# Variants of Sex Development

# 31

Isabel Viola Wagner and Olaf Hiort

## Contents



I. V. Wagner · O. Hiort (✉)
Department of Pediatric Endocrinology and Diabetology,
University Hospital of Schleswig-Holstein, Hospital for Childrens' and Youth Medicine, Lübeck, Germany
e-mail: IsabelViola.Wagner@uksh.de; olaf.hiort@uksh.de


E. Nieschlag et al. (eds.), *Andrology*, https://doi.org/10.1007/978-3-031-31574-9_31

**Abstract**

Variations (formerly "disorders") of sex development (DSD) describe congenital conditions with atypical constellations between the chromosomal, the gonadal, and the phenotypic sex. This definition encompasses a variety of clinical entities and is not strictly defined regarding its inclusive- and exclusiveness, because the classification allows for both a clinical and a (molecular-) genetic nomenclature and classification. In addition, the DSD complex is associated with a concept of clinical care and medical management, as affected persons may have a very special gender self-perception, which may impose a sociocultural challenge. In the following chapter, we therefore introduce the present classification and the current discussion about medical management before we explain defined conditions.

## 31.1 Introduction

The definition of variants (originally "disorders") of sex development (**DSD**) was established in 2005 at a consensus conference of major pediatric endocrine societies held in Chicago in order to deal with a mismatch between chromosomal, gonadal, and phenotypic sex. The intention behind this was to replace the previously used terms such as **intersexuality** or **(pseudo) hermaphroditism,** which were thought to be pejorative**.** However, it is precisely this that has sparked a social dispute, as the definition is imprecise and is also unrelated to a sociocultural context of the self-perception of those affected. Therefore, in many countries, especially in Western countries, a broader view has been established, which on the one hand is based on the biological definition, but on the other hand also associates DSD with a special, medically oriented care concept within the social context of gender diversity.[1]

## 31.2 Nomenclature and Classification

The definition of DSD allows for the inclusion of a broad spectrum of possible biological variants of sex development. According to current knowledge, DSD is characterized by a dynamic in the developmental biological processes of sex development. These in turn allow a range of expression of sex and gender differentiation. What is known is the initial omnipotence of the embryo, which does not prescribe any differentiation or categorization of sex. Only with the **differentiation and development of the gonadal system** under the control of a complex genetic regulation into a testicular or ovarian orientation are the main directions "male" or "female" predetermined. With further development, a **dominance of testicular hormone synthesis is** assumed, which causes the formation of the internal and external genital structures via transcriptional regulation. Ultimately, these regulatory events allow a high degree of variability, which can also be seen within the reproductively relevant categories of "male" and "female." DSD as variants of sex development therefore has diverse characteristics, which will certainly also have to be defined more accurately in the future.

### 31.2.1 The Classification of DSD

Consideration of the biological **classification of DSD** arose from the understanding of the genetic and hormonal cascades of sex development in their spatiotemporal context and should allow a diagnostic classification. It does not serve the gender categorization of affected persons, which must follow individually, considering a variety of other biological-medical but also sociocultural aspects.

The classification follows the developmental biological understanding that the usual direction is given with the determination of chromosomal sex. Thus, a classification is first made into the categories of numerical chromosomal inconsistencies and **46,XX or 46,XY DSD**. According to this classification, Klinefelter syndrome and Ullrich-Turner syndrome also belong to the group DSD forms even though Klinefelter syndrome is a particularly frequent variant (see Chap. 21) and certainly both Klinefelter and Ullrich-Turner syndromes require different approaches to medical care than is assumed for many other forms of DSD. Other numerical abnormalities include mixed gonadal dysgenesis with **45,X/46,XY karyotype** and the rare cases of **46,XX/46,XY chimerism**.

Within the numerically inconspicuous categories 46,XX and 46,XY DSD, a distinction is then made between congenital abnormalities of gonad development and tangible

[1]For reasons of better readability, the masculine form is used for personal names and personal nouns in this chapter. Corresponding terms apply in principle to all genders for the purpose of equal treatment.

disorders of androgen biosynthesis (with increased or decreased androgen formation) or androgen action. This subdivision allows future inclusion of new findings of molecular genetic or biochemical causes in the main categories. In addition, however, clinical descriptions of genital abnormalities not yet defined at the molecular genetic level, such as pronounced **hypospadias** or complex malformations such as **cloacal exstrophy,** are also permitted in each main category. Table 31.1 summarizes the classification according to consensus.

This categorization emphasizes that it has not yet been clearly defined whether every genital abnormality should be classified as DSD. For example, isolated unilateral undescended testis is certainly a genital abnormality, but is not counted as DSD according to current interpretation. Similarly, it is not clear to what degree hypospadias should be classified as DSD. On the other hand, a clear separation should be made from **gender dysphoria/transgender identity** entities. An update of the DSD consensus from 2016 draws the line at pronounced **hypospadias in combination with cryptorchidism** and gives the **frequency for DSD as** about 1:4000–5000 (Lee et al. 2016). However, this certainly does not provide satisfactory and exact clarification. Further consensus work is needed on this topic.

**Table 31.1** Classification of DSD (modified according to Hughes et al. 2006)

| DSD due to numerical aberrations of the sex chromosomes | 46,XY-DSD | 46,XX-DSD |
|---|---|---|
| **A: 47,XXY** | **A: Disorders of gonadal/testicular development** | **A: Disorders of gonadal/ovarian development** |
| Klinefelter syndrome and variants | • Ovotesticular DSD | • Gonadal dysgenesis |
| **B: 45,X** | • Complete or partial gonadal dysgenesis (e.g., SRY, SOX9, SF1, GATA4, WT1, DHH, WNT4 duplication, DAX1 duplication, etc.) | • Ovotesticular DSD |
| Ullrich-turner syndrome and variants | • Gonadal regression | • Testicular DSD (e.g., SRY+, SOX9 duplication) |
| **C: 45,X/46XY mosaic** | **B: Disorders of androgen biosynthesis or androgen action** | **B: Androgen excess** |
| Mixed gonadal dysgenesis | • Disorders of androgen biosynthesis | • Fetal androgen excess |
| **D: 46,XX/46XY** | – LH receptor mutations | – 3 β-Hydroxy-steroid dehydrogenase type-2 |
| Chimerism | – Smith-Lemli-Opitz syndrome | – 21-hydroxylase |
| | – Steroidogenic acute regulatory protein (STAR) | – P450 oxidoreductase |
| | – P450 side chain cleavage (SCC) | – 11 β-hydroxylase |
| | – 3 β-Hydroxysteroid dehydrogenase type-2 | – Glucocorticoid resistance |
| | – 17 α-hydroxylase/17,20-lyase | • Fetoplacental androgen excess |
| | – P450 oxidoreductase | – Aromatase deficiency |
| | – 17 β-Hydroxysteroid dehydrogenase type-3 | – P450 oxidoreductase |
| | – 5-Reductase type-2α | • Maternal androgen excess |
| | • Disorders of androgen action | – Virilizing tumor (luteoma) |
| | – Complete and partial androgen resistance | – Application of androgenic substances |
| | – Endocrine disruptors | **C: Other** |
| | **C: Other** | • Syndromal forms |
| | • Syndromal forms | – Cloacal malformations |
| | – Cloacal malformations | – Et al. |
| | – Aarskog syndrome | • Agenesis/ hypoplasia of the Müller structures (MURCS) |
| | – Hand foot genital syndrome (HOXA13) | • Vaginal atresia (McKusick-Kaufmann syndrome) |
| | – Robinow syndrome | • Labial synechiae |
| | – Et al. | • Etc. |
| | • Persistent Müllerian duct syndrome (AMH and AMH receptor disorders) | |
| | • Vanishing testis syndrome | |
| | • Isolated hypospadias | |
| | • Cryptorchidism | |
| | • Etc. | |

## 31.3 Clinical Examination and Medical Classification

Any suspicion of a congenital abnormality of sex development requires a systematic patient history, especially with regard to family characteristics, previous surgeries, and the onset and course of pubertal development. According to the classification, DSD covers a very **broad phenotypic spectrum of genital findings**, only some of which become conspicuous during childhood. These include the obvious findings of ambiguous genitalia at birth, or a deviation of the phallic structures from the usual reference, an unusual localization or absence of gonadal structures, an only partial fusion of the labioscrotal structures, and a deviating localization of the meatus urethrae. In the course of time, various scoring systems have been used for the complete recording of a genital finding in childhood, which, however, had the disadvantage that they could not be used for the range of gender expression, but were oriented toward a "male" or "female" gender. Thus, the **Prader score** describes genital abnormalities in 46,XX adrenogenital syndrome (Prader 1945) and the **Quigley score** describes the range of 46,XY androgen resistance (Quigley et al. 1995). Recently, this shortcoming was overcome by the European definition of an **External Genitalia Score (EGS),** which attempts to establish a common description (van der Straaten et al. 2020) (Table 31.2).

Another possibility for the clinical evaluation of a genital abnormality in childhood is the determination of the **anogenital distance**. It is considered a sensitive measure of androgen action during embryonic development and is sexually dimorphic. For this purpose, the distance of the anus from various genital structures is measured (Fig. 31.1).

