# 1 Genetics and Alterations in the Development of Male Reproductive System: Diagnosis and Clinical Management

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## 1.1 Physiology of Male Sex Determination and Sexual Differentiation

Humans are born with 46 chromosomes, including 22 pairs of autosomes and 1 pair of gonosomes. Sex chromosomes can be X or Y, to specify which sexual determination pathway will be initiated. Generally, most women have 46,XX karyotype and most men 46,XY, but there are a few exceptions, which involve the sex monosomies (45,X0 or 45,Y0), the sex polysomies (i.e., 47,XXX, 47,XYY, or 47,XXY), translocations, rearrangements of chromosomes, or mutations of different genes.

Sex determination is regulated by the temporospatial expression of many different genes with critical dosage effects. Gonadal development is a particularly complex process, involving many genetically regulated, well-synchronized developmental steps, to balance opposing signals and to equilibrate cell proliferation and apoptosis.

Gonadal development starts in the fetal life, around the fourth gestation week, with the formation of the undifferentiated urogenital ridges, containing two different duct systems at the same time, the mesonephric (Wolffian) and the paramesonephric (Müllerian) ducts (Fig. 1.1). They are developed from the intermediate mesoderm, and a week later, they are colonized by primordial germ cells. Genes involved in the formation of bi-potential gonads belong to two groups: homeobox genes and transcriptional factors.

While the expression patterns of genes in the undifferentiated stage of gonadal development are the same in male and female, starting from the 41st gestational day, the gene expression profile will radically change in the two sexes.

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C. Foresta, D. Gianfrilli (eds.), *Pediatric and Adolescent Andrology*, Trends in Andrology and Sexual Medicine, https://doi.org/10.1007/978-3-030-80015-4_1

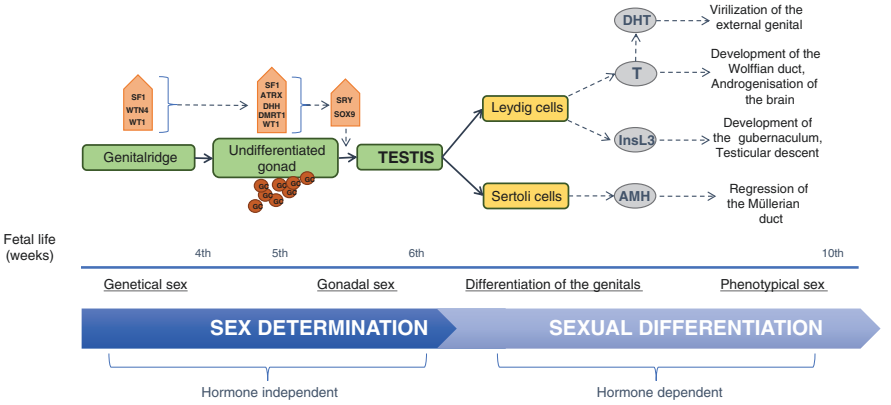


**Fig. 1.1** Schematic figure illustrating the processes involved in male sexual development

In fact, the presence/absence of the Y chromosome-linked *Sex Determining gene on Y* (*SRY*) gene will determine the fate of the bi-potential gonadal precursors, i.e., to become either testes or ovaries. *SRY*, which contains 1 single exon, conserves 79 amino acid domains with similarities to a type of DNA-binding domain, the so-called high-mobility group (HMG) box. Apart from *SRY*, the HMG box containing protein family (SOX) involves other 20 transcription factors.

The first step of male gonadal development is linked to the upregulation of SRY expression. The exact mechanism by which the upregulation occurs is still not fully clarified, but a number of factors have been identified as modulators of the *SRY* expression. Among them, some are affecting the *SRY* expression positively, and some negatively. A positive regulator is the Wilms tumor 1 (WT1) protein and specifically its isoform WT1(KTS +), i.e., the protein containing three amino acids, Lys-Thr-Ser (KTS). This gene encodes a transcription factor that takes part in cellular development and cell survival and is also considered as a tumor suppressor. The transcripts of ***WT1*** are expressed in the primordial gonads and subsequently in the Sertoli cells of the testicles or in the granulosa and epithelial cells of the ovary. ***WT1*** is expressed in the same cell lineage, before, during, and after *SRY* expression. This protein has several isoforms, and one of them plays a role in the undifferentiated gonad, whereas the other is essential for testis determination. The two isoforms are the results of alternative splicing at the end of exon 9, and they only differ by the absence (-KTS) or presence (+KTS) of three amino acids. The +KTS isoform upregulates SRY expression through its binding to SRY mRNA, functioning as a post-transcriptional stabilizer. Other positive transcription factors are the *GATA4* with a co-factor, termed as *Friend of GATA2* (*FOG2*), and the *MAP3K4* gene, member of the mitogen-activated protein kinase (MAPK) pathway. *MAP3K4* pathway regulates the phosphorylation of *GATA4* and allows its binding to the *SRY* promoter with subsequent stimulation of *SRY*. *Steroidogenic factor 1* (*SF-1*; alias *NR5A1*) is known as a pivotal gene with multiple functions. It acts as an early modifier of the

expression of several genes required for the initiation and the maintenance of the male determination and differentiation pathways. In addition, it also takes part in the regulation of the hypothalamic-pituitary axis.

In the past, the female sex determination was supposed to be the default pathway in the absence of *SRY*. Several evidences indicate the existence of ovary-specific genes and pathways. These genes (*WNT4*, *RSPO1*, β-*catenin*, *FOXL2*) are also acting as negative modifiers of *SRY* expression.

Following the concerted action of positive modifiers of the *SRY* male-specific gene expression cascade, the testis determination starts. *SRY* signaling in the bipotential gonad acts as an initiator of pre-Sertoli cell differentiation and seminiferous tubule formation through the activation of *SRY-Box 9* (*SOX9*). This gene is a key pro-testis gene for the development of the normal male gonad. *SOX9* is expressed at low levels in both female and male sex, and its upregulation starts immediately with *SRY* expression in Sertoli cells. Sertoli cells are crucial for physiological testis development and for the subsequent Leydig cell differentiation, which are cells producing an essential male hormone, testosterone. After SRY-induced expression of *SOX9*, this protein keeps up its own expression due to autoregulation and positive feedback loops with *fibroblast growth factor 9* (*FGF9*), which activates *FGF receptor 2* (*FGFR2*), and *prostaglandin D2* (*PGD2*), encoded by *PTGDS*. *SOX9* directly inhibits *β-catenin* and indirectly, through *FGF9* which inhibits another female-specific gene, *WNT4*. Additional sex determination gene in human, which activates *SOX9*, *SOX8*, and *PTGSD* and inhibits the ovary-promoting genes, is the *doublesex gene in Drosophila and Mab3-related transcription factor 1* (*DMRT1*). One of the most important negative modifiers of *SOX9* expression is the *nuclear receptor DSSAHC critical region on the X chromosome 1* (*DAX1*, alias *NR0B1*) gene. It is predicted that yet unknown genes and co-factors contribute to the regulation of *SOX9*.

In conclusion, during sex determination, a complex interplay occurs between female and male fates, through a series of opposing feedback loops. Among them, the most crucial antagonism is between the *SRY/SOX9/FGF9* and *WNT4/RSPO1/β--catenin* signaling pathways [1].

Sex determination is followed by sexual differentiation. This process is characterized by sex steroid hormone secretion by the developing gonads, specific to the phenotypical sex, leading to the proper differentiation of the internal and external genitalia.

Differentiated Sertoli cells produce the anti-Müllerian hormone (AMH), which forces the regression of the Müllerian duct, while testosterone produced by Leydig cells induces the differentiation of the Wolffian duct derivatives into the epididymis, the vas deference, and the seminal vesicles. Furthermore, testosterone is converted—due to steroidogenic enzymes—into dihydrotestosterone (DHT), which is crucial for the normal development of the external genitalia and for virilization. The multifunctional *NR5A1* stimulates the expression of insulin-like polypeptide 3 (INSL3) together with maternal hCG and fetal LH. INSL3 is involved in the abdominal phase, whereas testosterone is necessary for the inguinal phase of testes descent.

## 1.2 Pathophysiology of Male Sex Determination and Sexual Differentiation

According to the Chicago Consensus Statement by Lee et al. (2006, 2016), disorders of sex development (DSD) are defined as congenital conditions within which the development of chromosomal, gonadal, or anatomic sex is abnormal [2, 3]. DSD can derive from defects in pathways related to sex determination or sex differentiation with a broad phenotypic spectrum.

