Genetic Testing Of XY Newborns With A Suspected Disorder Of Sex Development

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Abstract

Purpose of review

The current review focuses on the neonatal presentation of disorders of sex development, summarise the current approach to the evaluation of newborns and describes recent advances in understanding of underlying genetic aetiology of these conditions.

Recent findings

Several possible candidate genes as well as other adverse environmental factors have been described as contributing to several clinical subgroups of 46, XY DSDs. Moreover, registry-based studies showed that infants with suspected DSD may have extra-genital anomalies and in 46, XY cases, being small for gestational age (SGA), cardiac and neurological malformations are the commonest concomitant conditions.

Summary

Considering that children and adults with DSD may be at risk of several co-morbidities a clear aetiological diagnosis will guide further management. To date, a firm diagnosis is not reached in over half of the cases of 46, XY DSD. Whilst it is likely that improved diagnostic resources will bridge this gap in the future, the next challenge to the clinical community will be to show that such advances will result in an improvement in clinical care.

Keywords

Ambiguous genitalia, DSD, newborn, genetics, diagnostic yield

Introduction

Disorders of sex development (DSDs) is a collective term for a group of relatively rare congenital conditions that are associated with an alteration in chromosomal, gonadal, or anatomic sex [1]. Atypical genitalia at birth are the commonest manifestation of DSD and in epidemiological studies, this may occur in approximately 1 in 300 births [2] although true genital ambiguity requiring comprehensive medical assessment may only occur in 1 in 4500 live births [3]. Registry based studies show that over three quarters of cases of atypical genitalia present with a hypospadias [2], have a 46, XY karyotype [4] and are raised as boys [5]. In addition, it is likely that more infants with this presentation will be raised as boys in the future [6] and long-term management of these boys will require a detailed knowledge of the underlying pathological diagnosis [7]. However, systematic and thorough investigations in these boys with a 46, XY karyotype reveal endocrine abnormalities in only a quarter of cases whilst molecular genetic assessment may reveal a molecular genetic cause in almost half, depending on the extent of genetic analysis [8-10]. Thus, as a group, 46, XY neonates with atypical genitalia represent the greatest challenge in terms of diagnosis and long-term management. Whilst clinical guidelines stress the importance of an integrated multidisciplinary approach for the assessment and management of these conditions [1, 11], rapid advances in genetic knowledge as well as technology are altering the stepwise investigational strategies that have traditionally been employed in this field [12, 13]. This review will focus on the neonatal presentation of DSD and summarise the current approach to the evaluation of these children.

Clinical presentation of a newborn with DSD

A thorough initial evaluation of an affected newborn including a family history, pregnancy history and an assessment of feeding, electrolyte and blood sugar abnormalities is an important first step. Unlike the cases that present late, when the diagnosis of DSD is suspected by a disorder of

puberty, in neonates the classical presentation includes the presence of atypical genitalia and, in some cases, associated anomalies. Features of atypical genitalia include clitoromegaly or posterior labial fusion in genitalia that are otherwise 'apparently female' and bilateral cryptorchidism, microphallus, hypospadias, or bifid scrotal folds in an otherwise 'apparently male' infant [1]. In addition to a thorough examination and palpation of the gonads, the phenotype of the involved neonate can be more comprehensively assessed by using scoring systems. While the Prader scale is primarily employed to assess the extent of virilization of the female genitalia in congenital adrenal hyperplasia (CAH), the external masculinization score (EMS) is often used as a standardized tool to guide the need for investigations [11, 14]. However, such objective scores as well as the appearance of the external genitalia do not seem to play a critical role in guiding sex of rearing as evident from registry-based studies [6, 15].

Infants with suspected DSD may often have extra-genital anomalies and in 46, XY cases, cardiac and neurological malformations may be identified in 20% of cases [4]. However, the most common associated condition is being small for gestational age (SGA) which has been reported in almost quarter of cases [4]. The highest frequency of concomitant conditions was in those with gonadal development disorders. Although the occurrence of extra-genital abnormalities may be associated with the severity of under-masculinization [16] no correlation was made between the presence of variants in *AR* and SGA [17]. In fact, the presence of SGA is more likely in those who may have been labelled as PAIS (partial androgen insensitivity syndrome) on phenotype but do not have a confirmed diagnosis on *AR* analysis [17]. Thus, initial evaluation and further comprehensive clinical assessment can guide complementary diagnostic procedures.