Some affected persons do not come to medical attention until adolescence or even adulthood. In this case, special attention should focus on the sex-related physiognomy and on the (secondary sex characteristic) hair structure and its distribution pattern, breast development, and fat distribution. In general, a **complete physical examination is** always part of a clinical diagnosis when a variant of sex development is suspected, which should describe any atypical findings, since some forms of DSD are associated with other malformations of the skeletal system, the internal organs, especially the heart and kidneys, or the nervous system.

Adults with DSD have been paid little attention in terms of their healthcare needs. However, the European study DSDLife, as well as those nationwide studies of the German network "Intersexuality"(from 2003 to 2008), found a high dissatisfaction with the medical care of 46.XY DSD people (Thyen et al. 2014). This may be due to the special issues and needs of this patient group. Among other things, this concerns **hormone substitution** during puberty and adulthood. There is little reliable data on this. Recently, we were able to show that in 46,XY women with complete androgen resistance, therapy with both estrogens and androgens can be considered safe, but testosterone therapy given at the usual male substitution dose leads to a significant improvement in libido (Birnbaum et al. 2018). Whether and what conditions need to be considered for the initiation of puberty as well as hormone replacement in adults living in the male gender is currently unresolved. Similarly, questions about **fertility options have** not yet been addressed or have only been addressed hypothetically. In 2015, the German Medical Association issued a detailed statement on this issue (Bundesärztekammer 2015). Currently, guideline-based care for individuals with DSD in Germany is being addressed by the Federal Ministry of Health and the quality indicators for the provision of specialized centers are to be developed and reviewed in a "DSD-care" research project (Jürgensen et al. 2021).

In addition, challenging disease entities sometimes occur, e.g., prostatitis is conceivable in a 46,XY woman with an androgen biosynthesis defect. How should both medical and holistic care be provided in such cases? A frequently discussed topic is the possible tumor formation and its status in 46,XY DSD. Only sparse epidemiological data are available

**Table 31.2** The "External Genitalia Score" describes the clinical findings at five anatomical sites of the genitals: the extent of the labioscrotal fusion, the length of the genital tubercle (GT), the position of the urethral opening, and the localization of the right and left gonads

| External genitalia score | Labioscrotal fusion | Length of the genital tubercle (mm) | Urethral opening | Right gonad | Left gonad |
|---|---|---|---|---|---|
| 3 | Merged | >31 | At the top of the GT | | |
| 2.5 | | 26–30 | Coronary | | |
| 2 | | | On the shaft | | |
| 1.5 | Partial fusion posterior | 21–25 | At the base of the GT | Labioscrotal | Labioscrotal |
| 1 | | 10–20 | Labioscrotal | Inguino-labioscrotal | Inguino-labioscrotal |
| 0.5 | | | | Inguinal | Inguinal |
| 0 | Not merged | <10 | Perineal | Not palpable | Not palpable |

The final score is calculated from the sum of the points from all five findings
*EGS,* external genitalia score; *G,* genital tubercle
According to van der Straaten et al. (2020)

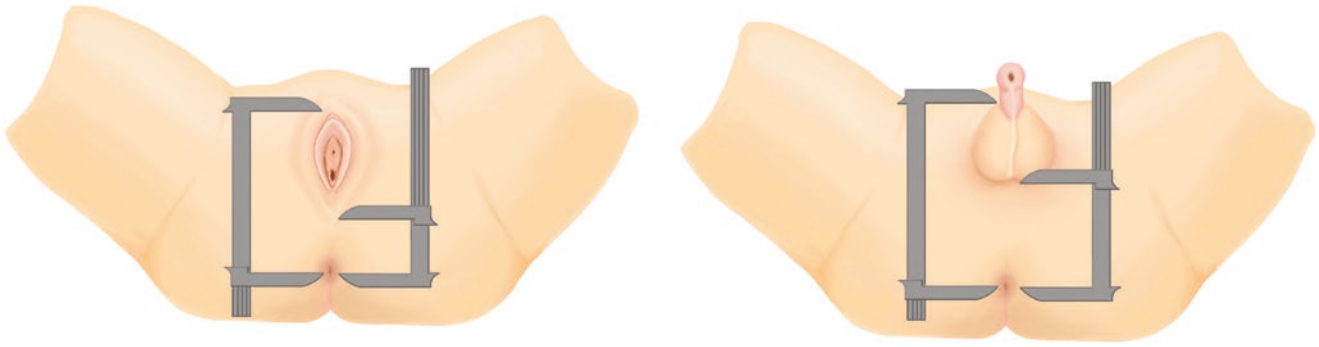

**Fig. 31.1** Measurement of the anogenital distance (Thankamony et al. 2016). For this purpose, the distance from the anus to the posterior commissure of the labioscrotal folds is measured, as well as the distance from the posterior commissure to the root of the phallus. The greater the ratio of the lower to the upper measurement, the more androgen effect can be assumed. (drawn by Cand. Med. Mirkka Hiort)

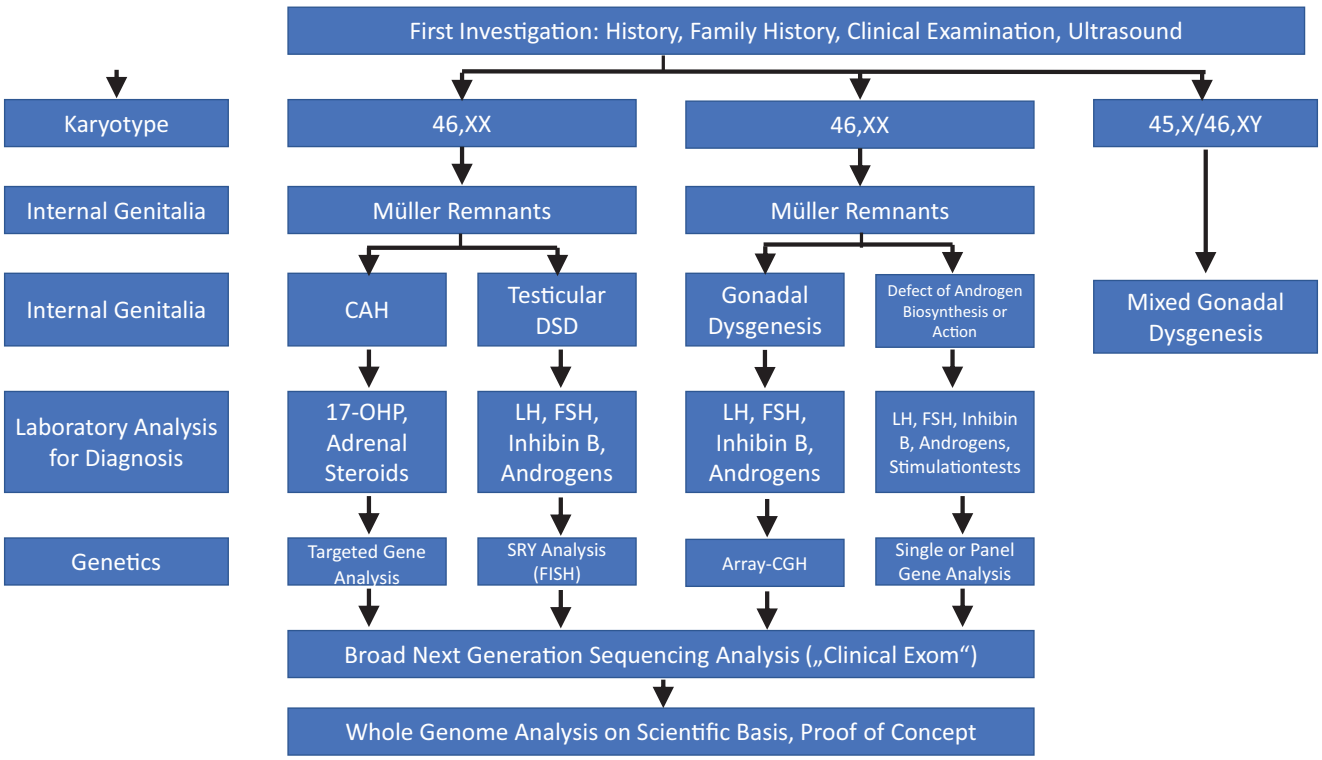


**Fig. 31.2** Linking clinical, biochemical, and (molecular) genetic diagnostics in DSD (from Hiort et al. 2019)

on this. Even if tumors are described, their status is usually unclear and not considered life-threatening. Very recently the German Federal Cabinet banned **any gender reassignment surgery** up to the age of consent.

In the following, we will discuss the individual entities of DSD in the context of this book. The linkage of clinical, biochemical, and (molecular) genetic diagnostics is shown in Fig. 31.2.

## 31.4 Structural Chromosomal Abnormalities

Klinefelter's syndrome is dealt with in Chap. 21. Similarly, the Ullrich-Turner syndrome itself does not play a significant role here in a textbook of andrology. Affected individuals have a female habitus and are cared for by gynecologists and endocrinologists because of ovarian insufficiency.