The phenotypic representation of both 46,XY and 46,XX DSD patients depends on the etiology of the disorder and may range from female through male with ambiguous genitalia to male with normal virilization. Depending on the karyotype, disorders of gonadal development may be associated with testicular DSD (T-DSD), ovo-testicular DSD (OT-DSD), and complete/partial gonadal dysgenesis (GD) or be part of rare syndromic forms. Disorders related to androgen biosynthesis and action can be observed in both 46,XX and 46,XY DSD, whereas persistent Müllerian duct syndrome affects 46,XY DSD patients.

## 1.3 Clinical Manifestations of Mutations in Genes Involved in Sex Determination

### 1.3.1 46,XX Testicular/Ovo-Testicular DSD

The T/OT-DSD phenotype is largely dependent on the etiology of the disease. The 46,XX male syndrome—first reported by De la Chapelle et al. in 1964 [4]—is a rare, heterogeneous clinical condition with an incidence of about 1:20,000–25,000 male neonates [5]. The majority of 46,XX T-DSD cases are SRY+, with completely differentiated male external and internal genitalia in 85% of cases. Those who are *SRY* negative have ambiguous genitalia and poor virilization and OT-DSD. The incidence of OT-DSD is about 1:100,000 births, and children are usually reared as male due to the size of the phallus. OT-DSD is characterized by the presence of both ovarian and testicular tissues in the same gonad or less commonly in different gonads. Since the ovarian portion of OT is often functionally normal, breast development at puberty can occur due to follicular growth and estradiol production. Additional phenotypical alterations in 46,XX DSD are micropenis, hypospadias, cryptorchidism, and gynecomastia, due to atypical hormonal signaling. Short stature is a common remark, due to the absence of Y chromosome-linked growth control genes (Table 1.2). A constant clinical manifestation in these subjects is azoospermia with high FSH value. It is due to the lack of Y chromosome-linked AZF regions, where essential genes for sperm production are harbored. Depending on the entity of Leydig cell

dysfunction, testosterone level may range from normal to low, but in the majority of cases, LH is high.

**Genes Involved in "Male Pathway"**

- Sex Determining gene on Y (*SRY*)
  During paternal meiosis, the translocation of *SRY*-containing segments of the Y chromosome onto the X chromosome is the suggested genetic mechanism leading to the large majority (in about 90%) of XX male cases. These 46,XX male, SRY+ patients have usually small stature, gynecomastia, male external/internal genitalia, and decreased testis volume [1].
- SRY-Box genes (*SOX*)
  SRY-negative (SRY–) 46,XX male syndrome can be caused by autosomal gene mutations/over-expression, a gain of function in key testicular pathway genes, which are causing testis differentiation. Ectopic expression, achieved via duplication of ***SOX9***, is the most common mechanism in this group of patients. In addition, rarely, duplications of ***SOX3*** and ***SOX10*** have also been described both in mouse model and in human 46,XX male cases [6]. Both genes present high homology with *SOX9*; hence, the proposed pathogenic mechanism is based on their increased expression which mimics *SOX9* gene's function, leading to the initiation of testis development. These are extremely rare genetic defects with a broad phenotypic spectrum. The affected patients may present with either normal male external/internal or ambiguous genitalia, with T or OT-DSD.
- Fibroblast growth factor 9 (FGF9)
  Large duplication of the ***FGF9*** gene has been identified in a 46,XX *SRY*-negative male patient. This gene takes part of the male sex determination pathways; consequently, its increased dosage explains the observed male phenotype in the absence of *SRY*. The affected patient had male external genitalia with hypospadias and small testes [7].

**Genes Involved in the "Female Pathway": *R-Spondin 1* (*RSPO1*) and *Wnt Family Member 4* (*WNT4*)**
Loss-of-function mutations in the female pathway are associated with some exceptional forms of 46,XX sex reversal. Mutations in ***RSPO1*** and ***WNT4*** are related with rare autosomal recessive syndromes in human. Besides the abnormal gonadal phenotype, ***RSPO1*** is associated with palmoplantar hyperkeratosis (PPK) and predisposition to squamous cell skin cancer, whereas ***WNT4*** with SERKAL syndrome (SEx Reversal, Kidneys, Adrenal, and Lung dysgenesis) [8]. In addition, ***WNT4*** mutation was identified in a 46,XX DSD female patient, presented with primary amenorrhea, absence of Müllerian structures, unilateral renal agenesis, and clinical signs of androgen excess [1].

**Mutations in *NR5A1 Gene,* Involved in Both "Male" and "Female Pathways"**

- Nuclear receptor subfamily 5 group A, member 1 (*NR5A1* alias *SF-1*)
  Mutations in this gene represent one of the most frequently found genetic causes of DSD [9]. Since mutations within this gene can disrupt both the testicular and ovarian pathways, they are related to various gonadal development disorders, depending on the karyotype and other phenotypic modifiers (e.g., additional genetic variants). The clinical characteristics of ***NR5A1*** mutation carriers in 46,XY individuals could vary from oligo−/azoospermia to complete sex reversal (OT-DSD). While, at birth, patients show severely undervirilized external genitalia, the majority of them undergo spontaneous virilization during puberty, thanks to conserved Leydig cell function. However, oligozoospermic carriers are at potential risk for late-onset testosterone defect and progressive decrease of sperm production. For these reasons, they should cryopreserve sperm and perform regular endocrine follow-up. In the more severe phenotypes, in about 24% of cases, Müllerian duct derivatives are retained as a consequence of disturbed AMH production in the Sertoli cells. On the other hand, in 46,XX individuals, the phenotypic spectrum includes primary ovarian insufficiency (POI) and severe forms such as T-DSD or OT-DSD. Atypical genitalia are the most frequent presentation ranging from female genitalia to micropenis and penoscrotal hypospadias. In the minority of OT-DSD cases, gonads are not palpable, and the uterus or hemiuterus is present.

### 1.3.2 46,XY Gonadal Dysgenesis/Ovo-Testicular DSD

Impaired gonadal development in 46,XY karyotype is the consequence of mutations, causing under-expression or over-expression of different sex determination genes. It can result in a heterogenic group of conditions, which are characterized by a varying degree of abnormally configured and differentiated gonads, called as gonadal dysgenesis (GD). In 46,XY complete gonadal dysgenesis (CGD, alias Swyer syndrome or pure gonadal dysgenesis), the disrupted Sertoli cell formation and testis differentiation lead to inadequate levels of testosterone and AMH, hence disrupting the formation of the Wolffian ducts and internal genitalia and driving to Müllerian duct development. Therefore, normal female external genitalia with normal uterus and fallopian tubes can be seen, but the gonadal tissue is replaced with functionless, fibrous tissue, termed as streak gonads. These tissue clumps replace the testicular tissue and might vaguely resemble to ovarian stroma without follicles. In partial gonadal dysgenesis (PGD), the original tissue is retained to some extent. GD may occur unilaterally or bilaterally. Typical clinical findings are the delayed/absent puberty, lack of secondary sex characteristics, and infertility, due to primary amenorrhea (Table 1.1 and Fig. 1.2). The incidence of 46,XY CGD is in approximately 1:80,000 live births and together with 46,XY PGD is 1:20,000 live births. Depending on the underlying gene defect, 46,XY GD may also occur along with other pathologies and syndromes. The etiology is defined only in about 30–40% of cases and it can be due to *SRY*, *MAP3K1*, *SOX9* and other pro-testis gene defects as well as duplication of pro-ovary genes:

**Table 1.1** 46, XY due to disruption of genes involved in sex determination or steroidogenesis

| | Pathology | Pathophysiology | Genetics | External genitalia | Internal genitalia | Possible associated clinical findings |
|---|---|---|---|---|---|---|
| SEX DETERMINATION | **XY-female syndrome (complete form: Swyer sdr)** | Altered sex determination | Mutations/ deletions, which cause under-expression of: *SRY, SOX9, MAP3K1, NR5A1* (alias *SF1*), *CBX2, DHH, WT-1, DMRT1, GATA4, FOG2, FGFR2, ATRX;* Duplications, which cause over-expression of: *WNT4*, *RSPO, DAX1* (alias *NR0B1*); or unknown genetic factors | 46,XY: Female external genitalia | 46,XY: Normal uterus, fallopian tubes, with (complete/partial) gonadal dysgenesis (non-functional gonads) | Delayed/absent puberty, lack of/sparse secondary sex characteristics, lack of breast development, infertility (primary amenorrhea); osteoporosis/osteopenia; high risk of gonadal germ cell tumors; *WT-1:* Wilm's tumor; *DHH*: polyneuropathy; Syndromes: *SOX9*: Campomelic Dysplasia; *WT-1:* WAGR, Frasier and Denys-Drash sdr; *DMRT1:* Human 9p monosomy sdr; *GATA4* and *FOG2:* congenital heart defects; *FGFR2:* Craniosynostosis sdr; *ATRX*: ATR-X sdr; |

(continued)

