Understanding the genetic aetiology in patients with XY DSD

S. F. Ahmed†*, A. Bashamboo‡, A. Lucas-Herald†, and K. McElreavey‡
†Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK, and‡Human Developmental Genetics, Institut Pasteur, Paris, France

Background: Disorders of sex development (DSD) consist of a wide range of disorders and are commoner in those with an XY karyotype. In over half of these cases who have a 46,XY karyotype and who are raised as boys, the underlying aetiology remains unclear.

Areas of agreement: Identification of the underlying genetic abnormality may predict long-term outcome. However, genetic abnormalities that are associated with XY DSD manifest themselves with a wide range of phenotype. To understand the aetiology as well as the phenotypic variation, there is a need to harness the advanced genetic technology that is now available.

Areas of controversy: The point at which genetic analysis should be undertaken in the course of investigations is unclear. In addition, there is little agreement on the most effective approach for genetic analysis that will be of clinical benefit to the patient.

Areas timely for developing research: There is a need to understand and improve the clinical utility of genetic analysis in the clinical setting of the patient with a suspected DSD. This will be even more important when parallel gene sequencing identifies variations in multiple genes.

Keywords: genes/karyotype/sex development/gonad development

Introduction and background

Disorders of sex development (DSD) are a wide range of relatively rare conditions with diverse pathophysiology that most often present in the newborn or the adolescent periods and are defined as ‘congenital conditions in which the development of chromosomal, gonadal or anatomical sex is atypical’.1 Truly ambiguous genitalia on expert examination are a particularly challenging management problem in the newborn but this situation is relatively rare, reported to occur in ~1:4500 births.2
However, a concern about the development of the external genitalia may exist in 1 in 300 newborn infants and in over 80% of cases the affected infants are raised as boys and have a presumed or actual XY karyotype. In these boys, ∼50% will have a hypospadias where the urethral meatus is distal; in 35% the urethral meatus will be sited on the penile shaft and in the rest it is sited more proximal. The commonest known genetic condition that leads to 46,XX DSD is congenital adrenal hyperplasia (CAH) due to 21α-hydroxylase deficiency and this occurs in ∼1:14 000 infants. Rarer conditions include 46,XX testicular DSD which refers to a male with testes and male genitalia, and 46,XX ovotesticular DSD which refers to individuals that have both ovarian and testicular tissue in the gonads, usually as ovotestes but less commonly as separate gonads. 46,XX gonadal dysgenesis is very rare in childhood and may occur due to a mutation in a gene that controls ovarian development.

46,XY DSD is characterized by reduced androgenization and causes include complete gonadal dysgenesis (CGD) or partial gonadal dysgenesis (PGD) or a defect in androgen synthesis or action. In comparison with infants with 46,XX DSD, who are most likely to be investigated comprehensively and, in over 90% of cases, shall have CAH, many infants with 46,XY DSD and especially those who are raised as boys, are often investigated to a variable extent and the aetiology remains unclear in the majority. Girls with 46,XY DSD will most likely have androgen insensitivity syndrome (AIS), gonadal dysgenesis or a biochemical disorder of androgen synthesis. In 46,XY girls with a clinical diagnosis of AIS, over 80% may have a mutation in the AR gene. However, in 46,XY boys with a DSD, 20–30% may have a mutation in AR; 10% will have a mutation in NR5A1 and 5% a mutation in MAMLD1. Thus, the commonest broad group of affected infants are boys with 46,XYDSD, and in this large group, a confirmed genetic diagnosis remains elusive in 60–70% of the cases. The next broad group of infants are girls with XY DSD who have abnormal gonadal function or androgen synthesis and in the majority of these cases a confirmed genetic diagnosis is also unclear. In those with disorders of gonadal development, small deletions or duplications that are likely to be causative of the condition can be found in 25% of cases where other organ systems are affected, whereas they are only found in 6% of cases where only testis development was affected.

Other forms of DSD include cloacal extrophy and vaginal atresia, and may present on their own or as a part of other conditions such as X-linked lissencephaly, Smith-Lemli-Opitz syndrome, hand–foot–genital syndrome or genito-palato-cardiac syndrome. Indeed, hypospadias is a part of >100 known syndromes.

