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CLINICAL PRACTICE UPDATE

46,XY disorders of sex development (DSD)

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Summary

The term disorders of sex development (DSD) includes congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical.

Mutations in genes present in X, Y or autosomal chromosomes can cause abnormalities of testis determination or disorders of sex differentiation leading to 46,XY DSD. Detailed clinical phenotypes allow the identification of new factors that can alter the expression or function of mutated proteins helping to understand new undisclosed biochemical pathways. In this review we present an update on 46,XY DSD aetiology, diagnosis and treatment based on extensive review of the literature and our three decades of experience with these patients.

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Introduction

Male phenotypic development can be viewed as a two-step process: (i) testis formation from the primitive gonad (sex determination) and (ii) internal and external genitalia differentiation due to factors secreted by the testis (sex differentiation). The first step is very complex and involves interplay of several transcription factors.[1–3] (Fig. 1). The second step, male sex differentiation, is a more straightforward process (Fig. 2).

The term disorders of sex development (DSD) includes congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. The terms 'male pseudohermaphroditism', 'intersex', 'sex reversal', that previously described the DSD, were potentially derogatory to the patients and the consensus on the management of intersex disorders recommended a new nomenclature that will be followed in this review.[4,5]

The 46,XY DSD are characterized by ambiguous or female external genitalia, caused by incomplete intrauterine masculinization, and the presence or absence of Mullerian structures. Complete absence of virilization results in normal female external genitalia and these patients generally seek medical attention at pubertal age, due to the absence of breast development and/or primary amenorrhoea. A classification of 46,XY DSD based on the disorder's aetiology is proposed in Table 1.

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46,XY DSD due to abnormalities of gonadal development

Gonadal agenesis

Total absence of gonadal tissue or gonadal streak has rarely been described in 46,XY subjects with female external and internal genitalia indicating the absence of testicular determination. The origin of this disorder remains to be determined. A defect in genes essential for bipotential gonad development is likely the cause of this disorder.

46,XY DSD due to gonadal dysgenesis

Complete and partial 46,XY gonadal dysgenesis

46,XY gonadal dysgenesis consist of a variety of clinical conditions in which the foetal gonad development is abnormal and encompasses both complete and a partial forms. The complete form is characterized by female external and internal genitalia, lack of secondary sexual characteristics, normal or tall stature without somatic stigmata of Turner syndrome and the presence of bilateral dysgenetic gonads. The partial form of this syndrome is characterized by impaired testicular development that results in patients with ambiguous external genitalia with or without Mullerian structures. Similar phenotypes can also result from a 45,X/46,XY karyotype.

46,XY gonadal dysgenesis is a heterogeneous disorder that results from *SRY* deletions or point mutations, dosage sensitive sex (DSS) locus duplication on X chromosome or mutations in autosomal genes. Mutations in *SRY* were found in < 20% of the patients with complete 46,XY gonadal dysgenesis. To date, more than 53 mutations have been identified within the *SRY*, and most of them (43 mutations), are located in the HMG box. Most of the mutations described in *SRY* are predominantly *de novo* mutations. However, some cases of fertile fathers and their XY affected children, sharing the same altered SRY sequence, have been reported.[6,7] In few of these cases, the father's somatic mosaicism for the normal and mutant SRY have been demonstrated.

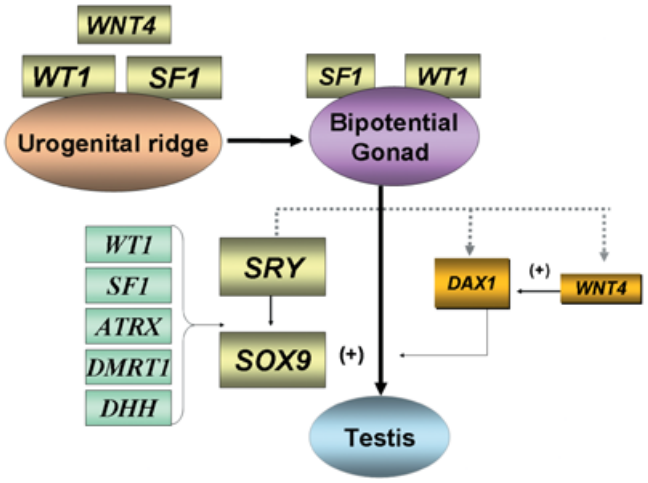

Fig. 1 Summary of the molecular events in sex determination indicating the genes in which molecular defects cause 46,XY DSD in humans. *SF1*, *WNT4* and *WT1* are expressed in the urogenital ridge whose development results in formation of the gonads, kidneys and adrenal cortex. *SF1* and *WT1* maintain their expression in the bipotential gonad and up-regulate *SRY* expression. *SRY* expression in pre-Sertoli cells initiates the male gonad development. SRY strongly up-regulates SOX9 in Sertoli cells. *DMRT1*, *ATRX* and *DHH* are also involved in testes determination. WNT4 activates *DAX1*. SRY down-regulates *WNT4* and *DAX*. Duplication in either *DAX1* (*locus* DSS) or *WNT4* antagonizes testis formation. On the other hand, *DAX1* regulates the development of peritubular myoid cells and the formation of testicular cords. Straight arrow: stimulatory effect; dotted arrow: inhibitory effect.

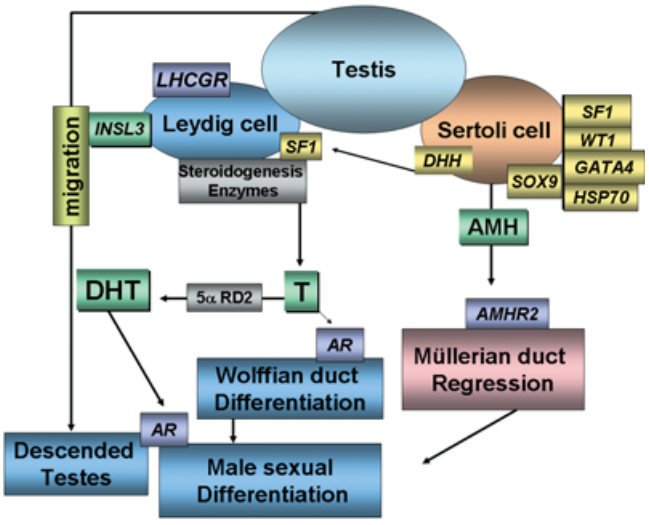

Fig. 2 Summary of the molecular events in sex differentiation indicating the genes in which molecular defects cause 46,XY DSD in humans. After testis determination, hormones produced by the male gonad induce the differentiation of internal and external genitalia acting on their specific receptor. The regulation of *AMH* gene requires cooperative interaction between SOX9 and SF1, WT1, GATA4 and HSP70 at the AMH promoter. Combinatorial expression of *DHH* and *SF1* is required for Leydig cell development. *SF1* regulates gonadal steroidogenesis. The Leydig cells also produce the INSL3 which causes the testes to descend to the scrotum.