**Table 1.1** (continued)

| | Pathology | Pathophysiology | Genetics | External genitalia | Internal genitalia | Possible associated clinical findings |
|---|---|---|---|---|---|---|
| SEXUAL DIFFERENTIATION | **Leydig cell hypoplasia** | Disruption of Leydig cell differentiation | Compound heterozygous or homozygous mutations of *LHCGR* | 46,XY: Complete: female external genitalia, +/− mild clitoromegaly or labial fusion; Partial: male external genitalia +/− micropenis, hypospadias | 46,XY: male gonads, +/− cryptorchidism | Complete form: absence of secondary sex characteristics, lack of breast development, infertility (primary amenorrhea); Partial form: partial virilization, fertility problems |
| | **7-DHC-R deficiency (Smith-Lemli-Opitz sdr)** | Disruption of cholesterol synthesis | Compound heterozygous or homozygous mutations of *DHCR7* | 46,XY: ambiguous genitalia, hypoplasic or bifid scrotum; micropenis and hypospadias/ female-like genitalia | 46,XY: male gonads, +/− cryptorchidism | Embryonic lethality; congenital malformations (holoprosencephaly, microcephaly, polydactyly, syndactyly) typical facial appearance, cardiovascular and gastrointestinal anomalies, renal agenesis, seizures, hypotonia, mental retardation; Normal puberty with normal hypothalamus, pituitary gland, and adrenal glands (HPA) axis function; in mild cases: fertility unknown |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **StAR deficiency** | Disruption of cholesterol transfer | Compound heterozygous or homozygous mutations of *STAR* | 46,XY: female or ambiguous genitalia with blind vaginal pouch/mild forms: micropenis | 46,XY: small testes, +/− cryptorchidism | lipoid CAH, salt wasting, hyponatremia, hypovolemia, hyperkalemia, acidosis, hyperpigmentation, death in infancy; no pubertal development; infertility |
| | **P450scc deficiency** | Impaired conversion of cholesterol into pregnenolone | Compound heterozygous or homozygous mutations of *CYP11A1* | 46,XY: female or ambiguous genitalia, undervirilization | 46,XY: male gonads, +/− cryptorchidism | Prematurity, severe forms: early-onset adrenal insufficiency without CAH, complete hypogonadism, milder forms: late-onset adrenal insufficiency without CAH, mild masculinization |
| | **POR deficiency** | Disrupted activity of P450 enzymes | Compound heterozygous or homozygous mutations of *POR* | 46,XY: ambiguous genitalia, undermasculinized external genitalia, hypospadias, micropenis | 46,XY: male gonads, +/− cryptorchidism | CAH; cortisol deficiency; arterial hypertension; delayed development; hypergonadotropic hypogonadism, PCOS; fertility unknown Antley Bixler Syndrome: skeletal malformations; intellectual disability, |

(continued)

**Table 1.1** (continued)

| | Pathology | Pathophysiology | Genetics | External genitalia | Internal genitalia | Possible associated clinical findings |
|---|---|---|---|---|---|---|
| | **3β-HSD2 deficiency** | Impaired aldosterone, cortisol and sex hormones production | Compound heterozygous or homozygous mutations of *HSD3B2* | 46,XY: ambiguous genitalia: micropenis, perineal hypospadias, bifid scrotum, blind vaginal pouch | 46,XY: male gonads | CAH with or without salt wasting; gynecomastia, fertility unknown |
| | **P450c17 deficiency (combined 17α-hydroxylase and 17,20-lyase deficiency)** | Disrupted conversion of pregnenolone into 17α-hydroxypregnenolone and DHEA | Compound heterozygous or homozygous mutations of *CYP17A1* | 46,XY:female-like or slightly virilized external genitalia, blind vaginal pouch; rarely ambiguous genitalia | 46,XY: hypoplastic male gonads; +/− cryptorchidism | Gynecomastia, sparse axillary/pubic hair; infertility (primary amenorrhea) arterial hypertension; hypokalemia |
| | **Isolated 17,20-lyase deficiency** | Enzymatic defect in testosterone biosynthesis | Compound heterozygous or homozygous mutations of *CYP17A1* | 46,XY: ambiguous genitalia with micropenis, perineal hypospadias; at puberty: poor virilization | 46,XY: hypoplastic male gonads, +/− cryptorchidism | Gynecomastia; normal glucocorticoid and mineralocorticoid production |
| | **17β-HSD3 deficiency** | Impaired conversion of androstenedione to testosterone | Compound heterozygous or homozygous mutations of *HSD17B3* | 46,XY: complete or predominantly female external genitalia, blind vaginal pouch, +/− bifid scrotum; at puberty: virilization | 46,XY: male gonads, +/− cryptorchidism | Gynecomastia; social sex change; normal glucocorticoid and mineralocorticoid production, fertility problems |

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|---|---|---|---|---|---|---|
| | **5α-RD2 deficiency** | Impaired conversion of testosterone to DHT | Compound heterozygous or homozygous mutations of *SRD5A2* | 46,XY: At birth: almost normal female genitalia/ ambiguous genitalia +/− blind vaginal pouch, bifid scrotum /undervilirized male genitalia +/− hypospadias or isolated micropenis; at puberty: virilization | 46,XY: male gonads; +/− prostate hypoplasia; cryptorchidism | Normal testosterone dependent secondary sexual characteristics at puberty (social sex change), fertility problems |
| | **Androgen Insensitivity syndrome (AIS): CAIS—Morris sdr; PAIS—Reifenstein sdr; MAIS—Mild Androgen Insensitivity sdr** | Androgen insensitivity | Mutations of AR (X chromosome linked) | 46,XY: **CAIS**: female phenotype **PAIS**: undervirilized male genitalia: micropenis, severe hypospadias, bifid scrotum; **MAIS**: normal male genitalia | 46,XY: **CAIS**: testes with cryptorchidism; **PAIS:** +/− (bilateral) cryptorchidism; **MAIS:** predominantly normal male gonads | **CAIS**: absent axillary/ pubic hair; infertility (primary amenorrhea); **PAIS**: gynecomastia; infertility (azoospermia); **MAIS:** fertility problems |
| | **Persistent Müllerian Duct Syndrome (PMDS)** | Impaired synthesis or action of AMH | Compound heterozygous or homozygous mutations of *AMH* or *AMHR2* | 46,XY: normal male appearance | 46,XY: Both female (uterus, fallopian tubes, upper vagina) and male internal gonads; +/− cryptorchidism | Fertility problems |

**Abbreviations**: *AMH* Anti-Müllerian hormone, *AMHR2* Anti-Müllerian hormone receptor type 2 gene, *AR* Androgen receptor, *CAH* Congenital adrenal hyperplasia, *CAIS* Complete androgen insensitivity syndrome, *PAIS* Partial androgen insensitivity syndrome, *MAIS* Mild androgen insensitivity syndrome, *PCOS* Polycystic ovary syndrome, *POR* Cytochrome P450 oxidoreductase, *P450scc* Cholesterol side-chain cleavage enzyme, *sdr.* syndrome, *SRY* Sex determining gene on Y chromosome, *StAR* Steroidogenic acute regulatory protein, *3β-HSD2* 3β-hydroxysteroid dehydrogenase type II, *5α-RD2* 5α-reductase type II, *7-DHC-R* 7-Dehydrocholesterol reductase enzyme, *17β-HSD3* 17 β-hydroxysteroid dehydrogenase type III