Identification of an underlying cause can help with treating any coexisting hormone deficiencies and can help with anticipating any other immediate or long-term health concerns. In boys and men with XY DSD...
there is some evidence that the long-term developmental outcome may be dependent on the underlying genetic diagnosis.\textsuperscript{11,12} Thus, knowing the cause not only helps with explaining the condition to the parents and the growing child but also allows the clinician and the patient and their family to plan for the future. An improved knowledge of DSD can also provide novel insights into aetiology of other much more common reproductive disorders such as male and/or female infertility.\textsuperscript{13,14}

Genomic studies of human patients presenting with DSD have revealed an increasing number of genes important for sex determination. In addition, murine studies have extended our understanding of the mechanism of action of these genes. Understanding the molecular processes of testis development may also shed light on male infertility. However, despite these recent advances, with the exception of patients presenting with errors of testosterone biosynthesis, the genetic cause of 46,XY DSD remains unknown in the majority of cases. Basic research that defines the molecular mechanisms of sex determination and differentiation must be integrated into clinical practice leading to more accurate diagnosis and prognosis, and may assist in providing appropriate knowledge-based options and therapies for the clinical management of DSD.

**Biology of sex development**

The urogenital ridge, the common precursor of the urinary and genital systems, develops at $\sim$4 weeks post-fertilization in the human embryo as a thickening of the mesodermicmesonephros covered by coelomic epithelium. As shown schematically in Fig. 1, at 5 weeks the human gonadal ridge is formed, which is bipotential and can develop into either an ovary or a testis. In the human and mouse, several genes including NR5A1, WT1, EMX2, CBX2 and PBX1 are required for the formation of the bipotential gonadal ridge.\textsuperscript{15,16} At 7 weeks in the XY gonad, SRY is expressed in pre-Sertoli cells, possibly initiated by GATA4/FOG2/NR5A1/WT and this results in the upregulation of SOX9 expression that is further augmented by the synergistic action of SRY and NR5A1, leading to the initiation of definitive Sertoli cell differentiation.\textsuperscript{15,16} Once SOX9 levels reach a critical threshold, several positive regulatory loops are initiated, including autoregulation of SOX9 expression and formation of feed-forward loops via FGF9 or PGD2 signalling, which is required for the maintenance and sustained function of Sertoli cells.\textsuperscript{15,16} During the testicular development, SOX9 functions by regulating the production of anti-Müllerian hormone (AMH) from Sertoli cells, and possibly by repressing genes involved in ovarian development such as Wnt4 and Foxl2.\textsuperscript{14,16–18} The DMRT1 transcription factor may also be involved in this process. Human 9p deletions removing DMRT1
are associated with 46,XY gonadal dysgenesis and, in mice, \textit{Dmrt1} is expressed and required in both germ cells and Sertoli cells of the testis.\textsuperscript{19} The exact role of \textit{DMRT1} in mammalian sex determination has been unclear but the loss of \textit{Dmrt1} in mouse Sertoli cells, even in adults, activates \textit{Foxl2} and reprogrammes Sertoli cells into granulosa cells.\textsuperscript{20}

Once formed Sertoli cells induce the development of foetal Leydig cells, via a hedgehog signalling pathway, which at 8–9 weeks of development produce androgens and insulin-like factor 3 (INSL3).\textsuperscript{21} Testosterone and AMH cause the regression of Müllarian structures and differentiation of the Wolffian duct into the epididymis, vas deferens and seminal vesicles, whereas INSL3 is required for testicular descent. In the XX gonad, the absence of SRY, resulting in the inability of SOX9 expression to reach a critical threshold, together with the expression of factors such as RSPO1/ WNT4 signalling, FST and FOXL2 lead to formation of the ovary, at least in part through the suppression of the activity of ‘testis’ genes.\textsuperscript{17,18}

These data, together with the role of \textit{Dmr1} in the suppression of female differentiation in the testis, suggest that the maintenance of somatic sex identity of the gonad as either male or female is achieved by the suppression of the alternate state. It therefore would appear that at least in mice, the primary sex-determining decision is not final but is maintained in the adult by suppressing the opposing sex differentiation programmes.