Table 1. Classification of 46,XY DSD

46,XY DSD due to abnormalities of gonadal development
Gonadal agenesis
Gonadal dysgenesis – complete and partial forms
Embryonic testicular regression syndrome
Gonadal dysgenesis associated with syndromic phenotype
46,XY DSD associated with cholesterol synthesis defects
Smith-Lemli-Opitz syndrome
46,XY DSD due to testosterone secretion defects
Impaired Leydig cell differentiation (*LHCGR* defects)
Complete and partial forms
Enzymatic defects in testosterone synthesis
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
Defects in androgen action
Androgen insensitivity syndrome
Complete and partial forms
Persistence of Müllerian ducts syndrome
Defect in AMH synthesis
Defect in AMH receptor
Congenital non-genetic 46,XY DSD
Maternal intake of endocrine disruptors
Associated with impaired prenatal growth
Ovotesticular 46,XY DSD
Non-classified forms
Hypospadias
46,XY gender identity disorders
Male to female transsexualism

A recent study describes a remarkable family pedigree across four generations with multiple affected family members, of both sexes, with variable degrees of gonadal dysgenesis. The phenotypic mode of inheritance was strongly suggestive of X-linkage.[8] In this report, a fertile woman had a 46,XY karyotype in peripheral lymphocytes, mosaicism in cultured skin fibroblasts (80% 46,XY and 20% 45,X) and a predominantly 46,XY karyotype in the ovary (93% 46,XY and 6% 45,X). She gave birth to a 46,XY daughter with complete gonadal dysgenesis. The range of phenotypes observed in this unique family suggests a new mechanism which predisposes to chromosomal mosaicism.[8]

Embryonic testicular regression syndrome

Embryonic testicular regression syndrome has been considered part of the clinical spectrum of partial 46,XY gonadal dysgenesis. Most of the patients present ambiguous genitalia or severe micropenis

associated with complete regression of testicular tissue in one or both sides. The dysgenetic testes showed disorganized seminiferous tubules and ovarian stroma with occasional primitive sex cords devoid of germ cells; primordial follicles are sometimes observed in the streak gonad in the first years of life.[9] Familial cases have been reported with variable degrees of sexual ambiguity, but the nature of the underlying defect is still unknown. Recently, a novel heterozygous missense mutation (V355M) in *SF1* gene was found in one boy with a micropenis and testicular regression syndrome.[10]

Gonadal dysgenesis associated with syndromic phenotype

There are several syndromes associated with 46,XY gonadal dysgenesis in humans, caused by mutations in genes involved in gonadal determination. They will be described according to the time of gene expression in gonadal determination.

46,XY DSD due to underexpression of WT1

The Wilms' tumour suppressor gene (*WT1*) encodes a zinc-finger transcription factor involved in the development of the kidneys and gonads. *WT1* is located on 11p13. *WT1* mutations impair gonadal and urinary tract development and cause three syndromes: WAGR, Denys-Drash and Frasier syndromes.

WAGR syndrome is characterized by Wilms' tumour, aniridia, genitourinary abnormalities mental retardation and obesity. Heterozygous deletions of *WT1 and contiguous* genes, such as *PAX6,* are the cause of this syndrome.

Denys-Drash syndrome is characterized by dysgenetic 46,XY DSD associated with early-onset renal failure (diffuse mesangial sclerosis) and Wilms' tumour development in the first decade of life. Gonadal development is impaired to variable degrees, resulting in a spectrum of 46,XY DSD. Heterozygous missense mutations in the zinc finger encoding exons (DNA-binding domain) of *WT1* cause this syndrome.

Frasier syndrome is characterized by a female to ambiguous external genitalia phenotype in 46,XY patients, renal failure in the second decade of life, streak gonads and high risk of gonadoblastoma development. Constitutional heterozygous mutations in the *WT1* gene, almost all located at intron 9 (IVS9 + 4C > T mutation), are found in these patients. The *WT1* gene contains 10 exons, of which exons 1–6 encode a proline/glutamine-rich transcriptional-regulation region and exons 7–10 encode the four zinc fingers of the DNA-binding domain. There are four major species of RNA with conserved relative amounts, different binding specificities, and different subnuclear localizations, generated by two alternative splicing regions.[11] Splicing at the first site results in either inclusion or exclusion of exon 5. The second alternative splicing site is in the 3' end of exon 9 and allows the inclusion or exclusion of three amino acids lysine, threonine and serine (KTS) between the third and fourth zinc fingers, resulting in either KTS-positive or negative isoforms. Isoforms that only differ by the presence or absence of the KTS amino acids have different affinities for DNA and therefore possibly different regulatory functions.[12] The IVS9 + 4C > T mutation leads to a change in splicing resulting in deficiency of the usually more abundant KTS positive isoforms and reversal of the normal KTS positive : negative ratio, indicating that a precise balance between WT1 isoforms is necessary for normal WT1 function.[13]

46,XY DSD due to underexpression of steroidogenic factor-1 (NR5A1/SF1)

The steroidogenic factor 1 (SF1) or Nuclear Receptor Subfamily 5, Group A, Member 1; (*NR5A1*) is a member of the nuclear hormone receptor superfamily of transcriptional factors.

The first reported human case of *NR5A1/SF1* mutation, the heterozygous G35E in the DNA binding domain, was a 46,XY patient who presented female external genitalia and Müllerian duct derivatives associated with adrenal insufficiency.[14] Remarkably, the R92Q mutation in a highly conserved residue of the A-box of *NR5A1/SF1*, was described in the homozygous state in a 46,XY baby with female external and internal genitalia who also presented primary adrenal failure.[15] We identified a heterozygous frameshift mutation resulting from the deletion of eight nucleotides at position 2783 of *NR5A1/SF1* gene in a 46,XY 31-year-old-female patient with clitoromegaly, absence of Müllerian derivatives and gonadal tissue and normal adrenal function suggesting that SF1 transcription might have tissue-specific effects in humans.[16] After this first report, other cases with *NR5A1/SF1* mutations in the heterozygous state were described in a 46,XY DSD patients with normal adrenal function.[17,18] Most of the point mutations identified in *NR5A1/SF1* are located in the DNA-binding domain of the protein. The L437Q mutation, the first located in the ligand-binding region, was identified in a patient with a mild phenotype, a penoscrotal hypospadias; this protein retained partial function in several SF1-expressing cell lines and its location points to the existence of a ligand for SF1, which was considered an orphan receptor.[17] In 2007, sphingosine has been shown to be an endogenous ligand for SF1, antagonizing its capacity to increase CYP17 reporter gene activity.[19]

Recently, a new frameshift mutation in SF1 (c536delC) was described in two 46,XY newborn infants who presented ambiguous genitalia associated to a hormonal phenotype mimicking androgen insensitivity syndrome.[20]

In summary, these recent reports indicate that *SF1* mutations should be considered in patients with 46,XY DSD due to abnormalities of gonadal development with and mainly without adrenal failure.