### 31.4.1 Definition and Etiology

In this chapter, we would like to focus on the special features of numerical chromosomal abnormalities with a **45,X/46,XY mosaic karyotype,** which are important for andrology. The frequency of 45,X/46,XY mosaics is given as about 1:15,000 live births (Nielsen and Wohlert 1991). The 45,X cell line probably results from a loss of an inconspicuous or structurally altered Y chromosome during early meiosis, leading to the mosaic finding.

### 31.4.2 Diagnosis

Diagnosis is made by chromosomal analysis. In many cases, this may be an incidental finding in the differential diagnosis of Turner syndrome. The clinical picture can be highly variable and includes genital abnormalities, short stature, varying degrees of gonadal dysgenesis and function, and impaired fertility. Phenotypic variability ranges from a completely female phenotype with typical findings of the Ullrich-Turner syndrome to only mildly underandrogenized males. It is even likely that the majority of affected children will be born as inconspicuously male (Lindhardt Johansen et al. 2012). A relevant number of children have comorbidities such as kidney or heart malformations.

### 31.4.3 Therapy

From an andrological perspective, the risk of gonadal tumor formation, sex hormone substitution for puberty induction, and treatment of hypergonadotropic hypogonadism, as well as the assessment and treatment of fertility restriction are important. In a recently published study, patients with 45,X/46,XY mixed gonadal dysgenesis and male sex assignment were followed up (Ljubicic et al. 2019). This showed that about half of the children had a genital abnormality with hypospadias and undescended testes at birth, while the other half were initially classified as phenotypically normal males. While the majority entered puberty spontaneously, the children with genital abnormalities usually had relevant hypergonadotropic hypogonadism, which was classified as requiring treatment. Thus, in addition to possible surgical correction of the genitals, therapy usually also includes puberty induction and permanent substitution with testosterone when the individual lives as a male.

The **gonadal histology** shows a variable proportion of undifferentiated stroma, while a Sertoli cell-only syndrome may be present in the testicular portion. About a quarter of patients show spermatids; therefore, infertility or reproductive dysfunction is a common finding and problem. **Germ cell neoplasias** are seen in about 10% of the cases, mostly as germ cell neoplasia in situ, possibly also as a gonadoblastoma (Lindhardt Johansen et al. 2012). Therefore, regular ultrasound controls of the gonads should be carried out.

## 31.5 46,XX Men (with 21-Hydroxylase Deficiency)

### 31.5.1 Definition and Etiology

The **46,XX testicular form of DSD** is dealt with in another chapter (see Chap. 22). However, the 46,XX highly androgenized forms of other causes must also be considered here for differential diagnosis. These can be children with increased testosterone production due to an **aromatase deficiency or testosterone production during pregnancy** in the mother due to a luteoma. In these cases, a detailed history of the pregnancy should be taken to determine whether the mother also had hyperandrogenemia.

Another rare cause of a 46,XX DSD with marked androgenization and male-appearing external genitalia can occur in **21 hydroxylase deficiency.** The children have an inconspicuously male phallus, but the scrotum is empty and gonads cannot be detected. Abdominal ultrasound reveals typical female internal genitalia with uterus and ovaries.

### 31.5.2 Diagnosis

In many Western countries, the children are usually detected through newborn screening, but we ourselves were able to identify several **46,XX children with 21 hydroxylase deficiency** and male gender assignment very late, because either the screening findings were not understood by the parents and no clarification took place, or the parents had consciously decided for male gender assignment due to the clinical appearance of the external genitalia. In addition, we were able to identify children with a migration background in whose home countries no newborn screening takes place. Overall, however, male gender assignment in 46,XX children with adrenogenital syndrome is very rare and epidemiological studies do not exist.

### 31.5.3 Therapy

46,XX males with congenital adrenal hyperplasia require **lifelong substitution with hydrocortisone and fludrocortisone**. This treatment is important to treat the adrenal insufficiency, but at the same time the adrenal hyperandrogenemia is reduced. Therefore, for these individuals, if the male sex assignment is maintained, adequate testosterone therapy must be given in adolescence. This simultaneously leads to

the absence of menstruation and suppression of ovarian function. In this respect, surgical removal of the internal genital structures is not absolutely necessary. The procedure is usually analogous to the treatment of transmen.

## 31.6 46XY-DSD

### 31.6.1 Gonadal Dysgenesis

#### 31.6.1.1 Definition and Etiology

The term **gonadal dysgenesis** defines a group of genetically caused conditions affecting gonadal sex differentiation. Mutations, deletions or duplications of transcription factors and genes of gonadal differentiation can lead to complete or partial gonadal dysgenesis. In case of complete gonadal dysgenesis, there are no functional gonads; instead, there are connective tissue **streak gonads (string gonads)** usually without residual function. A streak gonad is histologically characterized by stromal tissue, i.e., the absence of both germ cells and Sertoli/granulosa cells derived from the germ strands. In partial gonadal dysgenesis, hormone-active tissue is still partially present, which can lead to virilization in children with a 46,XY karyotype.

**Mutations in genes** responsible for early gonadal differentiation, such as *WT1* and *NR5A1*, and genes responsible for testicular differentiation, such as *SRY, DMRT, SOX9, DHH, ATRX,* and *ARX*, have been identified as a possible cause for DSD (Hughes 2008; Hughes et al. 2006). Mutations in some of these genes lead to syndromal forms of gonadal dysgenesis. For example, mutations in the *WT1* gene are also associated with nephropathy and in the *NR5A1* gene with adrenal insufficiency. Mutations in the *SOX9* gene lead to campomelic dysplasia, in the *DHH* gene to neuropathy, in the *ATRX* gene, and in the *ARX* gene to mental retardation.

#### 31.6.1.2 Diagnosis

In gonadal dysgenesis, apart from patients with confirmed 45,X/46,XY karyotype, the diagnosis can only be confirmed definitely by **laparoscopy with gonadal biopsy.** In gonadal dysgenesis with a 46,XX karyotype, LH and FSH are elevated, and estrogen and progesterone levels are decreased. Sometimes it is a familial disorder. The endocrine situation in gonadal dysgenesis with 46,XY karyotype is identical to the 46,XX variant. Diagnostically gonadotropins are markedly elevated, and Sertoli cell markers like Inhibin B or AMH are low. The testosterone concentration is often too low for males and too high for the female reference range. After hCG administration, there is often a small but significant increase in testosterone levels. Laparoscopy mostly shows at least rudimentary remnants of the Müllerian ducts. Biopsies from the intra-abdominal streak gonads consist histologically almost exclusively of connective tissue, ovarian stroma-like tissue without evidence of germinal cells. The testicular biopsy shows isolated precursors of germinal cells with absent or barely present Sertoli cells and often Leydig cell hyperplasia. A chromosomal analysis must be carried out in any case. Mutations in the sex-determining genes must be specifically searched for Hughes (2008). Furthermore, anorchia or bilateral cryptorchidism may be considered as differential diagnoses; however, in these children the external genitalia are most often unequivocally male.

#### 31.6.1.3 Therapy

Adequate **hormone replacement therapy** must be started at the time of expected puberty. In the case of ambiguous genitalia, surgical corrections should consider the patient's gender identity (and are currently forbidden in minors in several countries). There is a **risk of** gonadal **tumors** (gonadoblastomas, seminomas, dysgerminomas, carcinoma in situ) in all patients who have a cell line with a Y chromosome (Savage and Lowe 1990). For this reason, the gonads of these patients should be monitored and, in any case, if regular checks of the gonads by ultrasound are not possible, surgical biopsy or removal must be discussed. Infertility is not causally treatable. In countries with corresponding legal regulations, successful pregnancies have been achieved in patients with pure gonadal dysgenesis after in vitro fertilization with donor eggs (Kalra et al. 2019).

### 31.6.2 Gonadal Dysgenesis Due to SRY Mutation (Yp11.3)

In complete gonadal dysgenesis, a mutation in the SRY gene is present in approximately 10% of the cases. Complete gonadal dysgenesis with a karyotype of 46,XY was previously called **Swyer syndrome.** No androgens are produced during the embryonic period, so that affected individuals have **external female genitalia at** birth. Some cases present with slight clitoral hypertrophy. Since AMH production is absent, the Müllerian structures do not regress, so that the uterus and fallopian tubes persist. Later on, due to lack of sex steroids, there is increased height and at puberty the thelarche is absent and the menarche does not occur.

The **diagnosis** is usually not made until puberty, when adolescents present due to the lack of pubertal development. The XY karyotype and the proof of an existing uterus and fallopian tubes without ovaries on sonography indicate complete gonadal dysgenesis and warrant search for a mutation in the *SRY* or other developmental genes. The hormone status shows hypergonadotropic hypogonadism plus decreased AMH and inhibin B concentrations. There is a risk of degeneration of the gonads with increased risk of tumor formation such as gonadoblastoma or dysgerminoma.

### 31.6.3 Gonadal Dysgenesis Due to SF1/NR5A1 Mutations (9q33)

During embryonic development, the *SF1/NR5A1* gene is expressed in the gonads as well as in the adrenal glands, hypothalamus, and pituitary gland. Affected 46,XY individuals with a *NR5A1* **mutation** may rarely present with **adrenal hypoplasia**, which in the course of time usually leads to adrenal insufficiency (Achermann et al. 1999). The phenotype is usually externally female with persistent Müllerian structures and streak gonads. However, the phenotype is highly variable, and some or even the same mutations lead only to partial underandrogenization and partial or complete regression of the Müllerian structures.