Causes of 46, XY DSD

The causes of DSD should be considered through the prism of the pathogenesis of condition. According to the classification proposed in Chicago in 2005 [1], there are three major subgroups

of 46, XY DSDs: disorders of gonadal development, disorders of androgen synthesis and androgen action. The aetiology of DSD is multifactorial and the study of molecular mechanisms of sex development have revealed several possible candidate genes as well as other adverse environmental factors.

Disorders of Gonadal Development

46, XY disorders of gonadal development include the complete (CGD) and partial (PGD) forms of gonadal dysgenesis that are characterized by a variable presence of Müllerian and Wolffian ducts, variably functioning gonads and a spectrum of external genitalia from normal male to normal female genitalia. The development of the gonads throughout embryogenesis from the urogenital ridge is influenced by signalling pathways that lead to changing expression of genes involved [18]. The first testis-determining factor, the sex determining region Y (*SRY*), was discovered in 1990 [19] and to date, over 90 different mutations within this gene have been identified within the high mobility group (HMG) box domain [20] as well as beyond [21]. *SRY* variants cause CGD in less than 15% of cases [22] whereas the prevalence of this condition is only 1.2 per 100 000 [23]. A number of other genes have also been implicated in disorders of gonadal development, such as *SOX9, NR5A1, DAX1 (NR0B1), DHH, WT1, WNT4, GATA4, MAP3K1, DMRT1* and *WWOX* (Table 1). *SOX9* variants were detected in patients with gonadal dysgenesis and concomitant bone abnormalities due to the lack of chondrocyte-specific enhancer activity [78]. Although a small number of individuals were found to be carriers of variants in *DHH*, gonadal cancer was evident in almost 30% of them [60] and it was commonly associated with peripheral minifascicular neuropathy [61, 79, 80]. 46, XY PGD and CGD due to missense variants in *WT1* were recognised in Denys Drash syndrome [81] and concurrent renal abnormalities [82]. *NR5A1,* encoding the SF-1 protein, plays a pivotal role in the development of gonads and steroidogenesis. Phenotypes associated with *NR5A1* variants are highly diverse ranging from CGD with female external

genitalia and Müllerian remnants, severe adrenal insufficiency [40] to isolated glandular hypospadias with intact adrenal steroidogenesis, normal male genitalia with infertility as well as normal gonadal function with progressive deterioration in gonadal function [51, 83]. Thus, dysregulation of genetic pathways responsible for sex determination and steroidogenesis determines the complexity of the phenotypes in 46, XY gonadal dysgenesis.

Disorders of Androgen Synthesis

Disorders of androgen synthesis include luteinizing hormone receptor defects and defects in the testicular steroidogenesis pathway (Table 2). The gonadal expression of human lutropin-choriogonadotropin receptor gene (LHCGR) is stimulated by placental human chorionic gonadotropin (hCG) during the fetal period and results in increased testosterone synthesis and subsequent development of genitalia. Inactivating variants in *LHCGR* lead to Leydig cell insensitivity to hCG and luteinizing hormone (LH) stimulation [103] can lead to a variable level of undermasculinization including completely female external genitalia and a blind-ended vagina [104]. Androgen synthesis is impaired in cases of congenital hypogonadotropic hypogonadism and Kallman's syndrome and although this has usually been described in association with microphallus and cryptorchidism at birth [105], more recent reports suggest that variants in a number of hypogonadotropic hypogonadism genes have identified in cases of hypospadias [9].

Among all forms of 46, XY DSD, the genetic causes are clear for those presenting with enzyme deficiencies of 'classic' androgen biosynthesis pathways, including 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) or 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2) deficiency. Whilst the deficit of 17β-HSD3 may interfere only with androgen production and more often is detected because of virilization at puberty, 3β-HSD2 may affect all steroidogenic pathways and, therefore, results in severe salt-wasting and non-salt wasting forms of CAH and ambiguous genitalia in affected boys [106, 107]. Over 45 causative mutations have been reported

in *HSD17B3* and the prevalence has been reported about 1 per 150 000 [108]. The conversion of testosterone to dihydrotestosterone (DHT), the active androgen in peripheral target tissue, is regulated by the 'alternative' pathway and controlled by the members of the AKR1C family and 5α-reductase, type 1 enzyme encoded by *SRD5A1*. Splice site variants in *AKR1C2* and *AKR1C4* genes resulting in reduced function to about 10% of activity were reported by Fluck, et al. [102] in three previously described familial cases of 46, XY girls [109]. Among two known 5-alpha-reductase enzymes only expression of type 2 was detectable in different androgen-sensitive tissues [110] and over 70 missense mutations in SRD5A2 have been described as a cause of genital ambiguity in boys.