- Sex Determining gene on Y (*SRY*)
  In approximately 15–20% of affected patients, the cause is the mutation/deletion of the ***SRY*** gene on the Y chromosome. Most of the *SRY* mutations are located in the HMG box and are predominantly de novo mutations. Since the *SRY* gene is the initiator of male sex determination, the malfunction/absence of this gene results in a non-functional gonad with the consequent absence of testicular hormones. The phenotype is a female complete GD.
- Mitogen-Activated Protein Kinase Kinase Kinase 1 gene (MAP3K1)
  The dysregulation of MAPK pathway is implicated in the disrupted development of the gonads, in around 18% of cases. Deleterious mutations of ***MAP3K1*** can result in the under-expression of male pathway genes, since this gene has an important role to balance between *SOX9/FGF9* and *WNT/β-catenin* signaling. Carriers of ***MAP3K1*** mutations present female phenotype with GD.
- SRY-Box genes 9 (*SOX9*)
  As mentioned already, ***SOX9*** is expressed in the undifferentiated gonad of both sexes and is upregulated by the *SRY*; hence, it is evident that the loss-of-function mutations ca`n cause female sex development. This gene is also expressed in other tissues than the testis and involved in other processes, such as chondrogenesis, which can explain the consequent syndromic manifestation of ***SOX9*** mutations. Typically, campomelic dysplasia is associated with this gene mutation. This syndrome is characterized by distinctive facial features, severe skeletal malformations, and 46,XY GD.
- Wilms tumor 1 (*WT1*)
  The +KTS isoform plays a relevant role in testis determination. Diverse syndromes and diseases are associated with ***WT1*** deletions/mutations. The deletions may cause a group of pathologies, termed as WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation). Missense mutations in this gene can cause Denys-Drash syndrome, which is characterized by early diffuse mesangial sclerosis (DMS), Wilms tumor (nephroblastoma), and male pseudohermaphroditism (partial or complete gonadal dysgenesis) with a 46,XY karyotype. On the other hand, the absent production of +KTS due to mutations at the donor site of exon 9 leads to Frasier syndrome. Its typical manifestation is late-onset focal segmental glomerulosclerosis (FSGS) with complete gonadal dysgenesis, but without Wilms tumor.
- Other genes involved in 46, XY GD
  Mutations in other genes with essential function in male gonad development, such as ***DMRT1***, ***DHH***, ***CBX2***, ***NR5A1*** (alias ***SF-1***), ***GATA4***, ***FOG2***, ***FGFR2***, and ***ATRX***, have been rarely reported [10]. Some of them may cause syndromic form with severe anomalies. Mutations in ***DMRT1*** can cause human 9p monosomy syndrome, which is characterized by variable degrees of 46,XY GD, mental retardation, and craniofacial abnormalities. Mutation in ***FGFR2*** gene is associated with 46,XY GD and craniosynostosis. ATR-X syndrome, due to mutations of the ***ATRX*** gene, is associated with severe mental retardation, alpha thalassemia, typical facial appearance, skeletal malformations, and pulmonary, gastrointestinal, and urogenital anomalies.

- On the other hand, duplications in genes involved in the female pathway, such as ***DAX1*** (alias ***NR0B1***), ***WNT4***, and ***RSPO1***, have also been reported in the literature [10].

## 1.4 Clinical Manifestations of Mutations in Genes Involved in Sexual Differentiation

In contrast to the development of the female external genitalia, which does not require fetal ovarian hormones, the differentiation of male external genitalia requires the action of testosterone and its more potent derivative, DHT. Fetal Leydig cells produce testosterone staring from cholesterol through the sequential actions of various enzymes such as CYP11A1, CYP17A1, HSD3B2, and HSD17B3. In addition, adrenal gland also produces androgens. Noteworthy, some proteins are involved both in sexual determination and also in sexual differentiation, such as the above-mentioned *NR5A1* (alias *SF-1*), while others (e.g., *HSD3B2*, *HSD17B3*, *SDR5A2*) are expressed only during sexual differentiation.

Disorders associated with disrupted androgen production can arise from any step of testosterone synthesis in the testis or in the adrenal glands or from a defect in the conversion of testosterone to DHT in the peripheral tissues (e.g., genital organs and skin, prostate, hair follicles, liver, brain). Disorders of sexual differentiation show a broad spectrum of phenotypes, strongly depending from the underlying etiology. Frequent clinical finding is ambiguous external genitalia at birth; however, in milder forms, only undervirilized genitalia can be seen, which delays the diagnosis of DSD until puberty or even to adulthood (Tables 1.1, 1.2, 1.3 and Fig. 1.2).

### 1.4.1 Disorders of Androgen Biosynthesis: Reduced Androgen Levels

- 7-dehydrocholesterol reductase deficiency (Smith-Lemli-Opitz syndrome, SLOS) Loss-of-function mutations in the *DHCR7* gene can lead to an inborn error of cholesterol synthesis, due to 7-dehydrocholesterol reductase enzyme deficiency, which converts 7-dehydrocholesterol (7DHC) to cholesterol. The incidence of SLOS is approximately 1:20,000–40,000 per births, and it is most frequent in central and northern Europe. Its clinical manifestation is broad and variable, ranging from early embryonic lethality to different types of malformations and mental retardation postnatally. Typically, these patients may present holoprosencephaly, microcephaly (80% of cases) with a typical facial appearance, postaxial polydactyly of the hands or feet, syndactyly of the second and third toes (95% of cases), and short-proximally placed thumbs, as well as cardiovascular and gastrointestinal anomalies. Concerning genital development ambiguous genitalia, micropenis and hypospadias are observed in the majority of patients (70%) [11].

  *Hormonal diagnosis*: low plasma cholesterol and elevated plasma 7-dehydrocholesterol levels.

**Table 1.2** 46,XX DSD due to disruption of genes involved in sex determination or steroidogenesis

| | Pathology | Pathophysiology | Genetics | External genitalia | Internal genitalia | Possible associated clinical findings |
|---|---|---|---|---|---|---|
| SEX DETERMINATION | **XX-male syndrome (SRY+)** | Altered sex determination | *SRY* translocation | 46,XX: male external genitalia (85%), ambiguous genitalia | 46,XX: male gonads | Short stature, infertility (azoospermia) |
| | **XX-male syndrome (SRY-)** | | Duplications, which cause overexpression of: *SOX9*; *SOX3*; *SOX10*; *FGF9;* Mutations, which cause under-expression of*: RSPO1; WNT4; NR5A1* (alias *SF1*)*; WT-1;* | 46,XX: ambiguous genitalia, poor virilization, micropenis; penoscrotal hypospadias | 46,XX: testes/ ovotestis cryptorchidism | Short stature, gynecomastia, infertility (azoospermia); *RSPO1:* palmoplantar hyperkeratosis, Predisposition to squamous cell skin cancer; *WT-1:* Wilm's tumour; Syndromes: *WNT4:* SERKAL sdr, *WT-1:* WAGR sdr, Denys-Drash sdr, Frasier sdr |
| SEXUAL DIFFERENTIATION | **POR deficiency** | Disrupted activity of P450 enzymes | Compound heterozygous or homozygous mutations of *POR* | 46,XX: masculinized genitalia/ ambiguous genitalia/ clitoromegaly, labial fusion | 46,XX: female gonads | CAH; cortisol deficiency; arterial hypertension; delayed development; PCOS; unknown fertility, Antley Bixler Syndrome: skeletal malformations; intellectual disability, |
| | **21-OH deficiency** | Androgen excess | Compound heterozygous or homozygous mutations of *CYP21A2* | 46,XX: complete masculinization/ ambiguous genitalia/slight clitoromegaly | 46,XX: female gonads | CAH; failure to thrive; hyponatremia; hyperkalemia; acidosis; hypoglycemia; decreased height; precocious puberty; PCOS, excessive hair growth; acne; from normal to reduced fertility; |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **11β-OH deficiency** | Androgen excess | Compound heterozygous or homozygous mutations of *CYP11B1* | 46,XX: complete masculinization/ ambiguous genitalia/slight clitoromegaly | 46,XX: female gonads | CAH; low renin hypertension; hypokalemia; decreased height; precocious puberty; excessive hair growth; hirsutism; acne; PCOS, from normal to reduced fertility |

**Abbreviations**: *CAH* Congenital adrenal hyperplasia, *PCOS* Polycystic ovary syndrome, *POR* Cytochrome P450 oxidoreductase, *sdr.* Syndrome; *SRY* Sex determining gene on Y chromosome, *11β-OH* 11β-hydroxylase, *21-OH* 21-hydroxylase