Inhibin B and AMH are usually reduced. Some patients of neonatal age may already present with adrenal insufficiency. **Diagnosis is confirmed by** sequencing the *SF-1/NR5A1* gene.

Adrenal insufficiency must be treated with **glucocorticoids and mineralocorticoids.** There is a residual risk of gonadal tumor development. During puberty, sex hormone replacement therapy is almost certainly necessary.

### 31.6.4 Gonadal Dysgenesis Due to *WT-1* Mutations (11p13)

A mutation in the ***WT-1* gene (Wilms tumor suppressor 1 gene**) affects the development of gonads and kidneys with the risk of developing Wilms tumors and gonadoblastomas (in 46,XY patients). This form of gonadal dysgenesis can occur in 46,XX and in 46,XY individuals, as the gene exerts its effect at the level of the bipotent gonads. The androgen deficiency in 46,XY individuals leads to genital malformations, such as hypospadias. During puberty, secondary sexual characteristics are absent or diminished. Different gene alterations of the *WT-1* gene lead to different entities: such as Denys-Drash syndrome (with partial gonadal dysgenesis, Wilms tumors), Frasier syndrome (with complete gonadal dysgenesis and glomerulosclerosis leading to renal failure) or WAGR syndrome (with Wilms tumors, aniridia, atypical genitalia due to gonadal dysgenesis, and mental retardation). The diagnosis can only be confirmed by sequencing the *WT-1* gene.

### 31.6.5 Gonadal Dysgenesis Due to Deletion of the DMRT1 Gene Locus (9p-)

Deletion of sections of chromosome 9 (9p-) leads to a combination of 46,XY-DSD with androgenization deficiency of the external genitalia due to partial gonadal dysgenesis and is often associated with mental retardation. This can be accompanied by craniofacial abnormalities, heart defects, and kidney malformations, depending on the size of the rearrangement at this gene locus leading to a contiguous gene syndrome.

In suspicious cases, a chromosomal analysis or a FISH analysis should be performed. This can also be supported by an Array Comparative Hybridization Analysis (aCGH). A *DMRT-1* deletion is also associated with an increased risk of gonadoblastoma development.

### 31.6.6 *SOX9* (17q24), *DAX1* (Xp21.3), *DHH* (12q13.1), *WNT4* (1p35)

The SOX9 protein exerts its function immediately downstream of SRY and is thus of central importance for testis development. *SOX9* is expressed in embryonic Sertoli cells and in cartilage precursor cells. Thus, heterozygous mutations of *SOX9* lead to campomelic dysplasia and, due to gonadal dysgenesis, to 46,XY-DSD. Duplications of the X-linked *DAX-1* gene lead to suppression of normal testicular development and thus to impaired sex development due to gonadal dysgenesis. Rare mutations such as in the desert hedgehog (*DHH*) gene lead to gonadal dysgenesis with polyneuropathy. A duplication of *WNT4* can also lead to gonadal dysgenesis.

## 31.7 46,XY-DSD Caused by Defects in Androgen Biosynthesis

In a 46,XY variant of sex development, in contrast to gonadal dysgenesis, a failure of androgen biosynthesis is accompanied by normal Sertoli cell function. Therefore, persons with 46,XY DSD with a deficiency in androgen biosynthesis have absent Mullerian structures due to normal AMH secretion, but a diminished androgenization of the testosterone-dependent organ structures (external genitalia, seminal vesicles, prostate). The first steps of steroid hormone synthesis involve adrenals and gonads and can thus lead to a combination of DSD and adrenal insufficiency.

**Disruption of testosterone biosynthesis from cholesterol is possible at any level**. All these enzyme disorders are **inherited in an autosomal recessive manner**. In addition to obligate hypogonadism, adrenal insufficiency or mineralocorticoid excess may occur clinically, since 20,22-desmolase, 3ß-hydroxysteroid dehydrogenase, and 17α-hydroxylase are also involved in the synthesis of mineralocorticoids and glucocorticoids, respectively. Enzyme defects that selectively affect testosterone biosynthesis are the 17,20-desmolase defect and the 17β-hydroxysteroid dehydrogenase defect. The deficiency of 5a-reductase type 2 is special, because this enzyme is expressed in the peripheral target cell to convert

testosterone to dihydrotestosterone (DHT) prior to binding to the androgen receptor to facilitate androgen action.

At the time of expected puberty, testosterone and estrogen serum levels should be closely monitored in order to initiate hormone replacement therapy at an early stage if necessary. Depending on the phenotypic expression, estrogen substitution therapy or testosterone substitution is carried out for the rest of the patient's life. In order to preserve gonadal functionality in these patients, undescended testicles should be transferred to the scrotum at an early stage and closely monitored by palpation and sonography.

### 31.7.1 17ß-Hydroxysteroid Dehydrogenase Type 3 Defect (9q22)

The reduction of androstenedione to testosterone is catalyzed by the **17ß-hydroxysteroid dehydrogenase type 3 defect.** In both homozygous and compound heterozygous inactivating mutations, testosterone cannot be produced in sufficient quantities. Therefore, clinically individuals often present with only slightly androgenized genitalia at birth (Hiort et al. 2017). The testes are usually located inguinally, the Wolff derivatives are partially developed and the vagina is usually shortened and ends blind. During puberty, however, pronounced virilization may occur with phallic growth, vocal change and male body proportions develop. These changes can be so pronounced that in some cases a change to the male gender role takes place.

The **hCG test** may demonstrate an increased androstenedione-testosterone quotient (A:T > 1). To **confirm the diagnosis, the** HSD17B3 gene must be sequenced. Most of the time at least initially, the children grow up in a female gender role**.** The diagnosis is often not made until pubertal onset with **increasing virilization.** To gain time for diagnosis and decision-making, **puberty** can be **stopped with GnRH analogs**. If retention of the female gender role is desired, a gonadectomy can be performed after reaching consent age. If a change to the male sex role is preferred, hypospadias correction and penile reconstruction must be surgically performed. The risk of gonadoblastoma is classified as intermediate.

### 31.7.2 5α-Reductase Type 2 Defect (2p23) = Perineoscrotal Hypospadias with Pseudovagina

The so-called **perineoscrotal hypospadias with pseudovagina (PHP**) is a disorder of androgen action, however, not at the receptor level but at the metabolic level. All clinical manifestations of PHP are explained by a **deficiency of dihydrotestosterone** in the target cells of the genital tract. The basic defect lies in an impaired conversion of testosterone to DHT by the enzyme **(steroid) 5α-reductase** (Griffin et al. 1995; Imperato-McGinley 2002; Imperato-McGinley et al. 1982). Since DHT is responsible for virilization of the external genitalia, 46,XY newborns often have female external genitalia or—depending on the residual activity of 5α-reductase—signs of **virilization with clitoromegaly, hypospadias, and labioscrotal fusion.** Müllerian structures are absent due to the AMH effect of the testis, so that the urogenital sinus remains or a blind-ending pseudovagina results. In contrast, the Wolff ducts are formed due to testosterone action. The prostate, whose development is dependent on DHT, is localized as a rudiment dorsal to the urethra and remains small. The gonads are often located extra-abdominally, mostly in the inguinal canal or in the area of the labia majora. During puberty, the testosterone-dependent processes come into effect. Vocal change and the typical male muscle habitus occur, while DHT-dependent processes such as beard growth and acne are diminished. The hair on the head remains dense, the frontal hairline straight. At puberty, menarche and breast development do not occur, and there is a significant virilization. In many cases, a reorientation toward the male gender role has been observed (Hughes et al. 2006; Imperato-McGinley 2002; Imperato-McGinley and Zhu 2002).

There is a **positive family history in** about 40%, depending on the ethnic background. The condition is inherited in an autosomal recessive manner. In endocrinological diagnostics, an h**CG test** can be performed, whereby testosterone shows the expected increase, while DHT remains low. However, the specificity of this test is low**.** Blood estrogen levels, LH, and FSH concentrations are in the typical male reference interval or slightly elevated. A urine steroid profiling with gas chromatography mass spectrometry can also diagnose 5α-reductase type 2 deficiency. Genetic diagnosis is made by mutation detection in the corresponding gene, SRD5A2 (Audi et al. 2018; Kumar et al. 2019; Sinnecker et al. 1996).

**Treatment concepts** should be applied as for the 17ß-hydroxysteroid dehydrogenase type 3 defect, as DHT is currently not available for therapy. Surgical measures should only be carried out with great restraint and at the earliest, at the age of puberty.

### 31.7.3 Gonadotropin Receptor Mutations

Specific receptors mediate the effect of the gonadotropins LH and FSH on the Leydig and Sertoli cells, respectively. Such receptor mutations lead to functional changes in these cells with multiple clinical consequences. Basically, a distinction must be made between **inactivating and activating mutations.** While the former lead to a loss of function,

activating mutations cause a constitutive, i.e., autonomous activity of the target cells without the need for stimulation by LH or FSH. Overall, however, these clinical pictures are rare.