46,XY DSD associated with campomelic dysplasia (underexpression of SOX9)

SOX9, located on human chromosome 17q is a highly conserved HMG family member and it is also implicated in the sex-determination pathway. Heterozygous mutations in *SOX9* cause severe skeletal malformations (campomelic dysplasia) associated to dysgenetic 46,XY DSD in three-quarters of the affected patients.[21]

The external genitalia varied from normal male with cryptorchidism through ambiguous to female genitalia.

Other rare syndromes associated with 46,XY DSD due to gonadal dysgenesis

Underexpression of *DMRT1* and *DMRT2*, located at 9p24.3, *ATRX* (X-linked α-thalassaemia and mental retardation) located at Xq13

and *DHH* (Desert hedgehog) located at 12q12–q13.1, determine abnormalities in testis development and may be associated or not with other syndromic signs. The main characteristics of these syndromes are presented in Table 2.

46,XY DSD due to overexpression of DAX1

Genetic male patients with female or ambiguous external and internal genitalia, associated or not with mental retardation, cleft palate and dysmorphic face due to partial duplications of Xp have been described. A common 160-kb region of Xp21.2 containing *DAX1,* named DSS *locus* were duplicated in these patients.[22] Recently, a 637-kb tandem duplication on Xp21.2, that in addition to *DAX1* includes the four *MAGEB* genes was described in two sisters with isolated 46,XY gonadal dysgenesis and gonadoblastoma.[23] However, until now, there has not been a description of isolated *DAX1* duplication causing 46,XY DSD in humans, suggesting that other contiguous genes could be involved in X-dosage-sensitive 46,XY DSD.

46,XY DSD due to overexpression of WNT4

Wingless-type mouse mammary tumour virus integration site member 4 (*WNT4*) overexpression may be a cause of 46,XY DSD. A 46,XY newborn infant, with multiple congenital anomalies including bilateral cleft lips and palate, intrauterine growth retardation, microcephaly, tetralogy of Fallot, ambiguous external and internal genitalia, and undescended gonads consisted of rete testes and rudimentary seminiferous tubules, who carried a duplication of 1p31–p35, including *WNT4* gene, was reported.[24] *In vitro* studies suggest that *Wnt4 up*-regulates *Dax1* in Sertoli cells, resulting in an excess of *DAX1* expression and 46,XY DSD. Although, these studies suggest a role of *WNT4* overexpression in testicular dysgenesis a single patient with *WNT4* duplication and gonadal dysgenesis has been described in the literature.[24]

46,XY DSD associated with cholesterol synthesis defect

Smith-Lemli-Opitz syndrome (SLOS)

This disorder is caused by mutations in the sterol delta-7-reductase (*DHCR7*) gene, which maps to 11q12–q13. Typical facial appearance is characterized by short nose with anteverted nostrils, blepharoptosis, microcephaly, photosensitivity, mental retardation, syndactyly of toes 2 and 3, hypotonia and genital ambiguity.[25,26] Adrenal insufficiency can be present or evolve with time. Ambiguity of the external genitalia is a frequent feature of males (71%) and ranges from hypospadias to female external genitalia despite normal 46,XY karyotype and *SRY* sequence. Müllerian derivative ducts can also be present. The aetiology of masculinization failure in the SLO syndrome remains unclear. However, the description of patients with SLOS who present with hyponatraemia, hyperkalaemia, and decreased aldosterone : renin ratio suggest that the lack of substrate to produce adrenal and testicular steroids is the cause of adrenal insufficiency and genital ambiguity.[27]

Affected children present with low plasma cholesterol and elevations of plasma 7-dehydrocholesterol. Considering the relative high frequency of Smith-Lemli-Opitz syndrome, approximately 1 in 20 000 to 60 000 births, we suggest that at least cholesterol levels should be routinely measured in patients with 46,XY DSD.

A summary of the phenotypes of the disorders of sex differentiation are presented in Table 3.

46,XY DSD due to testosterone synthesis defects

46,XY due to impaired Leydig cell differentiation (complete and partial forms)

The inability of Leydig cells to secrete testosterone in 46,XY DSD results in failure of intrauterine and pubertal virilization. Both hCG and LH act by stimulating a common G-protein coupled receptor (*LHCGR*) and mutations in this gene cause Leydig cell hypoplasia.

In 1976, Berthezene *et al.* described the first patients with Leydig cell hypoplasia and subsequently other cases have been reported.[28–33] The study of 8 of our cases and review of the literature allowed us to delineate the characteristics of 46,XY DSD due to the complete form of Leydig cell hypoplasia as: (i) female external genitalia leading to female sex assignment (ii) no development of sexual characteristics at puberty, (iii) undescended testes slightly smaller than normal with relatively preserved seminiferous tubules and absence of mature Leydig cells (iv) presence of rudimentary epididymis and vas deferens and absence of uterus and fallopian tubes, (v) low testosterone levels despite elevated gonadotrophin levels, with elevated LH levels predominant over FSH levels, (vi) testicular unresponsiveness to hCG stimulation, and (vii) no abnormal step up in testosterone biosynthesis precursors.[33–36] Several different mutations in the LH receptor gene were reported in patients with Leydig cell hypoplasia.[29,34,35,37–39]

In contrast to the homogenous phenotype of the complete form of Leydig cell hypoplasia, the partial form can have a broad spectrum.[33,37–41] Most patients have predominantly male external genitalia with micropenis and or hypospadias. Testes are cryptorchidic or topic (testes in the scrotum). During puberty, partial virilization occurs and testicular size is normal or only slightly reduced, while penile growth is significantly impaired. Spontaneous gynaecomastia does not occur. Before puberty the testosterone response to the hCG test is subnormal without accumulation of testosterone precursors. After puberty, LH levels are elevated and testosterone levels are intermediate between those of children and normal males.