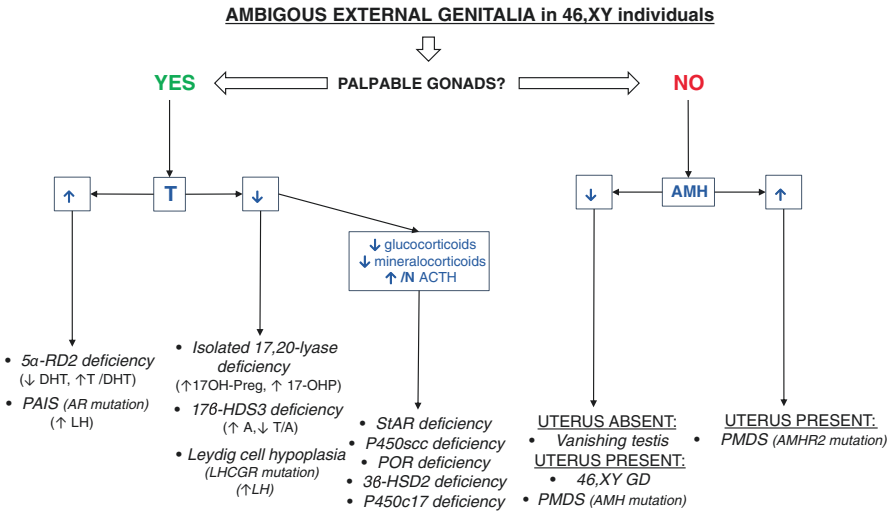


**Fig. 1.2** Hormonal features in 46,XY individuals with ambiguous external genitalia

- Congenital lipoid adrenal hyperplasia due to steroidogenic acute regulatory protein (StAR) deficiency
  Cholesterol transfer across the mitochondrial membrane to synthesize pregnenolone is mediated by the steroidogenic acute regulatory (StAR) protein. StAR phosphoprotein can be found in the mitochondria of the adrenal and gonadal cells. Mutations in the gene which encodes StAR result in lipoid congenital adrenal hyperplasia (lipoid CAH), the most severe form of CAH. It is an extremely rare, autosomal recessive condition, with a higher prevalence in Japanese, Korean, and Palestinian populations. The adrenal cortex and the gonads become engorged with cholesterol and cholesterol esters leading to mineralocorticoid, glucocorticoid, and sex steroid deficiency. The most severe form, adrenal insufficiency, manifests in salt wasting, hyponatremia, hypovolemia, hyperkalemia, and acidosis, which consequently lead to a life-threatening condition. Elevated adrenocorticotropic hormone (ACTH) levels due to intrauterine glucocorticoid deficiency can appear in generalized hyperpigmentation at birth. As far as the genital phenotype is concerned, in the case of complete defects, both 46,XY and 46,XX patients have female phenotype. 46,XY patients have testes, with or without cryptorchidism, and no Müllerian derivatives due to the presence of AMH. In the case of partial defect and 46,XY karyotype, genital ambiguity can occur.
  *Hormonal diagnosis*: elevated ACTH, renin and gonadotropin levels, with low basal values of glucocorticoids, mineralocorticoids, and androgens. In milder forms, partial steroid production can be measured.
- Cholesterol side-chain cleavage enzyme (P450scc) deficiency
  The following step of steroid biosynthesis is the conversion of the intramitochondrial cholesterol into pregnenolone by P450scc. This protein is encoded

by the *CYP11A1* gene and located in the mitochondria of the gonads and the adrenal glands. Compound heterozygous or homozygous mutations of *CYP11A1* gene can cause P450scc deficiency with a similarly severe phenotype like observed in patients with StAR loss-of-function mutations (congenital adrenal insufficiency with 46,XY DSD).

*Hormonal diagnosis*: high ACTH, renin, and gonadotropin levels, with undetectable or low values of glucocorticoids, mineralocorticoids, and androgens.

- Cytochrome P450 oxidoreductase (POR) deficiency

  POR is required for the activity of all P450 enzymes, such as P450c17, P450c21, and P450aro, which are involved in different steps of steroidogenesis. Homozygous or compound heterozygous mutations in the *POR* gene can lead to a rare form of CAH, due to combined deficiency of P450c17 and P450c21 and accumulation of steroid metabolites. It manifests in syndromic (Antley-Bixler syndrome) or non-syndromic form, with clinical features like cortisol deficiency, altered sex steroid synthesis, DSD, and skeletal malformations. The majority of individuals with POR deficiency have ambiguous genitalia at birth, which can occur in both sexes. 46,XX patients may present with masculinized genitalia, with enlarged clitoris and labial fusion, while 46,XY patients can present with undermasculinized external genitalia, with hypospadias and micropenis. In certain cases, complete sex reversal can occur. The unusual finding that both sexes can present with DSD suggests to an alternative pathway in human androgen synthesis, present only in fetal life, which explains the combination of antenatal androgen excess and postnatal androgen deficiency [12]. Typical congenital craniofacial and skeletal anomalies are, e.g., midface retrusion, craniosynostosis, hand and feet malformations, large joint synostosis, etc. Arterial hypertension may occur due to (mild) mineralocorticoid excess (Tables 1.1 and 1.2).

  *Hormonal diagnosis:* ACTH plasma concentration is normal or elevated, with normal or low cortisol serum concentration. Pregnenolone, progesterone, 17-hydroxypregnenolone (17OH-Preg), and 17-hydroxyprogesterone values are often elevated at baseline and/or after ACTH stimulation. Androgen serum concentration may be decreased and unresponsive to ACTH or hCG stimulation.

- 3β-hydroxysteroid dehydrogenase type II (3β-HSD2) deficiency

  The 3β-HSD enzyme has two isoforms, which share identity in around 93%. 3β-HSD1 is encoded by the *HSD3B1* gene and expressed mostly in the placenta and peripheral tissues. On the other hand, 3β-HSD2 is encoded by the *HSD3B2* gene and expressed exclusively in the adrenal glands, ovaries, and testes. 3β-HSD2 is not only essential for the synthesis of sex steroids acting on the conversion of dehydroepiandrosterone (DHEA) in androstenedione, but also for the synthesis of mineralocorticoids and glucocorticoids. *HSD3B2* mutations abolishing the enzyme activity lead to CAH with severe salt loss, whereas less severe mutations which reduce 3β-HSD2 enzyme activity result in CAH with mild or no salt wasting. In individuals with 46,XY karyotype, it leads to DSD, due to impaired androgen synthesis. 46,XY DSD patients present ambiguous genitalia,

characterized by micropenis, perineal hypospadias, bifid scrotum, and blind vaginal pouch. During puberty, gynecomastia is a common finding.
*Hormonal diagnosis*: The gold standard for biochemical diagnosis is measurement of the basal and post-ACTH serum 17OH-Preg and the 17OH-Preg/cortisol ratio. The serum levels of pregnenolone, 17OH-Preg, DHEA, and dehydroepiandrosterone sulfate (DHEAS) are elevated.

- P450c17 (combined 17α-hydroxylase and 17,20-lyase) deficiency
  The conversion of pregnenolone into 17α-hydroxypregnenolone and DHEA is catalyzed by the P450c17 steroidogenic enzyme. It is encoded by the *CYP17* gene, expressed in the endoplasmic reticulum of the adrenal cortex and of the gonads, and has both hydroxylation and lyase functions as well. Defect in the *CYP17* gene has been associated with combined 17α-hydroxylase and 17,20-lyase deficiency. Most of the affected subjects with 46,XY karyotype are phenotypically females with female-like or slightly virilized external genitalia, blind vaginal pouch, and cryptorchidism. Ambiguous genitalia have also been associated with this disease. The Wolffian derivatives are hypoplastic. During puberty, these patients usually present gynecomastia and sparse axillary/pubic hair. Commonly, they are assigned to female social sex and sought treatment due to primary amenorrhea or lack of breast development. High blood pressure and hypokalemia are frequent clinical findings.
  *Hormonal diagnosis*: typically, massive increase in the 17-deoxysteroid-corticosterone, deoxycorticosterone, and progesterone levels, with decreased aldosterone, cortisol, 17-OH-progesterone, androgen, and estrogen levels. Excessive production of deoxycorticosterone and corticosterone consequently leads to vascular hypertension, decreased levels of renin, and inhibition of aldosterone synthesis.
- Isolated 17,20-lyase deficiency
  Isolated 17,20-lyase deficiency due to mutation in *CYP17* gene may lead to diminished fetal testicular testosterone synthesis with intact cortisol production. 46,XY individuals affected by this disease have ambiguous genitalia with micropenis, perineal hypospadias, and cryptorchidism. During puberty, gynecomastia can occur.
  *Hormonal diagnosis*: elevated serum 17-OH-progesterone and 17OH-Preg levels, with low levels of androstenedione, DHEA, and testosterone.
- 17β-hydroxysteroid dehydrogenase type III (17β-HSD3) deficiency
  17β-Hydroxysteroid dehydrogenase (17β-HSD) acts on the final step of steroidogenesis. 17β-HSD enzyme has five isoenzymes. Most of them are involved in the estrogen balance, like type I, which is expressed in the ovary and converts estrone into estradiol. Type III (17β-HSD3) is an exception, because it is expressed exclusively in the testes and catalyzes the reduction of androstenedione to testosterone. Homozygous or compound heterozygous mutations in the *HSD17B3* gene cause the defect of 17β-HSD3 enzyme. It is also known as 17β-keto reductase deficiency and is characterized by impaired sex hormone production without impairing glucocorticoid and mineralocorticoid adrenal secretion. It is the most frequent disorder of androgen synthesis. 46,XY

newborns with 17β-HSD3 deficiency have complete or predominantly female external genitalia with a blind vaginal pouch, and the testes are often found in the inguinal canal or in a bifid scrotum. Despite the mainly female-like external genitalia, male internal genitalia, i.e., epididymides, vas deferens, seminal vesicles, and ejaculatory ducts, can be found. Most commonly, the affected males are raised as females. At the time of puberty, virilization occurs, and several patients with 17β-HSD3 deficiency change to male social sex. Testes can be safely maintained, as long as they are positioned inside the scrotum. The phenotype of the affected patients is clinically almost indistinguishable from other causes of 46,XY DSD such as partial androgen insensitivity syndrome (PAIS) or 5α-reductase type II deficiency (both of them will be discussed later), without biochemical assessment.