#### 31.7.3.1 LH Receptor Defect (2p21) or Inactivating LH Receptor Mutations

**Leydig cell hypoplasia or Leydig cell agenesis** is a very rare autosomal recessive disorder with an approximate incidence of 1:1,000,000. The name of the syndrome as Leydig cell agenesis is misleading in that Leydig cells are present but cannot develop due to **inactivating mutations of the LH receptor** and therefore lack of stimulation (Huhtaniemi and Alevizaki 2006). The expression of the phenotype depends crucially on the extent of intrauterine testosterone secretion. Depending on this, either: (1) a **male phenotype** with weak virilization and microphallus or (2) **hypogonadism** with delayed puberty or (3) a **46,XY variant of sex development** develops. Inactivating mutations of the LH receptor lead to an isolated disorder of gonadal testosterone production and are inherited in an autosomal recessive manner. LH and hCG cannot act on the Leydig cells, which negatively affect Leydig cell development, in the sense of hypoplasia. This results in reduced virilization at the 46, XY chromosome set. The phenotype ranges from almost male with micropenis to an external female phenotype. Wolff structures are usually rudimentary, Müllerian derivatives are absent due to normal AMH production in the Sertoli cells. The testes are located in the inguinal canal or scrotum.

One unique case of a patient with Leydig cell hypoplasia (LCH) type II caused by a genomic deletion resulting in the complete absence of exon 10 of the LH receptor (LHR) was published by Gromoll et al. Subsequently, hCG treatment resulted in an increase in testicular volume and the appearance of spermatozoa in the ejaculate after 16 weeks of treatment. The response to hCG indicates a possible dual mechanism of hormone binding and signal transduction for hCG and LH on a LHR that lacks exon 10 (Gromoll et al. 2000).

There is no adequate testosterone response in the **hCG test** (see Chap. 7). During puberty, the gonadotropins increase and show an increased response in the LHRH test. **Diagnosis is confirmed by a** biopsy, in which hypoplastic testicles can be detected, and by **sequencing the LH receptor gene**.

**Clinical management** should depend on the extent of virilization and age at diagnosis. Estrogen substitution will induce the development of female body shape and sexual characteristics. In partial forms with marked virilization, male sex assignment may also occur, which would involve therapy with testosterone and surgical corrective measures. The risk of gonadoblastoma is classified as very low.

#### 31.7.3.2 Activating LH Receptor Mutations

**Activating mutations of the LH receptors** cause LH-independent activity of the Leydig cells. This activation leads clinically to an **isosexual peripheral precocious puberty**, which usually manifests before the age of 4. There is usually a familial accumulation. The result is a noncentrally controlled continuous production of testosterone, also known as **testotoxicosis.** If left untreated, it leads to very early pubertal development and **short stature.** Activating LH receptor mutations have also been described in Leydig cell tumors.

The **treatment goal** is to temporarily **inhibit testosterone biosynthesis or block androgen receptors** so that normal pubertal development can occur after discontinuation of therapy and fertility is not affected. There are several treatment options: treatment with **ketoconazole** or a combination of **spironolactone and testolactone**. Also, treatments with cyproterone acetate and anastrozole have been described.

#### 31.7.3.3 Inactivating and Activating FSH Receptor Mutations

Inactivating mutations of the FSH receptor can lead to subfertility and infertility (Simoni et al. 1997) However, these are extremely rare and occur mainly in the Finnish population. Usually, the testicular volume is smaller and the fertility status variable.

Men with activating mutations of the FSH receptor are difficult to identify clinically because they do not show any particular phenotypic features. One case was discovered by showing intact spermatogenesis only with testosterone substitution despite hypophysectomy due to an adenoma (Simoni et al. 1997).

## 31.8 Disorders of Androgen Action

### 31.8.1 Definition and Etiology

**Sex differentiation** is the result of a cascade involving the interaction of regulatory genes, cellular and hormonal signals (see also Chap. 2). In the first phase of sex development, the developing gonads are initially omnipotent and both Wolffian and Müllerian ducts can be detected. In the typical male pathway, starting due to the effect of the SRY gene, the indifferent gonads differentiate into testes. The Sertoli cells start to produce the anti-Müllerian hormone, which causes regression of the Müllerian ducts which would otherwise develop into the uterus, tubes, and upper part of the vagina. The Leydig cells then begin to secrete testosterone, which stimulates the differentiation of the Wolffian ducts into ductus epididymis, vasa deferentia, seminal vesicles, and part of the prostate. Testosterone is then converted peripherally to dihydrotestosterone, which causes differentiation of the external genitalia

and stimulates the development of the prostate. Since androgens can only exert their effect when they bind to a functional receptor, **mutations of the androgen receptor gene cause** different degrees of **androgen insensitivity**. Androgen action is mediated by an intracellular steroid hormone receptor that functions as a transcription factor of androgen-regulated genes. Testosterone and DHT can bind to the androgen receptor via a ligand-binding domain. This is followed by translocation from the cytoplasm to the nucleus. Then a receptor dimerization occurs and subsequently binding to the promoter region of androgen-regulated target genes in conjunction with hitherto mostly unknown cofactors. This results in an increased or decreased transcription of the target genes, which unfold their biological effects after translation into their corresponding proteins.

The spectrum of androgen insensitivities ranges from a:

- Female phenotype with **complete androgen insensitivity (CAIS)** over
- ambiguous expression of the genitalia in **partial androgen insensitivity (PAIS)** up
- to a predominantly male phenotype with infertility in **minimal androgen insensitivity (MAIS).**

According to Quigley's classification, **seven different grades** are distinguished (Quigley et al. 1995). Grade 1 is characterized by a typical male appearance of the external genitalia and grades 6 and 7 by a typical female phenotype of the external genitalia, with grade 7 having a complete absence and grade 6 having sparse axillary and pubic hair in adolescence and adulthood. Grades 2–5 describe different degrees of virilization of the external genitalia.

### 31.8.2 Diagnosis

The diagnosis of androgen insensitivity is now typically confirmed by mutation detection in the androgen receptor gene in the context of a genetic test (Gottlieb et al. 2004; Hornig and Holterhus 2021). Nowadays, an AR-mutation-negative form of androgen insensitivity called AIS type II has been described, where the genetic origin is speculated to be in unknown cofactors of the androgen action cascade (Hornig et al. 2018).

These mutations can be divided into four different groups (Wieacker et al. 1998):

- Complete and larger partial deletions
- Deletions and insertions of a few nucleotides
- Point mutations that act as missense, nonsense or splice mutations
- Expansion of the CAG repeat in exon 1 in X-linked spinobulbar muscular atrophy

Almost all forms of inactivating mutations can be found in the androgen receptor, including point mutations, missense and nonsense mutations, splice-site mutations as well as deletions and insertions. They diminish the transcriptional regulation, DNA binding, and ligand binding. As a result, **the clinical picture is** also **very variable**. In about 1/3 of cases, new mutations are present in androgen resistance, and in 1/3 of cases the new mutations only occur postzygotically (Holterhus et al. 2001). Complete androgen resistance is almost always associated with a mutation in the androgen receptor, while only in 1/3 of cases a mutation is found in presumed partial androgen resistance. The majority of mutations are family-specific and only a few recurrent mutations have been described so far, so that complete gene sequencing is indicated for diagnosis.

**Androgen insensitivity is inherited in an X-linked recessive manner**. A carrier of an AR mutation passes on the mutated hereditary trait to half of the children with an XY karyotype. Androgen-binding studies, which have to be performed on genital fibroblasts after a skin biopsy, no longer play a significant role in confirming the diagnosis. The form of androgen resistance that was historically considered receptor-positive can now be explained by mutations in the DNA-binding domain, in which steroid binding is undisturbed, as expected. Additionally, androgen insensitivity can be identified by the APOD assay, which measures the expression pattern of an AR-regulated gene in genital skin fibroblast of an affected individual. Its combination with next-generation sequencing of the AR locus uncovered an AR mutation-negative, new class of androgen resistance, whose etiopathogenesis is currently unknown (Hornig et al. 2016).

### 31.8.3 Therapy

Therapy varies from individual to individual and depends on age and gonadal function. Usually, no therapy is necessary before puberty and until gonadectomy. Afterwards, hormone therapy with estrogens or testosterone can be indicated.

### 31.8.4 Complete Androgen Insensitivity Syndrome (CAIS)

The prevalence of **CAIS** is about 1:20,000 among individuals with an XY karyotype. Due to normal testis differentiation, the Sertoli cells produce AMH, so that **oviducts and uterus are absent,** resulting in a **blind-ending vagina.** The Leydig cells produce androgens with concentrations in the typical male range or even above. Due to the AR dysfunction, however, these androgens cannot act, so that Wolffian structures regress and a **maldescensus testis** results. In the

case of complete androgen resistance, no androgens can act prenatally either, so that an **external female phenotype** is present at birth. The testes may be located intra-abdominally, in the inguinal canal or in the labia majora. The probability of androgen insensitivity in phenotypically female children with inguinal hernia is 1–2% (Grumbach and Conte 1998). Surgical inguinal hernia repair not infrequently leads to recognition of the diagnosis during childhood.

