridisation (FISH) and more recently specific molecular studies to screen the presence of mutations or gene dosage imbalance.

In summary, 46,XY DSD secondary to defects in androgen production by the foetal testis show a variable phenotype, strongly depending of the particularly mutated gene. The predominant phenotype is female or poorly virilised external genitalia and absence of uterus and fallopian tubes. This phenotype frequently results in a female sex assignment at birth and an accurate diagnosis can avoid late problems to this patients.

Finally, it is important to keep in mind that patients with DSD of any aetiology should receive long-term care provided by multidisciplinary teams in centres of excellence with ample experience in this clinical management.

Practice points

- The SLOS is caused by an inborn error of post-squalene cholesterol biosynthesis. Considering the relative high frequency of this syndrome, we suggest that at least cholesterol levels should be routinely measured in patients with 46,XY DSD.
- All of the enzymatic defects that alter the normal synthesis of testosterone present an autosomal recessive mode of inheritance and genetic counselling is mandatory, since every additional sibling has a 25% chance of being affected with the same synthesis defect.
- In prepubertal individuals with androgen production defects, hCG stimulation test is essential for the diagnosis, since basal levels are not altered.
- Patients with 46,XY DSD of any aetiology should receive long-term care provided by qualified multidisciplinary teams in tertiary hospital

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Research agenda

- The absence of causative mutations in *LHCGR* in several patients strongly suspected to have Leydig cell hypoplasia, supported the idea that other genes should be implicated in the molecular basis of this disorder.

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