Disorders of Androgen Action

A resistance to androgen action in 46, XY has been defined as an androgen insensitivity syndrome (AIS) which has phenotypically consisted of complete (CAIS) and partial (PAIS) forms. The appearance of genitalia in PAIS may vary extensively from slightly atypical to almost female whereas CAIS is associated with completely female external genitalia which often results in a later presentation with primary amenorrhea in adolescent girls. Most genetic analyses reveal defects in both, DNA-binding and steroid-binding, functional domains of the coding region of androgen receptor gene (*AR*) as a cause of this condition [111-113] that results in reduced androgen binding activity. The *AR* locus is positioned on the X chromosome between Xq13 and Xp11 [114], and, therefore, the majority of variants are maternally inherited whilst about 30% are *de novo* [115]. Although the presence of inactivating variants in *AR* may be evident in over 80% of girls and women with CAIS [15, 116], *AR* variants in PAIS are much rarer. It is possible that in some cases, these variants may exist beyond the *AR* coding region [117]. It is also possible that androgen insensitivity may be due to a defect in the coactivators binding process to the *AR* [118]. However, there is a need to explore more effective methods of selecting cases that may display

androgen insensitivity. Whilst in the past this has involved assessment of AR binding in genital skin fibroblasts [119, 120] or measurement of circulating androgen responsive proteins in response to androgen stimulation [121, 122], in the future it may be possible to use other methods such as measurement of apolipoprotein D in genital skin fibroblasts [117] or assessment of changes in an androgen responsive transcriptome within circulating polymorphonuclear blood cells [123]. Variants in several other genes, such as *INSL3, AMH, AMHR2, MAMLD1, TAC3, WDR11, TACR3, HS6ST1, CHD7*, may also contribute to DSD [124].

Although the number of studies emphasizing the role of endocrine-disrupting chemicals in genital malformations have increased over the last decade, the epidemiological data are scarce [125]. Nevertheless, one study highlighted the risk of contact with hair cosmetics and veterinary insecticides during pregnancy [126]. Other studies concentrating on organic solvents have indicated the association between urinary tract anomalies including hypospadias and cryptorchidism in babies and maternal exposure to these chemicals [127, 128]. Rodent studies have reported a negative impact of the phthalate exposure on rat genital development [129-131]. Whilst the influence of environmental and occupational risk factors on prenatal gonadal and genital development cannot be underestimated, there is a need for further studies to understand the true risk that is posed by these environmental disruptors.

What should be done immediately

After initial examination, infants with suspected DSD require an extended clinical, biochemical, and genetic evaluation soon after birth in order to exclude life threatening conditions and confirm the karyotype. The initial diagnostic approach to an infant with suspected DSD has been outlined in detail [11]. Since girls with CAH will more likely be severely virilized it is important to measure serum plasma glucose, serum 17-hydroxyprogesterone (17-OHP), and serum concentration of sodium, potassium, chloride, and urea. However, biochemical changes may only

emerge after the third or fourth days of life for 17-OHP and electrolytes. Serum level of AMH and ultrasound examination can give an insight about the presence of testicular tissue and the latter can clarify the presence of Müllerian structures. A rapid quantitative fluorescent PCR should effectively detect Y chromosome fragments [132, 133] and will guide further investigations [11].