*Hormonal diagnosis*: low testosterone/androstenedione (T/A) and estradiol/estrone ratios, due to high serum levels of androstenedione and estrone, and low levels of testosterone. The cut-off value for T/A ratio is 0.8. In addition, hCG test has been proposed as a useful diagnostic tool, showing an increase of Δ4-A more than T [13].

### 1.4.2 Disorders of Androgen Biosynthesis: Androgen Excess

- 21-Hydroxylase (21-OHD) Deficiency

  The *CYP21A2* gene encodes the 21-hydroxylase enzyme (alias P450c21) which is responsible for the conversion of progesterone to deoxycorticosterone in the aldosterone biosynthesis pathway and 17α-hydroxyprogesterone to 11-deoxycortisol in the cortisol biosynthesis pathway. Severe or complete 21-hydroxylase (21-OHD) deficiency is the most common CAH. There are three forms of 21-OHD: (i) the classic salt wasting form, (ii) the classic simple virilizing form, and (iii) the non-classic form. The classic forms have an overall incidence in the Caucasian population of around 1:20,000–30,000 female newborns. The non-classic form is much more common, with an estimated 1:1000 frequency in the Caucasian population and even greater in other specific ethnic groups, such as Hebrew Ashkenazi (1:27), Hispanic (1:53), or Italians (1:300) [14]. Typical clinical findings in severe forms are hyponatremia, hyperkalemia, hyperreninemia, and hypovolemic shock. These patients can have a phenotype which is corresponding to the karyotype, but it can manifest with ambiguous genitalia or DSD as well. Excessive adrenal androgen production may lead from slight clitoromegaly to complete masculinization of the external genitalia in 46,XX individuals. In most severely virilized females, the social sex is often mistakenly assigned to male sex. Most 46,XY newborns affected by 21-OHD show normal appearance of the external genitalia at birth, and they have no clinical signs which could refer to their disease. Later in life, these patients may experience decreased height, precocious puberty, excessive hair growth, acne, and reduced fertility. Males with the non-classic type may present with early beard growth, enlarged penis, and small testes (Table 1.2).

*Hormonal diagnosis*: The excess 17-OH-progesterone, which represents the biochemical hallmark of this condition, is converted by the only available accessible pathway into potent androgens, such as testosterone and DHT. In addition, hyperandrogenism is due to the ready conversion of accumulated 17OH-pregnenolone to DHEA by *CYP17A1*.

- 11β-hydroxylase (11β-OHD) Deficiency
  *CYP11B1* gene serves for 11β-hydroxylase enzyme production, which is found in the adrenal glands, and converts deoxycorticosterone into corticosterone and 11-deoxycortisol into cortisol. Mutations of *CYP11B1* gene result in 11β-hydroxylase deficiency with CAH. Its estimated frequency is 1:100,000–200,000 newborns, and it is more common among Moroccan Jews living in Israel, affecting approximately 1:5000–7000 newborns. Among all CAH cases, around 5–8% is due to 11β-OHD [14]. The clinical manifestation of 11β-OHD is highly similar to 21-OHD, but with hypertension. The excess production of androgens leads to atypical sexual differentiation, especially in females. In the severe forms, 46,XX newborns can be markedly virilized, with ambiguous genitalia, but with normal internal female gonads. 46,XY fetuses typically show normal sexual development, often with megalopenis and precocious puberty (Table 1.2).
  *Hormonal diagnosis*: high levels of ACTH, 11-deoxycortisol, 11-deoxycorticosterone, DHEA, androstenedione, and testosterone.

### 1.4.3 Disorders of Testosterone Metabolism

- 5α-reductase type II (5α-RD2) Deficiency
  The conversion of testosterone to DHT is catalyzed by the 5α-reductase enzyme, which has two isoenzymes. Isoenzyme 1 is encoded by the SRD5A1 gene and expressed at low levels in embryonic tissues, whereas after birth and during later life, it is expressed in the brain, liver, and non-genital skin. Its role is not well defined yet. Isoenzyme 2, which is encoded by the SRD5A2 gene, is predominantly expressed in external genital tissues and in the prostate. DHT is specially needed for the physiological development of the prostate, penis, and scrotum. Homozygous or compound heterozygous loss-of-function mutations of the SRD5A2 gene lead to 5α-reductase type II (5α-RD2) deficiency, which is a rare autosomal recessive disorder, associated with 46,XY DSD. Patients with 5α-RD2 can have ambiguous genitalia, but also apparently normal female or undervirilized male genital phenotype at birth. In the case of female-like external genitalia, normal male internal reproductive structures are observed, often with prostate hypoplasia and undescended testicles. In subjects with predominantly male external genitalia, hypospadias or isolated micropenis has also been described. During puberty, patients exhibit virilization without breast development and change gender identity from female to male. It is pivotal to make the proper diagnosis in infancy by biochemical and molecular studies before gender assignment or any surgical intervention because these patients should be considered as males.

*Hormonal diagnosis*: in childhood low DHT serum levels, with elevated testosterone/DHT ratio after hCG stimulation test [13]. At puberty or in young adult men, the basal hormonal evaluation demonstrates normal/elevated serum testosterone levels with low DHT levels and normal/elevated testosterone/DHT ratio.

### 1.4.4 Disorders of Androgen Action

- Androgen Insensitivity syndrome (AIS): Morris Syndrome and Reifenstein Syndrome
  Mutations of the X chromosome-linked *androgen receptor* gene (*AR*) are associated with variable form of androgen insensitivity. Obviously, male phenotype could be seen only in milder forms of this condition, ranging from undervirilization to normal male genitals with diminished sperm production. The prevalence of AIS is depending whether the androgen insensitivity is complete (CAIS), partial (PAIS), or mild (MAIS). The prevalence of CAIS is 2–5/100,000 in subjects with 46,XY karyotype. CAIS (alias Morris syndrome) is associated with a female phenotype. Estrogen-dependent secondary sexual characteristics may develop in these subjects as a result of excess aromatization of androgens, but without pubic and axillary hair growth. The uterus, cervix, and proximal vagina are absent because of the action of AMH, produced by Sertoli cells during fetal life. PAIS is thought to be at least as frequent as CAIS. In PAIS (alias Reifenstein syndrome), the typical phenotype is micropenis with severe hypospadias, bifid scrotum, and cryptorchidism. In MAIS, the habitual clinical sign is infertility without associated genital anomalies.
  *Hormonal diagnosis*: Typical signs of hormone resistance are observed, i.e., serum testosterone values are either within or above the normal range for young or adult men, with inappropriately high LH levels. FSH and inhibin B levels are usually in the normal range. High androgen sensitivity index can be found (calculated as the product of serum testosterone levels multiplied by serum LH levels; e.g., high LH levels in the presence of relatively high testosterone levels might suggest mild resistance).

### 1.4.5 Disruption of Leydig Cell Differentiation

- Leydig cell hypoplasia
  Leydig cell differentiation from mesenchymal cells is essential for later testosterone synthesis in this cell type. Several genes are involved in the development of fetal Leydig cells, among them the *Desert Hedgehog* (*DHH*) and *NR5A1* (alias *SF-1*) genes. *DHH* is produced by the Sertoli cells and specifies the fetal Leydig cell (FLC) lineage in the primordial gonad through a paracrine signaling mechanism. *NR5A1* (alias *SF-1*) is expressed in Leydig cells, and based on mouse models, a combinatorial expression of *DHH* and *NR5A1* is needed for physiological

Leydig cell development and for the replacement of FLCs by adult Leydig cells. *NR5A1* is an essential protein for hormone biosynthesis. Leydig cells are stimulated by both hCG during fetal life and LH after birth. Both hormones act through the binding and activation of a common receptor (LHCGR), located in the cell membrane. Several different mutations in the *LHCGR* gene are also associated with 46,XY DSD due to Leydig cell hypoplasia.

It is a rare disorder with unknown prevalence. A typical clinical finding in patients with Leydig cell hypoplasia is the lack of intrauterine and pubertal virilization, due to the lack or to diminished testosterone secretion. In the case of a complete form, the typical phenotype is normal female external genitalia, leading to female sex assignment, despite the 46,XY karyotype. On the other hand, partial forms manifest with a wide spectrum of phenotypes. Most frequently, male external genitalia can be found with micropenis and/or hypospadias. Additionally, cryptorchidism may occur. During puberty, these subjects undergo partial virilization with mostly normal testis volume.

*Hormonal diagnosis*: The determination of serum testosterone level can be assessed by hCG stimulation test. These patients have subnormal testosterone response to hCG test prior puberty, whereas during and after puberty, the hCG test shows absent/impaired increase of all testicular androgens. Hormonal assessment shows elevated serum gonadotropin levels, with a clear predominance of LH over FSH levels.