Mutations in the *LHCGR* gene have also been identified in patients with the partial form of Leydig cell hypoplasia.[33,37–41]

Leydig cell hypoplasia has been found to be a genetic heterogeneous disorder as molecular defects in the *LHCGR* were ruled out through segregation analysis of a polymorphism in exon 11 of the *LHCGR*, as being responsible for Leydig cell hypoplasia in three siblings with 46,XY DSD. This study supports the idea that other genes must be implicated in the molecular basis of this disorder.[42]

We reported that 46,XX sisters of patients with 46,XY DSD due to Leydig cell hypoplasia, with the same homozygous mutation in the *LHCGR*, have primary or secondary amenorrhoea associated with infertility.[23]

Table 2. Phenotype of 46,XY DSD patients with mutations in genes involved in human male sex determination

External genitalia	Female/ambiguous				Ambiguous		Ambiguous/ male with cryptorchidism	Ambiguous or male	Female/ambiguous/male		Female or ambiguous or male with cryptorchidism
Testes	Dysgenetic		Absent, dysgenetic	Dysgenetic	Dysgenetic		Dysgenetic		Absent or dysgenetic	Dysgenetic	Absent, dysgenetic or hypoplastic
Müllerian duct derivatives	+	+/–	+/–	+/–	+/–	+	NR	NR	+/–	+/–	+/–
Other associated phenotypes	–	Mini fascicular neuropathy	+/– Adrenal failure	Frasier syndrome, (late-onset renal failure, gonado-blastomas)	Denis-Drash syndrome, (early-onset renal failure, Wilm's tumour)	*WAGR* syndrome, (mental retardation, Wilm's tumour, aniridia, renal agenesis or horseshoe)	Cleft lip and palate, tetralogy of Fallot, intrauterine growth retardation, microcephaly	Thalassaemia, severe psychomotor and mental retardation, dysmorphic face	Mental retardation, cleft palate, dysmorphic face, gonadoblastomas	Campomelic dysplasia	Craniofacial abnormalities, microcephaly mental retardation
Inheritance	AD	AD	AD/AR	AD	AD	AD	AD	X-linked	AD	AD	AD
Gene	*SRY*	*DHH*	*SF1*	*WT1*	*WT1*	*WT1*	*WNT4 (1p31–p35)*	*ATRX*	*DAX1 (DSS locus)*	*SOX9*	*DMRT1*
Molecular defect	Inactivating mutation			Splice variants	Inactivating mutation	Contiguous gene deletion	Gene duplication	Inactivating mutation	Gene duplication	Inactivating mutation	Contiguous gene deletion

NR, not reported; AD, autosomal dominant; AR, autosomal recessive.

Table 3. Phenotype of 46,XY DSD patients with mutations in genes involved in human male sex differentiation

External genitalia	Female			Female or ambiguous	Ambiguous						Ambiguous/micropenis	
Testes	Abdominal/inguinal				Abdominal/inguinal/topic							
Müllerian duct derivatives	Absent											
Clinical outcome	Gynecomastia, primary amenorrhea, scarce pubic hair	Adrenal failure, HH	HH	Arterial hypertension, gynecomastia, primary amenorrhea, scarce pubic hair	Gynecomastia, partial virilization	+/– Adrenal failure +/– Gynecomastia	Virilization at puberty +/– Gynecomastia	+/– Adrenal failure Antley–Bixler phenotype +/–	HH +/– Gynecomastia	Virilization at puberty, no gynecomastia	HH	Short stature, metabolic syndrome
Inheritance	X linked/ Somatic mosaicism	AR	AR	AR	X-linked/ somatic mosaicism	AR	AR	AR	AR	AR	AR	–
Gene	*AR*	*STAR CYP11A1*	*LHCGR*	*CYP17*	*AR*	*HSD3B2*	HSD17B3	*POR*	*CYP17*	*SRD5A2*	*LHCGR*	–
Disorder	CAIS	CAH due to StAR or P450scc deficiency	Complete form of Leydig cell hypoplasia	CAH due to 17α-hydroxylase and 17–20 lyase deficiency	PAIS	CAH due to 3β-HSD II deficiency	17β-HSD3 deficiency	POR deficiency	Isolated 17–20 lyase deficiency	5α-RD2 deficiency	Partial form of Leydig cell hypoplasia	Small for gestational age
Molecular defect	Inactivating mutation											Epigenetic defects

AR, Autosomal recessive; HH, Hypergonadotropic hypogonadism.

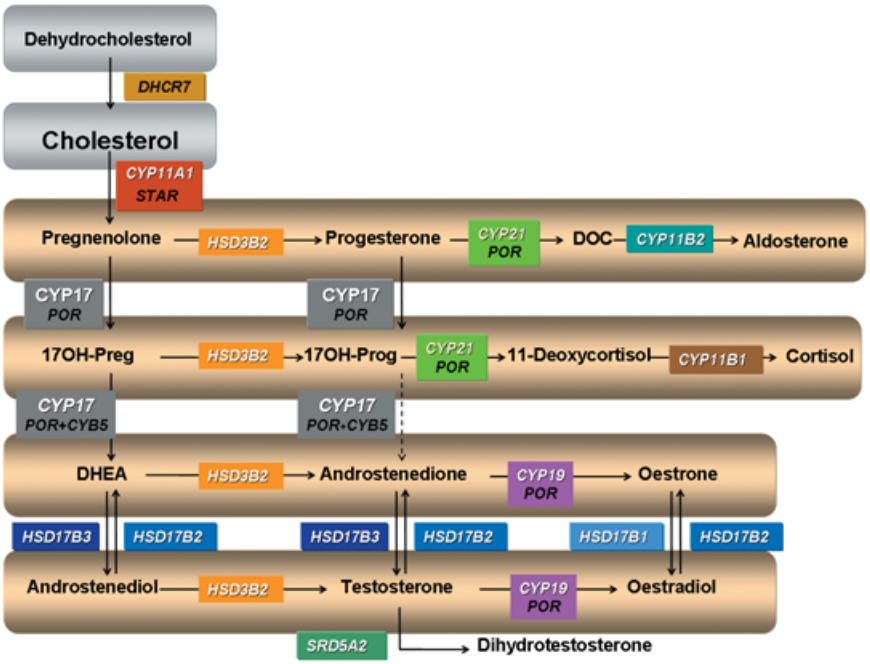

Fig. 3 Adrenal and testicular steroidogenesis displaying the genes in which mutations result in 46,XY DSD in humans.

46,XY DSD due to enzymatic defects in testosterone synthesis

Five enzymatic defects that alter the normal synthesis of testosterone have been described to date. All enzymatic defects present an autosomal recessive inheritance. Three of them are associated to defects in cortisol synthesis leading to congenital adrenal hyperplasia (CAH) (Fig. 3).

Defects in corticosteroid and testosterone synthesis

Congenital lipoid adrenal hyperplasia. In steroidogenic tissues, 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.