During puberty, testosterone is synthesized by the Leydig cells. Clinically primary **amenorrhea, absent (grade 7) or sparse (grade 6) axillary and pubic hair ("hairless women")** with normal **female breast development are** noticeable during puberty. The breast development might be explained by the sufficient estrogen concentrations due to the aromatization of testosterone by aromatase and the lack of androgen effect. Body size is increased compared to female siblings, probably as a result of delayed closure of the epiphyseal fossae (Han et al. 2008). The lack of androgen action causes a regulatory increase in LH, which stimulates Leydig cells. The resulting hyperplasia of Leydig cells may become visible as Pick's adenomas. Individuals with CAIS show female play behavior and a large proportion of the women who are diagnosed with CAIS show a **definite female gender identity**.

In some cases, CAIS can be **diagnosed in the newborn period** if testicles are palpable or an inguinal hernia occurs. If the diagnosis is not made at that period, it is usually made in **young adulthood when** menarche does not occur. Prepubertal patients usually have normally low LH and testosterone levels. In postpubertal patients, testosterone levels are in the typical male range and estradiol levels are higher than in men but lower than in females. LH can be markedly elevated, while FSH may be only slightly elevated or within the normal range (Doehnert et al. 2015). Inhibin B levels are in the pubertal male range but there is no suppression of AMH levels, which remain in the prepubertal male range, demonstrating the role of testosterone binding to its receptor for the negative regulation of AMH production (Johannsen et al. 2020). In CAIS, **no uterus** is detectable **sonographically.** The diagnosis is confirmed by **DNA sequencing of the androgen receptor gene**. However, the same mutations can be associated with very different degrees of reduced androgen effect, so that it is often not possible to make a precise prognosis about the course of the condition. In CAIS, there is a higher risk for malignant germ cell tumors (Hughes et al. 2006; Looijenga et al. 2007; Looijenga et al. 2019).

Among the tumors that do not originate from germ cells, Sertoli cell adenomas are more frequent than Leydig cell adenomas. It is proposed that morphological and histological evaluations of gonadal tissue, in combination with OCT3/4 and TSPY double immunohistochemistry and clinical parameters, are most informative in estimating the risk for germ cell tumor development in the individual patient, and might in future be used to develop a decision tree for optimal management of patients with DSD (Looijenga et al. 2007; Looijenga et al. 2019). Prophylactic gonadectomy has been recommended in complete androgen insensitivity syndrome (CAIS) because of an increased risk for the development of malignant germ cell tumors in the intra-abdominal gonads for many years (Doehnert et al. 2017). Because the tumor risk before puberty is very low, the timing of gonadectomy has been postponed to allow spontaneous puberty and involvement of the patients in important decisions affecting their body and health. Even after puberty, gonadectomy is still discussed controversially. Currently, the absolute malignancy risk for individuals with CAIS cannot be determined and endogenous hormone profiles show very specific features that influence bone health, psychosocial well-being, and many other aspects. For women with CAIS who wish to keep their gonads, we propose a biannual screening program which has to be evaluated in a prospective multicenter trial (Doehnert et al. 2017).

In CAIS, no **therapy** needs to be carried out until the onset of puberty, unless there is an inguinal hernia. The risk of degeneration of the testicles is considered to be rather low, so that the testicles should be left in situ for as long as possible. During puberty, this can lead to spontaneous pubertal development. The testicles then produce testosterone, which is partially aromatized into estrogens. In most cases, hormone replacement therapy is not necessary as long as the testicles remain in situ. However, an **imaging of the gonads** should be done every 6–12 months (Doehnert et al. 2017).

After gonadectomy, **hormone replacement therapy with** estrogens or testosterone (Birnbaum et al. 2018) is necessary. Corrective genital surgery, such as **vaginoplasty,** should ideally be performed after puberty and should be based on the individual wishes and needs of those affected. Patients mostly identify with the **female gender role** but there are some individuals that identify with the male gender identity (T'Sjoen et al. 2011).

### 31.8.5 Partial Androgen Insensitivity Syndrome (PAIS)

The **phenotype in partial androgen resistance** covers a broad spectrum corresponding to grades 2 to 5 according to the Quigley classification. This ranges from predominantly female phenotypes with clitoral hypertrophy, **ambivalent genital findings** with partial labioscrotal fusion to an almost male phenotype with hypospadias and micropenis. During puberty, there is then also increased aromatization, resulting in female body shapes and often a distressing and pronounced **gynecomastia**. **Axillary and pubic hairs** are present to a variable extent.

**Diagnostically,** PAIS usually shows ambiguous genitalia at birth. The hormone parameters in PAIS are comparable to those in CAIS. The hormone status shows **increased LH and testosterone,** as the regulatory circuit no longer functions due to the receptor defect. In PAIS, a molecular genetic diagnosis is only possible in 1/3 of the cases. In PAIS, the testicular tumor risk is obviously dependent on the localization of the testes. According to a consensus paper (Hughes et al. 2006), a **malignancy risk of** about 50% is cited for PAIS with intra-abdominal gonads, although this risk figure is based on a small number of patients. Gonadectomy at the time of diagnosis is recommended. In PAIS with scrotal gonads, a lower risk is assumed, although reliable quantification does not appear possible at present. In PAIS, the **risk of breast cancer is** also increased. In three patients with PAIS and breast cancer, a missense mutation in the DNA-binding domain was detected (Lobaccaro et al. 1993; Wooster et al. 1992).

**Treatment of PAIS** should be interdisciplinary, especially for grades 3 and 4. It should be based on the needs of the patient and consider, among other things, the risk of degeneration of the gonads and the surgical options in adulthood.

In PAIS, the measures depend on the degree of clinical virilization and the gender in which the child will be raised. Depending on the residual function of the androgen receptor, the response of the genital tissue to androgens during puberty may vary greatly. If gynecomastia is pronounced, plastic surgery should be offered if necessary. If patients have a male psychosexual orientation, therapy aims to influence the phenotype in a male direction. Surgical measures will therefore aim to correct hypospadias, cryptorchidism, and gynecomastia. After orchidectomy, exogenous sex hormone substitution must be carried out. In individual cases, an attempt can be made to achieve a certain intensification of androgenization through high-dose testosterone therapy. The risk of degeneration of the gonads is significantly higher than in CAIS. Therefore, regular checkups are necessary and possibly a gonadectomy during adolescence or adulthood.

### 31.8.6 Minimal Androgen Insensitivity Syndrome (MAIS)

**Minimal androgen insensitivity (MAIS)** corresponds to grade 1 according to Quigley. Except for a possible **micropenis,** the external genitalia are inconspicuously male. **Gynecomastia** typically occurs at puberty. There is infertility due to **azoospermia or severe oligozoospermia**. MAIS also includes male infertility due to a mutation in the AR gene. However, only a few AR mutations have been found in infertile males so far. Also, elongation of the variable polymorphic CAG trinucleotide region in the N-terminal end of the AR gene has been associated with MAIS and male infertility (Zitzmann et al. 2005). Endocrinological indications of MAIS are normal or increased testosterone concentrations with increased LH.

From a therapeutic point of view, fertility could be restored by androgen therapy if necessary.

## 31.9 Persistent Müllerian Duct Syndrome

The syndrome of **persistent Müllerian ducts** results from a **lack of AMH secretion or also action** (via the AMH type 2 receptor). Affected 46,XY individuals have unremarkable virilized genitals with normally developed Wolff ducts (ductus deferens, epididymis) and normally developed testes, which are usually located in the abdomen. However, despite the male phenotype, the uterus and fallopian tubes are present (Imbeaud et al. 1996).

Diagnostically, AMH concentrations that cannot be measured are found in AMH gene defects and normal or even increased AMH concentrations in AMH receptor defects.

The testicles should be orchidopexied. Laparoscopically, the Müllerian derivatives can be surgically removed, however, as the ductus deferens is usually adherent to the uterus, the surgical approach to testicular descent and uterine removal needs to be performed by an experienced surgeon (Josso et al. 2005).

## 31.10 Vanishing Testis Syndrome

In the **Vanishing testis syndrome,** no gonads can be detected with a chromosome set of 46,XY. The external genitalia usually correspond to a male phenotype with anarchy. This means that testicles must have been developed prenatally at least until the 12th week of pregnancy (see also Chap. 17), and their dysfunction is secondary.

Hormone measurements reveal **elevated gonadotropins** with very low to undetectable AMH and inhibin B levels. **Hypergonadotropic hypogonadism** is latest seen at puberty. There is no increase in testosterone in the **hCG test** (see Chap. 7). Laparoscopies sometimes show so-called "nubbins," "granules," and blind-ending blood vessels and ductus deferens. These have no risk of degeneration but are usually removed in the course of testicular prostheses. Puberty induction and hormone replacement therapy with **testosterone** must be given **lifelong.**

## 31.11 Ovotesticular DSD

### 31.11.1 Definition and Etiology

An **ovotesticular feature of** sex development (Hughes et al. 2006) is present when **ovarian and testicular tissues are** present **simultaneously.** The gonads can consist of ovotestes on one or both sides with both tissue parts. However, ovaries may also be present on one side and testes on the other**.**

The exact prevalence of the disease is not known, but no more than a few hundred patients have been described so far. Approximately **2/3 of all patients have a 46,XX karyotype, 10% have a 46,XY karyotype, and the** remaining patients have a **chromosomal mosaic** with at least one Y cell line. In a proportion of patients with an XX karyotype, SRY is detectable, in the presence of which the primarily undetermined gonad differentiates into a testis (McElreavey and Fellous 1997). Overall, this is a very rare DSD diagnosis.