Likelihood of finding an abnormality

Although a number of environmental exposures have been described as risk factors for genital malformations, the vast majority of aetiological studies in the field of DSD are being conducted to discover causative variants. Confirming a definitive diagnosis is one of the crucial diagnostic aspects for such type of conditions in order to predict co-morbidities and long-term outcomes [134, 135]. However, despite the existence of a wide range techniques available and a desire of clinicians to use them on a routine basis, the decision to perform these tests was reported to be restricted by geography or availability of the test, when the more extended analyses were accessible only through the research projects [13]. Although one study reported a diagnostic yield of 64% [136], most do not demonstrate such a high level of diagnostic yield. In a recent study published by Nixon, et al. [10] copy number variants (CNVs) identified using Comparative Genomic Hybridization or single gene variants detected by Sanger sequencing of seven DSD associated genes was present in about 50% of the cohort of boys with suspected DSD. Interestingly, despite the presence of a genetic abnormality, almost half of these patients had normal endocrine test results. Furthermore, the detection of CNV may be higher when investigating those with associated abnormalities. Another study reached a diagnostic yield of genetic abnormalities of almost 50% in 46, XY DSD using a massive parallel sequencing technology [9]. Currently, the known prevalence of genetic findings in XY DSD patients may principally depend on the extent of molecular genetic assessment [10]. High-throughput NGS technology has become available in many clinical centers and this may lead to a higher diagnostic

yield. However, it is likely that this will also place greater demands on careful and detailed phenotypic as well as bioinformatic analysis and will require close collaboration within a specialist multidisciplinary diagnostic team that consists of experts with a knowledge of the clinical field as well as complex biochemistry and molecular genetics.

Conclusion

In summary, DSD are a group of rare congenital conditions that commonly result in atypical appearance of genitalia or delayed/impaired puberty and an underlying causative diagnosis remain unclear in the majority of patients. In the long-term, children and adults with DSD may be at risk of several co-morbidities and a clear aetiological diagnosis will guide management. To date, this diagnosis is not reached in over half of the cases of 46, XY DSD. Whilst it is likely that improved diagnostic resources will bridge this gap in the future, the next challenge to the clinical community will be to show that such advances will result in and improvement in clinical care.

Key points

- Neonates affected by DSD usually present with atypical genitalia and, in some cases, associated anomalies and require a thorough evaluation
- Evaluation of a neonate with suspected DSD requires a systematic approach with a focus on first line investigations that ensure that the child is not at risk of any life-threatening events
- The aetiology of DSD is multifactorial and genetic abnormalities may be currently identifiable in around 50% of cases but this may depend on the extent of molecular genetic assessment
- Children and adults with DSD may be at risk of several co-morbidities and a detailed knowledge of the underlying genetic abnormality may guide management

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Conflicts of interest

The authors do not have a conflict of interest.

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Table 1. Genetic causes of 46, XY gonadal dysgenesis

Gene	Locus	Gene/Locus MIM number	Phenotypes	Comments
Sex-determining region Y (*SRY*)	Yp11.2	480000	46, XY CGD, 46, XY PGD or 46, XY woman with partial ovarian function	Most of the variants described were found in the HMG box domain [24], however, some variants at both 5' and 3' flanking sequences of *SRY* have been also identified [25-27]. A *de novo* Gln2X point variant was reported in a 28 year-old 46, XY woman with partial ovarian function [28]
			46, XX testicular DSD or 46,XX ovotesticular DSD	*SRY*-positive 46, XX karyotype in male may occur due to translocation of the gene to one of the X chromosome or autosome [29, 30]
SRY-BOX 9 (*SOX9*)	17q24.3–25.1	608106	46, XY CGD/PGD and campomelic or acampomelic dysplasia	Campomelic dysplasia (CD) was associated with 46, XY DSD in about 75% of patients [31]. CD is an autosomal dominant disorder due to loss-of-function mutations in *SOX9* [32]. Milder clinical variants of the disease and longer survival are typical for patients with translocation breakpoints [32-34]. Acampomelic dysplasia is a rare form of campomelic dysplasia, characterized by milder phenotype and absence of long bone curvature [31, 35]
			46, XX testicular DSD or 46, XX ovotesticular DSD	Interstitial chromosome duplications located around 600 kb upstream of *SOX9* [36]
Zinc finger protein, multitype 2 (*FOG2; ZFPM2*)	8q23.1	603693	46, XY PGD with congenital heart disease and bilateral clinodactyly of the 5th finger	Altered *FOG-2* expression due to *de novo* balanced t(8;10)(q23.1;q21.1) translocation [37]
			46, XY CGD with bilateral clinodactyly of the 5th finger and no heart disease	Single case of XY female with heterozygous c.1206T.A variant inherited from maternal grandmother [38]
			46, XY PGD with mental retardation, congenital heart disease, and Langer-Giedion syndrome	*De novo* chromosomal translocation: 46, XY t(8;18)(q22; q21) [39]
			46, XY PGD and autistic spectrum disorder	One *de novo* heterozygous (c.779G.A) as well as previously reported homozygous (c.1631G.A) missense variants of *FOG2* were found in 46XY female born from consanguineous marriage. Both parents had the c.1631G.A allele [38]
Nuclear receptor subfamily 5, Group A,	9q33	184757	46, XY DSD and adrenal insufficiency	Heterozygous loss-of-function variant in exon 3 of *NR5A1* reported. Rodent functional study using G35E mutant form revealed eliminated impaired binding of *NR5A1* to a canonical binding site [40]