Deficiency of the acute steroidogenesis regulatory protein (STAR). Lipoid adrenal hyperplasia is rare in Europe and America but it is the second most common form of adrenal hyperplasia in Japan. Affected subjects are phenotypic females or had slightly virilized external genitalia, irrespective of gonadal sex, with an enlarged adrenal cortex, engorged with cholesterol and cholesterol esters associated to severe adrenal insufficiency.[43] The disease was firstly attributed to P450scc deficiency, but most of the cases studied through molecular analysis showed an intact *P45011A* and *STAR* mutations were found in most of the affected patients.[43] Congenital lipoid adrenal hyperplasia in most Palestinian cases is caused by a founder c.201_202delCT mutation causing premature termination of the STAR.[44]

Deficiency of P450scc. It has been assumed that *CYP11A* mutations are incompatible with human term gestation, because P450scc is needed for placental biosynthesis of progesterone, which in turn, 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*.[45] Following this report, inherited CYP11A deficiency was described in a patient with congenital adrenal hyperplasia born to healthy parents.[46] More recently, mutational analysis of CYP11A1 was performed in 46,XY infants with adrenal failure and disordered sexual differentiation, and two infants had compound heterozygous mutations in CYP11A1.[47] Therefore, P450scc deficiency is a recently recognized disorder that may be more frequent than originally assumed.[47] The phenotypic spectrum of P450scc deficiency ranges from severe loss-of-function mutations associated with prematurity, complete underandrogenization, and severe, early-onset adrenal failure, to partial deficiencies found in children born at term with mild masculinization and late-onset adrenal failure. In contrast to congenital lipoid adrenal hyperplasia caused by STAR mutations, adrenal hyperplasia has not been reported in any of the six patients with P450scc deficiency.[47]

3β-hydroxysteroid dehydrogenase (3β-HSD) type II deficiency. Male patients with 3β-HSD type II deficiency present ambiguous external genitalia, characterized by micropenis, perineal hypospadias, bifid scrotum and a blind vaginal pouch associated or not with salt loss.[48]

Around 40 mutations in 3β-HSD type II gene (*HSD3B2*) have been described. Mutations that abolish 3β-HSD type II activity lead to CAH with severe salt-loss. Mutations that reduce type II activity lead to CAH with mild or no salt-loss, which in males is associated with 46,XY DSD.[48] Most of the patients were raised as males and they kept the male social sex at puberty. In one Brazilian family, two cousins with 46,XY DSD due to *HSD3B2* mutations were reared as females; one of them was castrated in childhood and kept the female

social sex; the other was not castrated and changed to male social sex at puberty.[7]

Male subjects with 46,XY DSD due to 3β-HSD type II deficiency without salt loss showed clinical features in common with the deficiencies of 17β-HSD3 and 5α-RD2.

P450c17 (17-hydroxylase and 17-20 lyase) deficiency. CYP17 is a steroidogenic enzyme that has dual functions – hydroxylation and as a lyase, and is located in the zona fasciculata and zona reticularis of the adrenal cortex and gonadal tissues. The first activity results in hydroxylation of pregnenolone and progesterone at the C (17) position to generate 17α-hydroxypregnenolone and 17α-hydroxyprogesterone, while the second enzyme activity cleaves the C(17)–C(20) bond of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone to form dehydroepiandrosterone and androstenedione, respectively. The modulation of these two activities occurs through cytochrome b5, necessary for lyase activity. The phenotype of 17-hydroxylase deficiency in most of the male patients is described as a female-like or slightly virilized external genitalia with blind vaginal pouch, cryptorchidism and high blood pressure, usually associated with hypokalaemia. Although cortisol synthesis is impaired in these patients, they do not present signs of glucocorticoid insufficiency, due to the elevated levels of corticosterone, which has a glucocorticoid effect.

Most of the male patients were reared as women and sought treatment due to primary amenorrhoea or lack of breast development. The phenotype is similar to 46,XX or 46,XY complete gonadal dysgenesis but the presence of systemic hypertension and absence of pubic hair in post pubertal patients suggests the diagnosis of 17α-hydroxylase deficiency.

The *CYP17* gene, which encodes the enzymes 17-hydroxylase and 17-20 lyase, is located at 10q24.3. Several mutations in the *CYP17* have been identified in patients with both 17-hydroxylase and 17,20 lyase deficiencies.[49–51]

Defects in testicular steroidogenesis

P450c17 (17,20 lyase activity) deficiency. A clear molecular evidence of isolated 17,20 lyase deficiency existence was first demonstrated in two Brazilian 46,XY DSD patients with clinical and hormonal findings indicative of isolated 17,20-lyase deficiency.[49,52] These patients harbour homozygous missense mutations in *CYP17*. Both mutations alter the electrostatic charge distribution in the redox-partner binding site leading to a selective electron transfer lost for the 17,20-lyase reaction.

46,XY DSD due to 17β-HSD type III deficiency. 46,XY DSD results from mutations in the HSD17B3 gene that encodes the 17β-HSD3 isoenzyme.[53]

Patients present female-like or ambiguous genitalia at birth, with the presence of a blind vaginal pouch, intra-abdominal or inguinal testes. Most affected males are raised as females and may change to male gender role behaviour at puberty.[54,55] Virilization in subjects with 17β-HSD3 deficiency occurs at puberty.[54] A limited capacity to convert androstenedione into testosterone in the foetal extragonadal tissues may explain the impairment of virilization of the external genitalia in the newborn. The phenotype is quite variable in 17β-HSD3 deficiency and some patients were previously diagnosed as partial androgen insensitivity syndrome (PAIS).[55]

The disorder is due to homozygous or compound heterozygous mutations in *HSD17B3* and several mutations have been reported.[7,53]

Altered steroidogenesis due to disrupted electron donor proteins

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

P450 oxidoreductase (POR) deficiency

CAH due to apparent combined P450C17 and P450C21 deficiency is a rare disorder. Affected girls and boys present ambiguous genitalia and can also present bone malformation as described in the Antley–Bixler syndrome (craniosynostosis, radiohumeral synostosis, femoral and ulnar bowing, joint contractures, arachnodactyly). Urinary steroid analysis indicates impaired C17 and C21 hydroxylation, suggesting concurrent partial deficiencies of P450C17 and P450C21. However, sequencing of the genes encoding these enzymes showed no mutations, suggesting a defect in a cofactor that interacts with both enzymes. POR is a flavoprotein that donates electrons to all microsomal P450 enzymes, including the steroidogenic enzymes P450c17, P450c21 and P450aro. The underlying molecular basis of congenital adrenal hyperplasia with apparent combined P450C17 and P450C21 deficiencies was defined in patients, who were compound heterozygotes for mutations in POR.[56,57] In a recent large survey of patients with Antley–Bixler syndrome, it was demonstrated that individuals with an Antley–Bixler-like phenotype and normal steroidogenesis have *FGFR2* mutations, whereas those with ambiguous genitalia and altered steroidogenesis have POR mutations.[58] To date, most patients with POR deficiency have also had the Antley–Bixler skeletal malformation syndrome. The mechanism by which POR deficiency yields the skeletal dysmorphology is unclear, but experiments with POR knockout mice suggest this could be associated with defective retinoid metabolism.[59]

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

A patient with type IV hereditary methaemoglobinaemia and with 46,XY DSD was described. The aetiology of 46,XY DSD in this patient was attributed to the cytochrome b5 defect as cytochrome b5 has been shown to participate in 17α-hydroxylation in adrenal steroidogenesis by serving as an electron donor.[7]

46,XY DSD due to defects in testosterone metabolism

5α-reductase type 2 (5RD2) deficiency

46,XY DSD results from mutations in *SRD5A2* gene, located at chromosome 2p23, which encodes the steroid 5α-reductase 2 isoenzyme.[60]

Affected patients present ambiguous external genitalia, micropenis, normal internal male genitalia, prostate hypoplasia and testes with normal or reduced spermatogenesis, scarce facial and body hair and absence of acne and temporal male baldness. The testes are usually located in the inguinal region, suggesting that DHT influences testis migration to the scrotum.[7,61] Virilization appears at puberty without the development of significant gynaecomastia. The main differential diagnosis of 5α-RD2 deficiency is 17β-HSD3 deficiency and PAIS, although in these two disorders gynaecomastia is common.