### 1.4.6 Persistent Müllerian Duct Syndrome (PMDS)

Mutations in the gene encoding for AMH or for its receptor (AMHR-II) cause decreased levels or impaired action of AMH leading to the failure of Müllerian duct regression. Persistent Müllerian duct syndrome (PMDS) is an extremely rare condition with around 150 documented cases globally. It is also called as hernia uterine inguinale, because it is often diagnosed accidentally during hernia repair or during orchidopexy (type of surgical intervention, which brings the undescended testis into the scrotum). 46,XY carriers bear both male external genitalia and gonads (often undescended), with female internal genitalia (uterus, fallopian tubes, and upper vagina). PMDS is a complex and anatomically variable disease classified in three main phenotypic categories, depending on the position of the testes and the uterus. In the "female" type (60–70% of patients), the testes and epididymes are connected to the fallopian tubes in the abdomen and are in analogue positions to the ovaries. In the "hernia uterine inguinale" type (20–30% of patients), one of the testes may be found in the hernia sac or in the scrotum, while the other is located in the abdomen. Transverse testicular ectopia (TTE) refers to the condition, when the two testes are in the same hernia sac along with the uterus and uterine tubes. It occurs in about 10% of cases [15].

*Hormonal diagnosis*: show a corresponding picture of testicular failure, with high FSH and low testosterone levels. Mutated *AMH* leads to decreased AMH level. On the other hand, in the case of AMH receptor mutation, the AMH level is high. There is a testosterone response to hCG stimulation test (Table 1.3).

**Table 1.3** Hormone parameters in DSD according to the etiology

| FSH | LH | T | Additional relevant biochemical/hormonal features | | Diagnosis |
|---|---|---|---|---|---|
| ↑ | ↑ | ↓ | | ↓ AMH | **XY- gonadal dysgenesis/ ovo-testicular DSD (impaired sex determination)** |
| ↑ | ↑/N | ↓/N | | – | **XX- testicular/ ovo-testicular DSD (impaired sex determination)** |
| N | N/↑ | ↓ | ↑ ACTH ↓ glucocorticoids | ↓ cholesterol, ↑ 7-dehydrocholesterol, ↓mineralocorticoids | **7-DHC-R deficiency[a] (Smith-Lemli-Opitz sdr)** |
| ↑ | ↑ | ↓ | | ↓mineralocorticoids, ↑ renin | **StAR deficiency[a]** |
| ↑ | ↑ | ↓ | | ↓mineralocorticoids ↑ renin | **P450scc deficiency[a]** |
| ↑ | ↑ | ↓ | | ↑ pregnenolone, ↑ progesterone, ↑ 17OH-Preg, ↑17-OH-Progesterone | **POR deficiency[a]** |
| ↑ | ↑ | ↓/N | | ↑ pregnenolone, ↑ 17OH-Preg, ↑DHEA, ↑ DHEAS | **3β-HSD2 deficiency[a]** |
| ↑ | ↑ | ↓ | | ↑ corticosterone, ↑17-deoxysteroid-corticosterone, ↑ progesterone, ↓ 17-OH-Progesterone, ↓ renin | **P450c17 deficiency[a] (combined 17α-hydroxylase and 17,20-lyase deficiency)** |
| ↑/N | ↓ | ↑ | | ↑ 17OH-Preg, ↑ 17OH-Progesterone, ↓mineralocorticoids, ↑ DHEA | **21-OH deficiency[a]** |
| ↑/N | ↓ | ↑ | | ↑ 11 deoxycortisol and 11 deoxycortisosterone, ↑ DHEA | **11β-OH deficiency[a]** |
| ↑ | ↑ | ↓ | | ↑ 17OH-Preg, ↑ 17-OH-Progesterone, ↓ androstenedione, ↓ DHEA | **Isolated 17,20-lyase deficiency** |
| ↑ | ↑ | ↓ | | ↑ androstenedione, ↑ estrone; T/Androstenedione ↓ | **17β-HSD3 deficiency** |
| N | ↓/N | ↑/N | | ↓ DHT; T/DHT↓ | **5α-RD2 deficiency** |
| ↑/N | ↑ | ↑/N | | – | **Androgen Insensitivity syndrome** |
| ↑ | ↑↑ | ↓/N | | – | **Leydig cell hypoplasia** |
| ↑ | ↑ | ↓ | | *AMH* mutation: ↓ AMH, *AMHR2* mutation: ↑AMH | **Persistent Müllerian Duct Syndrome (PMDS)** |

**Abbreviations:** *ACTH* Adrenocorticotropic hormone, *AMH* Anti-Mullerian hormone, *AMHR2* *Anti-Mullerian hormone receptor 2* gene, *DHEA* Dehydroepiandrosterone, *DHT* Dihydrotestosterone, *FHS* Follicle stimulating hormone, *LH* Luteinizing hormone, *N* Normal, *POR* Cytochrome P450 oxidoreductase, *P450scc* Cholesterol side-chain cleavage enzyme, *StAR* Steroidogenic acute regulatory protein, *T* Testosterone, *3β-HSD2* 3β-hydroxysteroid dehydrogenase type II, *5α-RD2* 5α-reductase type II, *7-DHC-R* 7-Dehydrocholesterol reductase enzyme, *11β-OH* 11β-hydroxylase, *17β-HSD3* 17 β-hydroxysteroid dehydrogenase type III, *17OH-Preg* 17-hydroxypregnenolone, *17-OH-Progesterone* 17-hydroxyprogesterone, *21-OH* 21-hydroxylase

[a]Depending on the severity of the enzymatic defect ACTH, glucocorticoids and mineralocorticoids maybe normal

## 1.5 Diagnosis and Clinical Management of DSD Patients

DSD is a rare endocrine disease with low number of affected individuals for each etiological category. This peculiarity together with the highly heterogeneous phenotypes implies that the diagnosis is complex and requests special expertise. The diagnostic work-up of this condition includes extensive **physical examination**, with **assessment of the genitals**, **biochemical** and **genetic analyses**, **imaging studies**, and in some cases **surgical exploration** (e.g., laparoscopy, gonadal biopsy) for the confirmation. Biochemical assessment is highly informative about the underlying pathology, and it is essential in order to plan appropriate hormonal treatments. If it is possible, chromatographic and mass spectrometric methods are recommended for exact steroid hormone measurements, although these methods are not yet widely available and standardization between laboratories is an issue. The ideal diagnostic evaluation should include genetic analyses since the identification of the genetic defect implies the possibility of individualized patient's care. The genetic diagnosis is achieved in the majority of 46,XX males, whereas in 46,XY DSD patients, the underlying genetic cause remains unknown in about 70% of cases. Regular follow-up is advised to these patients due to the frequent occurrence of comorbidities and increased incidence of malignancies.

The results of the diagnostic tests need to be discussed within a multidisciplinary team in order to provide a holistic approach to the affected individuals. The management is based on the strict collaboration of endocrinologists, urologists, gynecologists, andrologists, clinical geneticists, psychologists, nurses, and social workers.

***Key Points in the General Clinical Approach*** *Medical treatment options:* Gonadal function is compromised in the large majority of cases. Therefore, **sex hormone therapy** is necessary for pubertal induction and for hormone replacement therapy (HRT) during life. HRT serves to enhance gender identity and to induce or reinforce secondary sex characteristics, as well as for general well-being and to avoid cardiovascular and bone comorbidities (osteopenia/osteoporosis) later in life. It is important to state that both gender identity and sexual orientation seem to be determined during fetal development, under the influence of sex hormone production by the developing gonad. This is especially relevant in pathologies such as 5α-RD2 and 17β-HSD3 deficiency, where virilization occurs at puberty (if the gonads were not removed in childhood) with the consequent female-to-male social sex change. These patients, during their fetal life, are exposed to normal testosterone levels which leads to the virilization of the brain. Hence, if female sex is assigned, it will likely lead to distress related to gender dysphoria [16, 17]. It is therefore extremely important to diagnose this genetic defect as early as possible in order to avoid the removal of the testes and raise these individuals as boys [18].

HRT is based on estrogen or testosterone substitution, depending on the patients' social sex. Pubertal induction should be performed usually at the age of 10–12 in girls and 11–13 in boys, and the standard therapeutic regimen should simulate physiological puberty. In the case of patients with female gender identity, with or

without uterus, the hormonal substitution starts with low-dose estrogen (e.g., 0.07–0.15 mg/day orally), and this dose should be gradually increased every 6 months over 2–3 years. The maintenance dose is, for example, 0.625 mg/day of conjugate estrogen, with additional progesterone substitution (e.g., 50 mg/day, from the first to the 12th day of the month) to induce menstruation. Progesterone therapy is not required in patients without uterus. Low-dose transdermal estrogen treatment is also a viable option for HRT. Anti-androgens are used as adjuvants to estrogen, especially in the reduction of male sexual characteristics and the suppression of testosterone to female levels [10].