Member 1 (*NR5A1*)			46, XY CGD or PGD or testis with ambiguous external genitalia with normal adrenal function	Loss of function variants in *NR5A1* 46, XY DSD gonadal dysgenesis and/or ambiguous external genitalia in up to 20% of all cases [41-44]
			46, XY hypospadias and microphallus	Single case of XY patient bearing heterozygous *NR5A1* variant (p.Arg281Pro) associated with altered Sertoli cell function [45]
			46, XY bilateral anorchia and microphallus	1 case reported, a novel heterozygous partial loss of function mutation (V355M) in *NR5A1*was reported in a boy with a micropenis and testicular regression syndrome [46]
			46, XY hypospadias	Single case with isolated glandular hypospadias and normal testis within the scrotum [47]
			46, XX primary adrenal failure	1 case reported, heterozygous p.Arg255Leu mutation with apparently normal functioning ovaries in a 14-month-old girl without further follow-up description [48]
			46, XX primary ovarian insufficiency	Phenotypes ranging from ovarian dysgenesis to premature ovarian failure reported [49, 50]
			46, XY spermatogenic failure with normal male external genitalia	Most patients are moderate/severe oligospermic or azoospermic, may have risk of testes deterioration [51, 52]
			46, XX testicular DSD or 46,XX ovotesticular DSD	Heterozygous missense variant (p.Arg92Trp) in *NR5A1* was reported to be found in 3 46,XX males with testes and 2 46,XX females with ovotestes as well as in 46, XY female with PGD [53]
GATA-binding protein 4 (*GATA4*)	8p23.1–p22	600576	46, XY PGD and minor systolic murmur; 46, XY PGD with azoospermia and no heart disease; 46, XY micropenis and minor systolic murmur	Missense variant in *GATA4* (p.Gly221Arg) was reported in a familial case of 46, XY DSD associated with congenital heart disease [54]
Wilms' tumour gene 1 (*WT1*)	11p13	607102	46, XY CGD with progressive glomerulopathy and high risk of gonadoblastoma development (Frasier Syndrome)	Point variants in the donor splice side in intron 9 of *WT1* cause an imbalance in the expression of KTS isoforms [55]
			46, XY CGD/PGD early-onset renal failure and Wilms' tumour (Denys-Drash syndrome)	Most of the variants localized in exons 8 and 9. Unusual case with no nephropathy by 31 months of life bearing heterozygous missense variant in exon 7 (c.905G>T) and a splicing variant in exon 6 (IVS6-1G>T) reported [56, 57]
Desert hedgehog (*DHH*)	12q13.12	605423	46, XY PGD and peripheral minifascicular neuropathy	Homozygous missense variants in exons 1 and 2 of the *DHH* [58-60]
			46, XY CGD	Homozygous variants in the mature amino-terminal and carboxyl-terminal domains of the DHH protein [61, 62]
Chromobox homolog 2, *Drosophila*	17q25.3	602770	46, XY girl with normal female internal and external genitalia, normal ovaries (FSH levels elevated)	Single case report with two heterozygous variants: p.Pro98Leu inherited from the father and p.Arg443Pro inherited from mother [63]