The genital phenotype is highly variable and can range from predominantly male to ambivalent genital findings to female external genitalia. At birth, 90% of patients with ovotesticular disorders of sex development present with ambiguous **genitalia.** The remaining 10% of cases have unequivocally female or male external genitalia. Due to the asymmetry of the gonads, asymmetrical findings for the labioscrotal folds are also found, and the position of the gonads is also variable.

Depending on the proportion of ovarian or testicular tissue and thus their local AMH and testosterone secretion, different findings are found for Wolff and Müllerian structures. The end of the vagina and a rudimentary uterus are often present. In up to 50% of those affected, inguinal hernias develop, which is sometimes the first reason for the diagnosis. Hormone production during puberty often leads to virilization and gynecomastia (caused by androgens and estrogens). In a study by Melardi et al., puberty was evaluated in 20 patients, being spontaneous in 12 of them. Four patients with partial gonadectomy in infancy were able to enter female puberty spontaneously. It was observed that patients who preserved gonadal tissues were able to enter puberty spontaneously more often (Kilberg et al. 2019; Melardi et al. 2020). About half of the phenotypically female patients have a menstrual cycle, and pregnancies have also been described in individual patients with a 46.XX or 46.XX/46.XY karyotype (Narita et al. 1975). In the ovotestes, normal spermatogenesis does not develop, whereas in the testes, spermatogenesis can be observed in every tenth patient.

### 31.11.2 Diagnosis

Gonadotropins may be normal or elevated. The extent of estrogen and progesterone concentration depends on a possible ovarian cycle. The functional detection of testicular tissue is achieved by the hCG test and of ovarian tissue by the hMG test. To assess testicular function, the determination of inhibin B and AMH is also useful. The diagnosis can ultimately only be made by biopsy of seminiferous tubules, follicles, and ovarian stroma in the gonads. Karyotyping should be performed in all cases. For differential diagnose, the different forms of 46,XY- and 46,XX-DSD can be considered.

### 31.11.3 Therapy

Gender assignment in ovotesticular disorders of sex development must consider a variety of aspects. For ethical and legal reasons, interventions and irreversible measures should be postponed as far as possible so that those affected can decide for themselves. The malignant degeneration tendency of the gonads in ovotesticular disorders of sex development is 10% in cases with a Y chromosome and 4% in patients without a Y chromosome, i.e., significantly lower than in gonadal dysgenesis. The decision to extirpate the gonads must therefore not only take the karyotype of the patient into consideration but also, in particular, the fertility status and hormonal status of the patient. If the gonads are left in place, close ultrasound monitoring of the gonads is recommended. If the gonads are removed, lifelong estrogen or androgen replacement therapy must be carried out according to the phenotype.

**Key Points**

- DSD includes both a biological definition of variants of sex development and a concept of care for affected people, some of whom describe themselves as intersexual.
- The classification follows a biological ordering principle, but does not specify a gender assignment.
- Individuals with a 45,X/46,XY karyotype can have a very variable clinical picture, from completely male to completely female.
- 21-Hydroxylase deficiency with 46,XX DSD and marked androgenization may present with male-appearing external genitalia and inconspicuous female internal genitalia with uterus and ovaries. Diagnosis is made by newborn screening. 46,XX males with congenital adrenal hyperplasia require lifelong hormone replacement therapy with hydrocortisone and fludrocortisone.

- Gonadal dysgenesis is the term used to describe the incomplete development of the gonads. A distinction is made between "mixed gonadal dysgenesis" with a 45,X/46,XY karyotype and "partial gonadal dysgenesis" with a 46,XY karyotype with androgenization (Andrade et al. 2019). The cause has been identified as mutations in genes responsible for early gonadal differentiation such as *WT-1* and *NR5A1*, and those such as *SRY*, *DMRT, SOX9,* and *DHH,* which are instrumental in testicular differentiation. The diagnosis is made by laparoscopy, gonadal biopsy, and endocrine and genetic analysis.
- In case of a 46,XY disorder of androgen biosynthesis, female or ambiguous external genitalia are formed due to the disturbance of androgen production. The cause is either defects in steroid biosynthesis, which can then also lead to adrenal insufficiency requiring treatment, or mutations in the gonadotropin receptors. In the course of the disease, a gonadectomy and hormone replacement therapy must usually be carried out.
- Mutations in the androgen receptor lead to a disturbance of the androgen effect. A distinction is made between complete androgen insensitivity (CAIS) and ambiguous genital manifestations in partial androgen insensitivity (PAIS) and a predominantly male phenotype with infertility in minimal androgen insensitivity (MAIS). The gonads should be examined regularly by ultrasound due to the risk of degeneration, but should be left in situ as long as possible to benefit from their own hormone production.
- An ovotesticular feature of sex development is present when ovarian and testicular tissue are present simultaneously. The clinical phenotype, hormone concentration, and gonadal function are highly variable, so that individual treatment concepts must be developed for each patient.

## References


Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL (1999) A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet 22:125–126. https://doi.org/10.1038/9629

Andrade JGR, Fabbri-Scallet H, Dos Santos AP, Cools M, Werner R, Hiort O et al (2019) Clinical findings and follow-up of 46,XY and 45,X/46,XY testicular dysgenesis. Sex Dev 13:171–177. https://doi.org/10.1159/000504239

Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM (2018) The E. U. C. A. GENETICS IN ENDOCRINOLOGY: approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST action BM 1303 'DSDnet'. Eur J Endocrinol 179:R197–R206. https://doi.org/10.1530/EJE-18-0256

Birnbaum W, Marshall L, Werner R, Kulle A, Holterhus PM, Rall K et al (2018) Oestrogen versus androgen in hormone-replacement therapy for complete androgen insensitivity syndrome: a multicentre, randomised, double-dummy, double-blind crossover trial. Lancet Diabetes Endocrinol 6:771–780. https://doi.org/10.1016/S2213-8587(18)30197-9

Bundesärztekammer (2015) Bundesärztekammer: Stellungnahme zu "Versorgung von Kindern, Jugendlichen und Erwachsenen mit Varianten/Störungen der Geschlechtsentwicklung (Disorders of Sex Development, DSD)." Dtsch Artzebl A598/B-510/C-498

Doehnert U, Bertelloni S, Werner R, Dati E, Hiort O (2015) Characteristic features of reproductive hormone profiles in late adolescent and adult females with complete androgen insensitivity syndrome. Sex Dev 9:69–74. https://doi.org/10.1159/000371464

Doehnert U, Wunsch L, Hiort O (2017) Gonadectomy in complete androgen insensitivity syndrome: why and when? Sex Dev 11:171–174. https://doi.org/10.1159/000478082

Gottlieb B, Beitel LK, Wu J, Elhaji YA, Trifiro M (2004) Nuclear receptors and disease: androgen receptor. Essays Biochem 40:121–136. https://doi.org/10.1042/bse0400121

Griffin JE, McPhaul M, Russell DW, Wilson JD (1995) The androgen resistance syndromes: steroid 5a-reductase 2 deficiency, testicular feminization, and related disorders. In: Scriver CE, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, vol 2, 7th edn. McGraw Hill, New York, pp 2967–2998

Gromoll J, Eiholzer U, Nieschlag E, Simoni M (2000) Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. J Clin Endocrinol Metab 85:2281–2286. https://doi.org/10.1210/jcem.85.6.6636

Grumbach MM, Conte F (1998) Disorders of sex differentiation. In: Williams textbook of endocrinology, 9th edn. Sanders, Philadelphia, pp 1303–1426

Han TS, Goswami D, Trikudanathan S, Creighton SM, Conway GS (2008) Comparison of bone mineral density and body proportions between women with complete androgen insensitivity syndrome and women with gonadal dysgenesis. Eur J Endocrinol 159:179–185. https://doi.org/10.1530/EJE-08-0166

Hiort O, Marshall L, Birnbaum W, Wunsch L, Holterhus PM, Dohnert U, Werner R (2017) Pubertal development in 17beta-hydroxysteroid dehydrogenase type 3 deficiency. Horm Res Paediatr 87:354–358. https://doi.org/10.1159/000453613

Hiort O et al (eds) (2019) Paediatric endocrinology and diabetology, holterhus and hiort: peculiarities of sex development. Springer Verlag, Cham

Holterhus PM, Bruggenwirth HT, Brinkmann AO, Hiort O (2001) Postzygotic mutations and somatic mosaicism in androgen insensitivity syndrome. Trends Genet 17:627–628. https://doi.org/10.1016/s0168-9525(01)02485-4

Hornig NC, Holterhus PM (2021) Molecular basis of androgen insensitivity syndromes. Mol Cell Endocrinol 523:111146. https://doi.org/10.1016/j.mce.2020.111146