There are more than 50 families with this disorder described in several parts of the world. In a few 46,XY DSD patients due to 5-α RD2 deficiency diagnosed by clinical and hormonal findings no mutations were identified in the *SRD5A2*.[7]

Most of the patients are reared in the female social sex due to the impairment of external genitalia virilization, but many patients, who have not been submitted to orchiectomy in childhood, undergo male social sex change. In our cohort of 30 cases of 46,XY DSD due to 5-α-RD 2 deficiency from 18 families, all but two subjects were registered in the female social sex. Fourteen patients changed to a male gender role, two of them at prepubertal age, nine at pubertal age and two at adult age. No correlation was observed between the mutation, T : DHT ratio and gender role in these families. Two siblings carrying the same mutation presented a different gender role.[62] Ten cases are adults now and nine of them are married. All patients report male libido and sexual activity although the small penis size can make intercourse difficult. Most of the patients have retrograde ejaculation and highly viscous semen due to rudimentary prostate and underdeveloped seminal vesicles and they need *in vitro* fertilization to have children. Three of our patients adopted children, while in two cases *in vitro* fertilization using the patients' sperm cells resulted in successful pregnancies.[62]

Fourteen patients kept the female sexual identification. Among them, three were castrated in childhood and 11 of them developed virilization signs at puberty. From the 10 adult female patients, now aged 20 to 47 years, none of them got married, but eight of them have satisfactory sexual activity. Therefore, the patients who changed to male sex are more socially adapted than the ones that keep the female social sex. Small penis size is their main problem.[61,62]

46,XY DSD due to defects in androgen action

Androgen insensitivity syndrome is classified as the complete form (CAIS) when there is an absolute absence of androgen action and as the partial form (PAIS) when there are variable degrees of androgen action impairment. Prenatal diagnosis of CAIS can be suspected when a 46,XY foetus presents with female genitalia on prenatal ultrasound. At prepubertal age, an inguinal hernia in girls can indicate the presence of testes and at puberty, complete breast development and primary amenorrhoea associated with reduced or absent pubic and axillary hair suggest CAIS. Gonadectomy should be performed because of the increased risk of testicular tumours, although a recent study showed that tumour risk is low in CAIS patients before and during puberty.[63] We favour prepubertal gonadectomy, after diagnosis, and then induction of puberty with oestrogens at the appropriate age. This approach diminishes the time that the girl has an inguinal mass and surgery is better handled psychologically by a young child than an adolescent. However, the optimal timing for gonadectomy in CAIS patients is controversial and some authors advise to postpone gonadectomy until after spontaneous breast development at puberty.[64] In our experience, breast development is similarly obtained with endogenous oestrogenization or with pharmacological replacement.

Female relatives on the maternal side of the patient can be studied for the mutation of an index case, and if the carrier status is identified genetic counselling should be performed.

Whereas the clinical picture of CAIS is quite homogeneous, the phenotype of PAIS is quite variable and diagnostic confusion with other causes of 46,XY DSD is frequent.[65]

In AIS patients final height is intermediate between mean normal male and female and decreased bone mineral density in the lumbar spine have been demonstrated.[66]

The androgen receptor (*AR*) gene is located at Xq11–12. Mutations in the *AR* gene are found in most of the subjects with CAIS and in several patients with PAIS.[67–69] The different frequencies of mutations found in *AR* (28–73%) are related to case selection. In our experience, selecting patients with normal basal and hCG-stimulated testosterone levels and steroid precursors, gynaecomastia at puberty, and, in prepubertal patients, and a family history suggestive of X-linked inheritance, results in the identification of mutations in 73% of the families.[68]

More than 300 different *AR* mutations have been described and are listed in a database: <http://androgendb.mcgill.ca/>. *AR* mutations are transmitted in an X-linked recessive manner in 70% of the cases, but in 30%, the mutations arise *de novo.* Sporadically, somatic mosaicism can happen when *de novo* mutations occur after the zygotic stage.[70] The identification of carriers of mutations in the *AR* is of clinical importance for genetic counselling. A patient with a CAIS phenotype was described as bearing a normal *AR*, but studies in genital skin fibroblasts revealed that transmission of the activation signal by the AF-1 region of the AR was disrupted, suggesting that a coactivator interacting with the AF-1 region of the AR was lacking in this patient.[71]

Recently, a new mechanism for regulating steroid hormone receptor activity has been proposed, involving FKBP52, a co-chaperone of the steroid receptor complex, phosphorylation of which can potentiate steroid receptor function.[72,73]

Patients with CAIS were raised as females and maintained female gender. Most of the patients with PAIS who were raised as females maintained a female social sex after postpubertal age, despite clitoral growth and partial virilization. In our experience, all 5 cases with PAIS kept the female social sex.[68] This is in distinct contrast to some other forms of 46,XY DSD such as 5-reductase 2 deficiency and 17β-hydroxysteroid dehydrogenase III deficiency in which several affected 46,XY individuals raised as females undergo a change to male social sex at puberty.[54,61] The impairment of androgen action in subjects with PAIS is probably similar during embryogenesis and puberty, whereas the action of androgens is stronger at puberty in subjects with enzymatic defects, because of alternate pathways and maturation of isoenzymes. There is an overlap in phallus length at the time of diagnosis in postpubertal PAIS subjects with female and male social sex, suggesting that sex assignment at birth and sex of rearing were more important than phallus size for development of gender

identity.[68] In another study, either male or female sex of rearing lead to successful long-term outcome for the majority of the 39 subjects with 46,XY DSD, 14 of them with PAIS, 5 living as men and 9 as women.[69]

Persistent müllerian duct syndrome

Defect in AMH synthesis

Defect in AMH receptor type 2. The development of female internal genitalia in a male individual is due to the incapacity of Sertoli cells to synthesize or secrete bioactive anti-Müllerian hormone (AMH) or to alterations in the hormone receptor. Persistent Müllerian duct syndrome (PMDS) phenotype can be produced by a mutation in the gene encoding anti-Müllerian hormone or by a mutation in the AMH receptor. These two forms result in the same phenotype and are referred to as type I and type II, respectively.[74] PMDS is a heterogeneous disorder that is inherited in a sex-limited autosomal recessive manner.