polycomb class (*CBX2*)				
Alpha thalassemia/mental retardation syndrome X-linked (*ATRX*)	Xq13.1-q21.1	300032	46, XY PGD/CGD with developmental delay and microcephaly and apparent absence of a-thalassemia	Affected XY members of a large pedigree had variable gonadal phenotypes from CGD to hypospadias in 80% of cases [64]. A hemizygous missense variant of uncertain clinical significance (p.G1900C) have been reported [65]
Mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*)	5q11.2	600982	46, XY CGD and 46, XY PGD	No concomitant anomalies reported; familial and sporadic variants in *MAP3K1* result in altered MAP kinase signalling pathway and are the commonest cause of the GD in 46, XY individuals [66, 67]
Testis-specific Y-encoded-like protein 1 (*TSPYL1*)	6q22.1	604714	46, XY PGD and viscero-autonomic dysfunction in early life, followed by death before age 12 months due to abrupt cardiorespiratory distress (Sudden infant death with dysgenesis of the testes syndrome)	Twenty-one affected individuals among the Old Order Amish were reported. Homozygous frameshift variant (457_458insG) causing premature truncation of the *TSPYL* at codon 169 revealed. All parents of affected children were carriers of the same heterozygous mutation [68]
Aristaless-related homeobox (*ARX*)	Xp21.3	300382	Variable degree of genital ambiguity and a broad spectrum of neurocognitive disorders (X-linked lissencephaly, microcephaly, agenesis of the corpus callosum, neonatal-onset intractable epilepsy, hydranencephaly, temperature dysregulation, chronic diarrhoea)	Carriers of non-conservative missense variants within the homeobox of *ARX* seem to be less severely undermasculinized than those individuals who owned premature termination mutations [69]
WW domain containing oxidoreductase *(WWOX)*	16q23.3-q24.1	605131	Variable phenotypes from 46, XY male with micropenis, hypospadias and descended testes to 46, XY PGD	Heterozygous deletion within the *WWOX* reported [70]. Duplication Phenotype and genetic findings in patients with Variants of unknown significance in WWOX were identified in two undervirilized 46, XY males and 46,XX female with primary amenorrhea and hypergonadotropic hypogonadism [65]
Duplication 1p35	1p35	603490	Variable phenotypes from 46, XY male with cryptorchidism to 46, XY CGH	Overexpressed *WNT-4* results in an XY female phenotype due to up-regulation of *DAX1* [71]
Deletion 9p24.3	9p24.3	154230	46, XY CGD/PGD with craniofacial dysmorphism, psychomotor delay and various congenital malformations (Deletion 9p syndrome)	Variable size of causal deletions underlies different phenotypes [72]
Duplication Xp21.2	Xp21.2	300018	46, XY CGD and 46, XY PGD associated with or without multiple congenital anomalies	Large duplications on the X chromosome overlapping *DAX1 (NR0B1)* reported [73, 74]

Deletion 10q26.1	10q26.1	609625	Variable degree of genital ambiguity from 46, XY male with urogenital anomalies to 46, XY CGD	*EMX2*$^{-/-}$ mice exhibits an absence of kidneys, ureters, gonads, and genital tracts [75]. Several cases of 10q microdeletion encompassing *EMX2* associated with genital anomalies have been reported [76, 77]

Table 2. Genetic causes of 46, XY disorders of androgen synthesis

Gene	Locus	Gene/Locus MIM number	Phenotypes	Comments
Luteinizing hormone/chorionadotropin receptor *(LHCGR)*	2p16.3	152790	Leydig cell hypoplasia; the 46, XY phenotypes spectrum ranges from normal-appearing female external genitalia to hypoplastic male external genitalia or hypospadias	*LHCGR*, activated by the placental hCG during embryologic and fetal life, induces Leydig cell proliferation and initiates testosterone synthesis. Variants in the *LHCGR* arise from the impaired processes of hormone binding or signal transduction [84, 85]
Steroid 5-alpha-reductase 2 *(SRD5A2)*	2p23.1	607306	5-alpha-reductase type 2 deficiency; affected males have normal male internal reproductive structures and external ambiguous genitalia, urogenital sinus, blind ending vagina, hypoplastic prostate. The testes are either in the labia, or inguinal canals or intra-abdominal	Enzyme converts testosterone to DHT which is responsible for the growth and differentiation of penis and scrotum, as well as the maturity of male secondary sexual characteristics during puberty. Most *SRD5A2* variants are autosomal recessive [86]
Steroidogenic acute regulatory protein *(StAR)*	8p11.23	600617	Lipoid CAH; Female external genitalia, rarely ambiguous or male. Adrenal failure, salt-losing crisis in the first 2 months of life. Rare cases with milder presentation in late infancy	A severe defect in fetal conversion of cholesterol to pregnenolone results in disrupted adrenal and gonadal steroidogenesis. Homozygotes or compound heterozygotes variants. Milder phenotype due to partial biological activity of mutated proteins [87, 88]
7-Dehydrocholesterol reductase *(DHCR7)*	11q13.4	602858	Smith-Lemli-Opitz Syndrome; variable phenotype including facial abnormalities, metabolic errors, intellectual disability, hypotonia, anomalies of the heart, lungs, brain, limbs, genitalia and kidneys	Enzyme converts 7-dehydrocholesterol to cholesterol, required for testosterone biosynthesis. Rare autosomal recessive variants, most of them are missense [89]
Cytochrome P450, subfamily XIA, polypeptide 1 *(CYP11A1)*	15q24.1	118485	From normal female to ambiguous genitalia with blind vaginal pouch in 46, XY individuals; early-onset or later-onset adrenal failure; prematurity	The conversion of cholesterol to pregnenolone is regulated by CYP11A1 encoding the cholesterol side chain cleavage enzyme (P450scc). The enzymatic block results in glucocorticoids, mineralocorticoids, and sex steroids deficiency. Cases with partial enzyme deficiency and late-onset adrenal failure reported [90, 91]