Hornig NC, Ukat M, Schweiker HU, Hiort O, Werner R, Drop SL et al (2016) Identification of an AR mutation-negative class of androgen insensitivity by determining endogenous AR activity. J Clin Endocrinol Metab 101:4468–4477. https://doi.org/10.1210/jc.2016-1990

Hornig NC, Rodens P, DorrH HNC, Kulle AE, Schweikert HU et al (2018) Epigenetic repression of androgen receptor transcription in mutation-negative androgen insensitivity syndrome (AIS type II). J Clin Endocrinol Metab 103:4617–4627. https://doi.org/10.1210/jc.2018-00052

Hughes IA (2008) Disorders of sex development: a new definition and classification. Best Pract Res Clin Endocrinol Metab 22:119–134. https://doi.org/10.1016/j.beem.2007.11.001

Hughes IA, Houk C, Ahmed SF, Lee PA, LWPES Consensus Group; ESPE Consensus Group (2006) Consensus statement on management of intersex disorders. Arch Dis Child 91:554–563. https://doi.org/10.1136/adc.2006.098319

Huhtaniemi I, Alevizaki M (2006) Gonadotrophin resistance. Best Pract Res Clin Endocrinol Metab 20:561–576. https://doi.org/10.1016/j.beem.2006.09.003

Imbeaud S, Belville C, Messika-Zeitoun L, Rey R, di Clemente N, Josso N, Picard JY (1996) A 27 base-pair deletion of the anti-mullerian type II receptor gene is the most common cause of the persistent mullerian duct syndrome. Hum Mol Genet 5:1269–1277. https://doi.org/10.1093/hmg/5.9.1269

Imperato-McGinley J (2002) 5alpha-reductase-2 deficiency and complete androgen insensitivity: lessons from nature. Adv Exp Med Biol 511:121–131.; ; discussion 131–124. https://doi.org/10.1007/978-1-4615-0621-8_8

Imperato-McGinley J, Zhu YS (2002) Androgens and male physiology the syndrome of 5alpha-reductase-2 deficiency. Mol Cell Endocrinol 198(1–2):51–59. https://doi.org/10.1016/s0303-7207(02)00368-4

Imperato-McGinley J, Peterson RE, Gautier T, Cooper G, Danner R, Arthur A et al (1982) Hormonal evaluation of a large kindred with complete androgen insensitivity: evidence for secondary 5 alpha-reductase deficiency. J Clin Endocrinol Metab 54:931–941. https://doi.org/10.1210/jcem-54-5-931

Johannsen TH, Andersson AM, Ahmed SF, de Rijke YB, Greaves RF, Hartmann MF et al (2020) Peptide hormone analysis in diagnosis and treatment of differences of sex development: joint position paper of EU COST action 'DSDnet' and European Reference Network on Rare Endocrine Conditions. Eur J Endocrinol 182:P1–P15. https://doi.org/10.1530/EJE-19-0831

Josso N, Belville C, di Clemente N, Picard JY (2005) AMH and AMH receptor defects in persistent Mullerian duct syndrome. Hum Reprod Update 11:351–356. https://doi.org/10.1093/humupd/dmi014

Jürgensen M, Rapp M, Döhnert U, Frielitz FS, Ahmed F, Cools M, Thyen U, Hiort O (2021) Assessing the health-related management of people with differences of sex development. Endocrine 71:675–680. https://doi.org/10.1007/s12020-021-02627-y

Kalra R, Cameron M, Stern C (2019) Female fertility preservation in DSD. Best Pract Res Clin Endocrinol Metab 33:101289. https://doi.org/10.1016/j.beem.2019.101289

Kilberg MJ, McLoughlin M, Pyle LC, Vogiatzi MG (2019) Endocrine management of ovotesticular DSD, an index case and review of the literature. Pediatr Endocrinol Rev 17:110–116. https://doi.org/10.17458/per.vol17.2019.Kmv.Endocrineovotesticulardsd

Kumar A, Sharma R, Faruq M, Suroliya V, Kumar M, Sharma S et al (2019) Spectrum of pathogenic variants in SRD5A2 in Indian children with 46,XY disorders of sex development and clinically suspected steroid 5alpha-reductase 2 deficiency. Sex Dev 13:228–239. https://doi.org/10.1159/000509812

Lee PA, Nordenstrom A, Houk CP, Ahmed SF et al (2016) Global disorders of sex development update since 2006: perceptions, approach and care. Horm Res Paediatr 85:158–180. https://doi.org/10.1159/000442975

Lindhardt Johansen M, Hagen CP, Rajpert-De Meyts E, Kjaergaard S, Petersen BL, Skakkebaek NE et al (2012) 45,X/46,XY mosaicism: phenotypic characteristics, growth, and reproductive function—a retrospective longitudinal study. J Clin Endocrinol Metab 97:E1540–E1549. https://doi.org/10.1210/jc.2012-1388

Ljubicic ML, Jorgensen A, Acerini C, Andrade J, Balsamo A, Bertelloni S et al (2019) Clinical but not histological outcomes in males with 45,X/46,XY mosaicism vary depending on reason for diagnosis. J Clin Endocrinol Metab 104:4366–4381. https://doi.org/10.1210/jc.2018-02752

Lobaccaro JM, Lumbroso S, Belon C, Galtier-Dereure F, Bringer J, Lesimple T et al (1993) Androgen receptor gene mutation in male breast cancer. Hum Mol Genet 2:1799–1802. https://doi.org/10.1093/hmg/2.11.1799

Looijenga LH, Hersmus R, Oosterhuis JW, Cools M, Drop SL, Wolffenbuttel KP (2007) Tumor risk in disorders of sex development (DSD). Best Pract Res Clin Endocrinol Metab 21:480–495. https://doi.org/10.1016/j.beem.2007.05.001

Looijenga LHJ, Kao CS, Idrees MT (2019) Predicting gonadal germ cell cancer in people with disorders of sex development; insights from developmental biology. Int J Mol Sci 20:5017. https://doi.org/10.3390/ijms20205017

McElreavey K, Fellous M (1997) Sex-determining genes. Trends Endocrinol Metab 8:342–346. https://doi.org/10.1016/s1043-2760(97)00135-5

Melardi JW, Cunha DFS, Steinmetz L, Damiani D (2020) Puberty in patients with ovotesticular DSD: evaluation of 20 patients and review of the literature. Pediatr Endocrinol Rev 17:243–249

Narita O, Manba S, Nakanishi T, Ishizuka N (1975) Pregnancy and childbirth in a true hermaphrodite. Obstet Gynecol 45:593–595

Nielsen J, Wohlert M (1991) Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Hum Genet 87:81–83. https://doi.org/10.1007/BF01213097

Prader A (1945) For the development of the chorda dorsalis of humans. Bull Histol Appl Physiol Pathol Tech Microsc 22:138

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271–321. https://doi.org/10.1210/edrv-16-3-271

Savage MO, Lowe DG (1990) Gonadal neoplasia and abnormal sexual differentiation. Clin Endocrinol 32:519–533. https://doi.org/10.1111/j.1365-2265.1990.tb00893.x

Simoni M, Gromoll J, Nieschlag E (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocr Rev 18:739–773. https://doi.org/10.1210/edrv.18.6.0320

Sinnecker GH, Hiort O, Dibbelt L, Albers N, Dorr HG, Hauss H et al (1996) Phenotypic classification of male pseudohermaphroditism due to steroid 5 alpha-reductase 2 deficiency. Am J Med Genet 63:223–230. https://doi.org/10.1002/(SICI)1096-8628(19960503)63:1<223::AID-AJMG39>3.0.CO;2-O

van der Straaten S, Springer A, Zecic A, Hebenstreit D, Tonnhofer U, Gawlik A et al (2020) The external genitalia score (EGS): a European multicenter validation study. J Clin Endocrinol Metab 105:dgz142. https://doi.org/10.1210/clinem/dgz142

Thankamony A, Pasterski V, Ong KK, Acerini CL, Hughes IA (2016) Anogenital distance as a marker of androgen exposure in humans. Andrology 4:616–625. https://doi.org/10.1111/andr.12156

Thyen U, Lux A, Jurgensen M, Hiort O, Kohler B (2014) Utilization of health care services and satisfaction with care in adults affected by disorders of sex development (DSD). J Gen Intern Med 29(Suppl 3):S752–S759. https://doi.org/10.1007/s11606-014-2917-7

T'Sjoen G, De Cuypere G, Monstrey S, Hoebeke P, Freedman FK, Appari M et al (2011) Male gender identity in complete androgen insensitivity syndrome. Arch Sex Behav 40:635–638. https://doi.org/10.1007/s10508-010-9624-1

Wieacker PF, Knoke I, Jakubiczka S (1998) Clinical and molecular aspects of androgen receptor defects. Exp Clin Endocrinol Diabetes 106:446–453. https://doi.org/10.1055/s-0029-1212014

Wooster R, Mangion J, Eeles R, Smith S, Dowsett M, Averill D et al (1992) A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. Nat Genet 2:132–134. https://doi.org/10.1038/ng1092-132

Zitzmann M, Gromoll J, Nieschlag E (2005) The androgen receptor CAG repeat polymorphism. Andrologia 37:216. https://doi.org/10.1111/j.1439-0272.2005.00692.x