AMH is located in chromosome 19p13.3 and its protein product acts through its specific receptor type 2 (AMHR2) a serine/threonine kinase, member of the family of type II receptors for TGF-β-related proteins, to induce regression of the Mullerian ducts. Affected patients present a male phenotype, usually along with bilateral cryptorchidism and inguinal hernia. Leydig cell function is preserved, but azoospermia is common due to the malformation of *ductus deferens* or agenesis of epididymis.[74]

Mutations in *AMH* or *AMHR2* in similar proportions are the cause of approximately 85% of the cases of PMDS.[74]

Congenital non-genetic 46,XY DSD

Maternal intake of endocrine disruptors

The use of synthetic progesterone or its analogues during the gestational period has been implicated in the aetiology of 46,XY DSD. Some hypotheses have been proposed to explain the effect of progesterone in the development of male external genitalia, such as reduction of testosterone synthesis by the foetal testes, or a decrease in the conversion of testosterone into DHT due to competition with progesterone (also a substrate for 5α-reductase 2 action). The effect of oestrogen use during gestation in the aetiology of 46,XY DSD has not been confirmed to date. A recent study in Japanese subjects supports the hypothesis that homozygosity for the specific oestrogen receptor α 'AGATA' haplotype may increase the susceptibility to the development of male genital abnormalities in response to oestrogenic effects of environmental endocrine disruptors.[75] Exposure to residues of a fungicide (vinclozolin), either alone or in association with the phytoestrogen genistein (present in soy products) induce hypospadias in 41% of mice, supporting the idea that exposure to environmental endocrine disruptors during gestation could contribute to the development of hypospadias.[76]

Congenital non-genetic 46,XY DSD associated to impaired prenatal growth

Despite the multiple genetic causes of 46,XY DSD, around 30–40% of cases remain without diagnosis. Currently, there is a frequent, non-genetic variant of 46,XY DSD characterized by reduced prenatal growth and lack of clear evidence for any associated malformation or steroidogenic defect.

We have identified a pair of monozygotic twins (46,XY; identical for 13 informative DNA loci) born at term who were discordant for genital development (perineal hypospadias *vs.* normal male genitalia) and postnatal growth (low birth weight *vs.* normal birth weight).[77] The most plausible cause of incomplete male differentiation associated with early-onset growth failure is a post-zygotic, micro-environmental factor, as different DNA methylation patterns associated with silencing of genes important to sex differentiation has been shown.

Additionally, other studies in undetermined 46,XY DSD report that around 30% of cases are associated with low birth weight, indicating that adverse events in early pregnancy are frequent causes of congenital non-genetic 46,XY DSD.[78–80]

46,XY ovotesticular DSD

There are rare descriptions of 46,XY DSD patients with well characterized ovarian tissue with primordial follicles and testicular tissue, a condition that histologically characterizes ovotesticular DSD. The differential diagnosis of 46,XY ovotesticular DSD with partial 46,XY gonadal dysgenesis should be performed considering that in the latter condition there are descriptions of dysgenetic testes with disorganized seminiferous tubules and ovarian stroma with occasional primordial follicles in the first years of life.[9] To our knowledge there are no descriptions of an adult patient with 46,XY ovotesticular DSD with functioning ovarian tissue, as occurs in all 46,XX ovotesticular DSD. Therefore the diagnosis of 46,XY ovotesticular DSD is debatable.

Non-classified forms

Hypospadias

Hypospadias is a fairly common pathology and 40% of the cases are associated with other defects of the urogenital system. It is usually a sporadic phenomenon, but familial cases can be observed. The presence of hypospadias indicates that sometime during the *intra-uterus* life there was an alteration in testosterone secretion or action. Fukami *et al.* described mutations in an X-linked gene, *CXorf6* in three boys with penoscrotal hypospadias and micropenis.[81]

Testicular function should be assessed in the presence of penoscrotal hypospadias with decreased penis size to rule out causes such as defects in testosterone synthesis and action, which require hormonal treatment and genetic counselling in addition to surgical treatment.

46,XY gender identity disorders

Male to female transsexualism

Patients with male to female transsexualism manifest a profound and persistent desire to live and be accepted as a member of the female sex, usually accompanied by a sense of discomfort with their anatomic sex and the wish to have surgery and hormonal treatment

to make their body as congruent as possible with the female sex. In our experience, the first manifestations of gender identity disorders usually start during childhood.

Management of patients with 46,XY DSD

The treatment of 46,XY DSD patients requires an appropriately trained multi-disciplinary team. Early diagnosis is important for good outcome of the patients and should start with a careful examination of the newborn's genitalia at birth.

Psychological evaluation is of extreme importance in the treatment of DSD patients. Every couple that has a child with ambiguous genitalia must be assessed and receive counselling by an experienced psychologist, specialized in gender identity, who must act as soon as the diagnosis is suspected, and then follow the family periodically, more frequently during the periods before and after genitoplasty.[4,5,82]

Parents must be informed by the physician and psychologist about normal sexual development. A simple, detailed and comprehensive explanation about what to expect regarding integration into social life, sexual activity, requirement of hormonal and surgical treatment and the possibility or not of fertility according to the sex of rearing, should also be discussed with the parents, before the attainment of final social sex.

The determination of social sex must take into account the aetiological diagnosis, penis size, ethnic traditions, sexual identity and the acceptance of the assigned social sex by the parents. In case parents and health care providers disagree over the sex of rearing and psychological support was not able to change parents' choice, their opinion should always prevail to avoid ambiguous sex of rearing. The affected child and his/her family must be followed throughout life to ascertain the patient's adjustment to his/her social sex.[4]

Hormonal therapy

Female social sex

The purpose of the hormonal therapy is the development of female sexual characteristics and induction of menses in those patients where uterus is present.

Several oral and non-oral routes are available for oestrogen replacement in women, as well as different combinations and doses of progestins. Administration of oestrogen via the transdermal route avoids hepatic first-pass metabolism and therefore the high serum levels of oestrogen metabolites that are associated with oral administration. Patch formulations have traditionally been the most common form of transdermal oestrogen replacement. However, as patches may be associated with local skin reactions, gel formulations have been developed in an attempt to improve acceptability and compliance with transdermal oestrogen therapy. Patch and gel formulations are equally effective in improving bone mineral density, and the effects are comparable to those achieved by oral formulations.[83]

Several progesterone formulations (synthetic, natural, and micronized natural) and routes of administration (oral, intramuscular, intravenous, intravaginal, intranasal, transdermal and rectal) are available. Synthetic progestins are widely used but may produce a number of significant side effects, such as fatigue, fluid retention, lipid level alterations, dysphoria, hypercoagulant states, and increased androgenicity. Natural progesterone is reported to have milder adverse effects, depending on the route of administration. Micronized natural progesterone is available for oral administration, has better bioavailability and fewer side effects than natural progesterone, and is convenient to administer. Therefore, micronized natural progesterone appears to be a safe and effective alternative to synthetic and natural progesterone formulations for hormonal therapy replacement in female patients.[84]

The treatment must simulate normal puberty. We introduce low oestrogen doses (0·07–0·3 mg of conjugated oestrogen) at 9–11 years to avoid excessive bone maturation except in tall girls in whom adult oestrogen dose is indicated. After breast development is complete, adult oestrogen doses (0·625–1·25 mg/day of conjugated oestrogen) is maintained continuously and 5–10 mg of medroxyprogesterone acetate (from the 1st to the 12th day of each month) is added to induce menses. In patients without a uterus, oestrogen alone is indicated. In patients who wish to initiate sexual activity, dilation of the blind vaginal pouch with acrylic moulds[85] or surgical neovagina[86] promote development of a vagina adequate for sexual intercourse.