For patients with male gender identity, available options are intramuscular testosterone ester depot injections, oral testosterone undecanoate, and transdermal preparations. The initial dose of depot injections is 25–50 mg/month, while the maintenance dose in adults is 150–250 mg every 2–4 weeks or 1000 mg every 3 months [10]. The medical treatment for micropenis is high-dose testosterone therapy (e.g., 500 mg of testosterone cypionate injection/week). Maximum increase of penile length can be obtained after 6 months of therapy. Hormonal therapy is used for pubertal suppression in DSDs associated with precocious puberty.

Patients with CAH require lifelong **glucocorticoid** and often additional **mineralocorticoid replacement therapy**. In the case of SLOS, the primary therapeutic approach focuses on **cholesterol supplementation**, to enhance cholesterol production and/or accretion, and to decrease the accumulation of potentially toxic cholesterol precursors. The most widely accepted forms of dietary cholesterol supplementation are egg yolks and/or crystalline or powder form cholesterol suspension.

*Gender assignment:* Gender assignment is a difficult and critical decision in the management of DSDs. This process should be managed in reference centers by experienced teams. They should take into account, among others, cultural and religious factors and the impact of the decision on the adult life of the affected individuals (e.g., gender dysphoria, drastic surgical interventions, dissatisfaction with the appearance of genitalia). The current criteria for gender assignment are based on (i) the psychosexual outcomes in adults, (ii) the potential for fertility, and (iii) the available hormonal and surgical options [17].

*Surgical treatment options*: The aim is to repair sexual ambiguity, with the removal of the internal structures, which are inadequate for the patient's social sex and to construct a normal-looking, appropriate external genitalia. There is a growing tendency to postpone surgery, until the patient will be able to take part in the decision-making. If surgery is performed at early ages, mutilating and irreversible interventions should be avoided. Depending on the patients' desired gender, the surgical procedures consist of procedures like phalloplasty, scrotoplasty, resection of the vaginal pouch, proximal/distal urethroplasty, orchidopexy, vaginoplasty, excision of Müllerian duct remnants, and breast surgery. Apart from AIS patients, all ectopic male gonads are at increased risk for malignancies and need to be mobilized to an extra-abdominal, inguinal, or preferably scrotal position to allow monitoring by self-examination and imaging. If this relocation fails, surgical removal of the gonad should be considered. In the case

of 46,XY GD, gonadal streaks even more often become cancerous; therefore, they are usually removed surgically as the diagnosis occurs [19]. The timing of gonadectomy is controversial but will mainly depend on the anticipated malignancy risk of the gonad. As the risk is very low at pre-pubertal age, the final decision on gonadectomy usually can be deferred, if the gonad can be monitorized safely. In the case of undesired masculinizing or feminizing effects of sex steroids, which is in discordance with the social gender identity, prior intervention may be carried out. Correction of hypospadias is advised early, in the first 2 years of life.

*Fertility:* Rarely, DSD individuals with (partially) functional testicles may undergo **assisted reproductive techniques** (ART). For instance, **microdissection testicular sperm extraction** (micro-TESE) may offer possibility to find sperm in the testis. The harvested spermatozoa may be cryopreserved and used for later **intracytoplasmic sperm injection** (ICSI). If the atypical gonad does not contain gametes, ART with donor sperm can be taken into consideration. In the case of 46,XY DSD with female phenotype and with uterus, successful pregnancies following egg donation have been described. Adoption, as a final option for the couple, should be discussed with the patient and his/her partner.

## 1.6 Conclusions

The understanding of the genetic aspects and of the molecular mechanisms behind human sex development and DSD has been greatly improving over the past decades. It became clear that mutations in the same gene could be associated with a spectrum of phenotypes suggesting a role for modifier genes and oligogenic inheritance. In cases where clinical and biochemical exams are clearly indicating a distinct gene defect (e.g., 21-OHD, AIS), targeted gene sequencing is the usual approach. Following the diffusion of novel sequencing technologies (massive parallel sequencing), genetic testing based on gene panel is becoming the first-level test allowing the diagnosis of both monogenic and oligogenic cases. However, despite technological advances and progresses in our knowledge about the biology of DSD, still many new genes need to be discovered. The identification of the genetic basis for each DSD case is pivotal for the clinical management. Many gaps in our knowledge concerning this heterogeneous group of rare pathologies are currently addressed through large-scale networks. Thanks to the worldwide interaction between researchers and clinicians, major advancements in the management of social and clinical aspects are expected in the near future.

## References

1. Knarston I, Ayers K, Sinclair A. Molecular mechanisms associated with 46,XX disorders of sex development. Clin Sci. 2016;130(6):421–32.
2. Lee PA, Houk CP, Ahmed SF, Hughes IA. International consensus conference on intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. International consensus conference on intersex. Pediatrics. 2006;118(2):e488–500.

3. Lee PA, Nordenström A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al. Global disorders of sex development update since 2006: perceptions, approach and care. HRP. 2016;85(3):158–80.
4. de la Chapelle A, Hortling H, Niemi M, Wennström J. XX sex chromosomes in a human male. Acta Med Scand. 1964;175(s412):25–38.
5. Vorona E, Zitzmann M, Gromoll J, Schüring AN, Nieschlag E. Clinical, endocrinological, and epigenetic features of the 46,XX male syndrome, compared with 47,XXY Klinefelter patients. J Clin Endocrinol Metab. 2007;92(9):3458–65.
6. Baetens D, Verdin H, De Baere E, Cools M. Update on the genetics of differences of sex development (DSD). Best Pract Res Clin Endocrinol Metab. 2019;33(3):101271.
7. Chiang H-S, Wu Y-N, Wu C-C, Hwang J-L. Cytogenic and molecular analyses of 46,XX male syndrome with clinical comparison to other groups with testicular azoospermia of genetic origin. J Formos Med Assoc. 2013;112(2):72–8.
8. Baxter RM, Vilain E. Translational genetics for diagnosis of human disorders of sex development. Annu Rev Genomics Hum Genet. 2013;14:371–92.
9. Domenice S, Machado AZ, Ferreira FM, Ferraz-de-Souza B, Lerario AM, Lin L, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. Birth Defects Res C Embryo Today. 2016;108(4):309–20.
10. Domenice S, Arnhold IJP, Costa EMF, Mendonca BB. 46,XY disorders of sexual development. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al., editors. Endotext [Internet]. South Dartmouth, MA: MDText.com, Inc.; 2000. http://www.ncbi.nlm.nih.gov/books/NBK279170/.
11. Mendonca BB, Costa EMF, Belgorosky A, Rivarola MA, Domenice S. 46,XY DSD due to impaired androgen production. Best Pract Res Clin Endocrinol Metab. 2010;24(2):243–62.
12. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet. 2004;363(9427):2128–35.
13. Bertelloni S, Russo G, Baroncelli GI. Human chorionic gonadotropin test: old uncertainties, new perspectives, and value in 46,XY disorders of sex development. Sex Dev. 2018;12(1–3):41–9.
14. Baronio F, Ortolano R, Menabò S, Cassio A, Baldazzi L, Di Natale V, et al. 46,XX DSD due to androgen excess in monogenic disorders of steroidogenesis: genetic, biochemical, and clinical features. Int J Mol Sci. 2019;20(18)
15. Ren X, Wu D, Gong C. Persistent Müllerian duct syndrome: a case report and review. Exp Ther Med. 2017;14(6):5779–84.
16. Fisher AD, Ristori J, Fanni E, Castellini G, Forti G, Maggi M. Gender identity, gender assignment and reassignment in individuals with disorders of sex development: a major of dilemma. J Endocrinol Invest. 2016;39(11):1207–24.
17. Guerrero-Fernández J, Azcona San Julián C, Barreiro Conde J, de la Vega JA B, Carcavilla Urquí A, Castaño González LA, et al. Management guidelines for disorders/different sex development (DSD). Anales de Pediatría (English Edition). 2018;89(5):315.e1–315.e19.
18. Bertelloni S, Scaramuzzo RT, Parrini D, Baldinotti F, Tumini S, Ghirri P. Early diagnosis of 5alpha-reductase deficiency in newborns. Sex Dev. 2007;1(3):147–51.
19. Wolffenbuttel KP, Hersmus R, Stoop H, Biermann K, Hoebeke P, Cools M, et al. Gonadal dysgenesis in disorders of sex development: diagnosis and surgical management. J Pediatr Urol. 2016;12(6):411–6.