3-Beta-hydroxysteroid dehydrogenase 2 *(HSD3B2)*	1p12	613890	Salt-wasting and non-salt-wasting CAH with or without ambiguous genitalia in 46, XY patients. Gynaecomastia and usually normal masculinization at puberty	*HSD3B2* variants affect glucocorticoid and mineralocorticoid synthesis and impair steroidogenic pathway in both the adrenals and the gonads. Rare autosomal recessive disorder, nonsense and frameshift variants reported [92, 93]
Cytochrome P450, family 17, subfamily A, polypeptide 1 *(CYP17A1)*	10q24.32	609300	17α-hydroxylase and 17–20 lyase deficiency in XY patients: female or undervirilized external genitalia with cryptorchidism, hypoplastic internal male genitalia, gynaecomastia at puberty, arterial hypertension and hypokalemia. Isolated 17–20 lyase deficiency XY patients: ambiguous genitalia, micropenis, severe hypospadias and undescended testes	*CYP17* encoding cytochrome P450c17 is responsible for 17alpha-hydroxylase and 17,20-lyase enzymes synthesis. *CYP17A1* variants affect the synthesis of glucocorticoids and sex steroids whereas mineralocorticoid precursors are being overexpressed. Recessive homozygous and compound heterozygous variants reported [94]
17-Beta hydroxysteroid dehydrogenase III *(HSD17B3)*	9q22.32	605573	Normal female or various degrees of genital ambiguity and cryptorchidism in 46, XY patients	Autosomal recessive homozygous or compound heterozygous variants reported [95, 96]
Cytochrome P450 Oxidoreductase (*POR*)	7q11.23	124015	P450 oxidoreductase deficiency. In 46, XY boys phenotypes vary from slightly undermasculinized to ambiguous genitalia. Most patients have skeletal malformations that are similar to Antley Bixler syndrome	*POR* variants underlie steroidogenic cytochrome P450 enzymes defect. Genotype-phenotype correlations: mild degree of skeletal malformations was associated with compound heterozygous for missense variants, whereas severe forms carried a major loss-of-function defect in *POR* [97, 98]
Cytochrome b5, Type A *(CYB5A)*	18q22.3	613218	Isolated 17, 20 lyase deficiency. Variable phenotypes ranging from normal-appearing female external genitalia to hypoplastic male external genitalia or hypospadias. May be associated with excessive congenital methemoglobinemia	Optimal 17,20-lyase activity, an enzyme necessary for the production of sex steroids, depends on the activity of cofactor cytochrome b5 (CytB5). In isolated 17,20-lyase deficiency glucocorticoid synthesis is not affected. Homozygous nonsense and missense variants reported [99, 100]
Aldo-keto reductase family 1, members C2/4 *(AKR1C2 and AKR1C4)*	10p15.1	600450 and 600451	Undervirilized male external genitalia and cryptorchidism or completely female external genitalia without evidence of Müllerian structures	Human aldo-keto reductases AKR1C2 and AKR1C4 are involved in the synthesis of 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnane-20-one, a precursor of androsterone and DHT [101]. Heterozygous missense variants in the coding region of *AKR1C2* and a splicing variant in *AKR1C4* were reported in a 46, XY female individuals [102]