Male social sex

Several options are available for androgen replacement. Oral testosterone, intramuscular injections, subcutaneous implants and transdermal therapy have all been used.[87] Each mode of delivery has advantages and drawbacks and the choice between them will often depend on patient preference. Recent advances include the development of longer-acting intramuscular testosterone preparations, which offer more stable androgen levels with fairly infrequent injections.[88] Intramuscular depot injections of testosterone esters are widely used. We start testosterone replacement between 10 and 11 year, simulating normal puberty according to the child's psychological evaluation and height. The initial dose of depot injections of testosterone esters is 25–50 mg/month administered IM. The maintenance dose in an adult patient is 200–250 mg every 2 weeks. In male patients with androgen insensitivity, higher doses of testosterone esters (250–500 mg twice a week) are used to increase penis size and male secondary characteristics. Maximum penis enlargement is obtained after 6 months of high doses and after that, the normal dosage is re-instituted.[61,62]

The use of topic DHT gel is also useful to increase penis size with the advantage of not causing gynaecomastia and promoting faster increase of penis size as it is 50 times more active than testosterone. Considering that DHT is not aromatized, one would expect it to have no effect on bone maturation, allowing the use of higher doses than testosterone and consequently attaining a higher degree of virilization.

Surgical treatment

The aims of surgical treatment are to allow development of adequate external genitalia and removal internal structures that are inappropriate for the social sex. Patients must undergo surgical treatment preferably before 2 years of age, which is the time when the child becomes aware

of his/her genitals and social sex. Only skilled surgeons with specific training in the surgery of DSD should perform these procedures.[4,5]

For those 46,XY DSD children assigned female, laparoscopy is the ideal method for performing gonadectomy and for resection of internal organs if appropriate.[89]

Feminizing genitoplasty should provide an adequate vaginal opening into the perineum, create a normal-looking vaginal introitus, fully separate the urethral from the vaginal orifice, remove phallic erectile tissue preserving glandular enervation and blood supply, and prevent urinary tract complications.[90] The most reasonable procedure for clitoroplasty is based on the concept of maintaining the clitoral glans and sensory input, which facilitates orgasm. The use of an adequate size of tissue flap is mandatory in Y-V vaginoplasty, to avoid introital stenosis. Failure to interpose an adequate flap will result in persistent introital stenosis, requiring later revision. Vaginal dilation with acrylic moulds in patients with introitus stenosis has been shown to be an effective treatment choice when these patients wished to start sexual intercourse, resulting in good outcomes.[85] In our experience, the single-stage feminizing genitoplasty consisting of clitoroplasty with the preservation of dorsal nerves and vessels and ventral mucosa, vulvoplasty and Y-V perineal flap, followed by vaginal dilation with acrylic moulds, allowed good cosmetic and functional results.[90]

For those raised as males, surgery consists in orthophaloplasty, scrotumplasty with resection of vaginal pouch, proximal and distal urethroplasty and orchidopexy when necessary. Surgeries were performed in two or three steps in the patients with perineal hypospadias. The most frequent complication is urethral fistula in the penoscrotal angle and urethral stenosis that can occur several years after surgery. The results of surgical correction are good, from both the aesthetical and functional points of view in our series as well as in others.[4,5,69,91]

Most of our patients present satisfactory sexual performance as long as they present a penis size of at least 6 cm. New approaches, such as the use of donor-grafting tissue to elongate the urethra and penis may help these patients in the future.

Dysgenetic or undescended gonads and tumour development

Neoplastic transformation of germ cells in dysgenetic gonads (gonadoblastomas and/or an invasive germ cell tumour) occurs in 20–30% of 46,XY DSD patients[92] and is associated with the presence of Y chromosome or part of it. The presence of a well-defined part of the Y chromosome, known as the gonadoblastoma Y *locus* (*GBY*), is a prerequisite for malignant transformation. Among the genes located on the GBY region the TSPY seems to be the most significant candidate gene for tumour-promoting processes.[92]

Recently, the presence of undifferentiated gonadal tissue containing germ cells that abundantly express TSPY and OCT4 has also been identified as a gonadal differentiation pattern bearing a high risk for the development of gonadoblastoma.[92]

Spontaneous breast development suggests the presence of an oestrogen-secreting tumour (gonadoblastomas).

Bilateral gonadectomy should be performed in 46,XY patients before pubertal age to avoid degeneration of dysgenetic tissue, unless the gonad is functional and easily accessible to palpation and imaging studies, which should be performed yearly. A gonadal biopsy showing the presence of undifferentiated gonadal tissue or testicular tissue with OCT4-positive cells on the basal lamina suggests a high risk for germ cell tumours, whereas testicular tissue displaying delay in maturation of germ cells and stromal ovarian tissue can be safely be left *in situ*.[92]

The risk for germ cell tumours is increased in patients with undescended testes, including all other 46,XY DSD syndromes.[92] Although data are limited, in the androgen insensitivity syndrome the risk seems to be markedly higher in the partial form than in the complete form and tumour prevalence in AIS is markedly increased after puberty. On the other hand, series reporting other causes of undervirilized 46,XY patients and gonadal tumours are too small and do not allow any conclusions.

Fertility in patients with 46,XY DSD

Infertility is almost always present in 46,XY DSD patients due to impaired spermatogenesis secondary to undescended testes, perineoscrotal hypospadias or complications of genitourinary surgery such as urethroscrotal fistulas. In addition, very low semen volume and increased viscosity can preclude natural insemination. Currently, *in vitro* fertilization techniques has enabled some 46,XY DSD patients caused by 5-reductase-2 deficiency to produce offspring.[62,93]

The first reported case, a 46,XY DSD patient with 5-reductase-2 deficiency achieved biological fatherhood twice by intrauterine insemination with his sperm.[93]

The use of intrauterine insemination circumvented the difficulties with insemination consequent to the urethroscrotal fistulas and abnormal semen quality and resulted in two successful pregnancies. Thus, intrauterine insemination can be used in men with 5-reductase-2 deficiency who have adequate sperm counts and motility. It may even be successful in affected men with low sperm counts and diminished motility, because washing the semen before insemination eliminates from the sample many of the abnormal sperm.[93] In two of our patients with 46,XY DSD due to 5-reductase-2 deficiency *in vitro* fertilization techniques was used successfully.[62]

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