In the 46,XX female, the Wolffian duct regresses and the Müllarian duct is maintained and forms the oviduct, uterus, cervix and upper

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\textbf{Fig. 1} A summary of the molecular and genetic events in mammalian gonad determination and differentiation (see text for details).
part of the vagina. In 46,XY males the testosterone is converted to dihydrotestosterone (DHT) by both the classical pathway utilizing the enzyme 5-α-reductase and a ‘backdoor’ pathway.22 DHT stimulates the genital tubercle to differentiate into the anlage of the external genitalia and its activity is mediated by the nuclear transcription factor, the androgen receptor (AR) that has high affinity for DHT. The absence of androgens in female leads to the development of female genitalia. Although the general genetic framework of testis and male development is known, the contribution of these genes to DSD in human is not that well understood. Also, the molecular mechanisms involving the interaction of genes involved in testis development as well as the interacting signal transduction pathways remain poorly understood. Our knowledge of genetics and molecular mechanisms governing the development and function of the female reproductive system is still in its infancy. The use of novel omics technologies for analysing a large number of patients with DSD, and careful assessment of the resulting data sets may result in the identification of novel genetic factors influencing DSD formation as well as improving our currently poor understanding of genotype/phenotype relationships.

46,XY complete or partial gonadal dysgenesis

46,XY CGD is characterized by completely female external genitalia, well-developed Müllerian structures and a gonad composed of a streak of fibrous tissue. 46,XY PGD is characterized by partial testis formation, usually a mixture of Wolffian and Müllerian ducts and varying degrees of masculinization of the external genitalia. The SRY gene on the Y-chromosome determines testis formation in most mammalian species. A number of single gene defects as well as recurrent chromosomal anomalies have been identified in association with 46,XY CGD or PGD (Table 1). Examples of the latter include deletions of 9p24.3 and 10q26.1 as well as duplications of Xp21.2 and 1p35. In the majority of cases these deletions are associated with syndromic forms of gonadal dysgenesis and the candidate gene has been identified (Table 1).

Mutations involving the SRY gene account for 10–15% of all cases of 46,XY CGD and around 1% of cases of 46,XY PGD. Although most SRY mutations are de novo, some are inherited from an apparently normal and fertile father.23 Small deletions (<50 kb) both 5' and 3' to the SRY gene have also been reported.23,24 Mutations in the closely related gene SOX9 give rise to campomelic dysplasia (CD), a condition characterized by skeletal defects and a typical facial appearance.25,26 Approximately 75% of XY individuals diagnosed with CD present with either CGD or PGD. A murine testis-specific Sox9 enhancer
element has been mapped to a 1.4-kb core region termed TESCO located 10 kb upstream of Sox9. Both SRY and NR5A1 (see below) bind to the TESCO enhancer sequence in vivo, thereby upregulating SOX9 expression. Mutations involving TESCO may result in DSD due to dysregulation of SOX9 expression, although none have been reported to date. In addition, recent data suggest the presence of a human testis-specific enhancer element located 600 kb upstream of the human SOX9 gene since small deletions or duplications or of this region are associated with either XY or XX DSD, respectively.

A key factor regulating the function of hypothalamic–pituitary–gonadal axis is steroidogenic factor 1 (SF-1). SF-1, encoded by nuclear receptor subfamily 5, group A, member 1 (NR5A1), belongs to the subfamily of transcription factors known as nuclear receptor subfamily 5, group A, member 1. Mice lacking Nr5a1 have gonadal and adrenal agenesis, reduced gonadotrophin excretion, small spleens and anomalies of development of the ventromedial hypothalamus. As well as reproductive anomalies, Nr5a1−/− mice also exhibit obesity and anxiety. In the human, the first mutations identified in NR5A1 were associated with 46,XY gonadal dysgenesis and adrenal insufficiency. Further studies identified mutations in ∼15% of all 46,XY DSD cases with no apparent adrenal anomalies. The spectrum of 46,XY DSD phenotypes associated with NR5A1 mutations is broad and ranges from CGD to isolated glandular hypospadias as well as phenotypically normal male. NR5A1 mutations are also associated with both male and female infertility with normal development of the external genitalia. Approximately 1% of all cases of primary ovarian insufficiency and ∼4% of all men with severe spermatogenic failure carry mutations in NR5A1. Some of these cases may represent mild forms of gonadal dysgenesis, although there are currently no biomarkers available to specifically identify those infertile patients carrying pathogenic NR5A1 mutations. The observation that NR5A1 mutations contribute to a wide spectrum of human diseases, including common conditions such as infertility raises a number of important questions and challenges. What are the genetic modifiers that impart wide phenotypic variability in different individuals carrying the same variant of NR5A1? Furthermore, are individuals carrying these variants at risk for late-onset adrenal insufficiency or tumours? The range of human phenotypes associated with NR5A1 mutations may well expand as highlighted by Suwanai et al., who proposed a link between NR5A1 mutations and psychiatric symptoms, including excessive anxiety and/or depression in two familial cases of DSD. Performing a genetic screen for NR5A1 mutations is beneficial in cases of 46,XY DSD since there is evidence of a progressive degeneration of testicular tissue with age in those individuals carrying pathogenic NR5A1 mutations. Cryopreservation of gonadal tissue or serum may be considered...
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<tr>
<td><strong>Sex-determining region Y (SRY)</strong></td>
<td>480 000</td>
<td>Yp11.3</td>
<td>46,XY CGD, 46,XY PGD or 46,XY POF</td>
<td>Most mutations localized within the HMG domain. Interstitial deletions both 5' and 3' to the SRY gene associated with 46,XY DSD. A de novo Gln2X mutation was reported in association with irregular menses of a 28-year-old woman. Translocation of SRY to X chromosome or autosome.</td>
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<td>SRY-BOX 9 (SOX9)</td>
<td>608 106</td>
<td>17q24.3–25.1</td>
<td>46,XX testicular DSD or 46,XX ovotesticular DSD; Campomelic dysplasia</td>
<td>Rearrangements 5' and 3' to the SOX9 gene show milder phenotype compared with intragenic mutations. Rare clinical variant of campomelic dysplasia, characterized by the absence of long bone curvature. Large duplications (&gt;1 Mb) 5' to SOX9.</td>
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<td><strong>Nuclear receptor subfamily 5, Group A, Member 1 (NR5A1)</strong></td>
<td>184 757</td>
<td>9q33</td>
<td>46,XY DSD and adrenal insufficiency</td>
<td>First mutations human mutations resembled the mouse knockout phenotype. Single case with heterozygous p.Arg255Leu mutation with apparently normal functioning ovaries in a 14-month-old girl. Up to 15% of all cases of 46,XY DSD cases with gonadal dysgenesis and/or ambiguous external genitalia may carry NR5A1 mutations.</td>
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<td>GATA-binding protein 4 (GATA4)</td>
<td>600 576</td>
<td>8p23.1–p22</td>
<td>46,XY PGD and minor systolic murmur; 46,XY ambiguous external genitalia, azoospermia, normal heart; 46,XY micropenis and minor systolic murmur</td>
<td>1 case with heterozygous p.Arg281Pro mutation associated with altered Sertoli cell function. 1 case reported, twin brother with same mutation had normal gonadal development. Apparently normal testis development associated with severe-to-mild hypospadias. Variable phenotype—ovarian dysgenesis to premature ovarian failure. Most cases are azoospermic or severe oligozoospermic; several mutations in common with 46,XY DSD and/or 46,XX primary ovarian insufficiency. Familial case affected 46,XY DSD males together with 46,XX individuals having apparently normal ovarian function but 2/4 known XX carriers had CHD.</td>
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<td>Zinc finger protein, multitype 2 (FOG2; ZFPM2)</td>
<td>603 693</td>
<td>8q23</td>
<td>46,XY PGD and congenital secundum-type atrial septal defect</td>
<td>Single case with balanced t(8;10)(q23.1;q21.1) translocation; the chromosome 8 breakpoint falls within the FOG2 gene and is associated with a truncated FOG2 transcript.</td>
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<td>Wilms’ tumour gene 1 (WT1)</td>
<td>607 102</td>
<td>11p13</td>
<td>46,XY CGD/PGD or hypospadias with early nephrotic syndrome with diffuse mesangial sclerosis progressing rapidly to end-stage renal failure, and Wilms’ tumor; Denys—Drash syndrome (DDS) 46,XY CGD with late-onset renal disease with focal glomerular sclerosis</td>
<td>In most cases associated with heterozygote mutations in exons 8 and 9 that encode zinc fingers 2 and 3 of the protein. DDS associated with donor splice site mutations at the exon 9 boundary; in XX individuals ovarian development and function appears normal.</td>
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<td>Desert hedgehog (DHH)</td>
<td>605 423</td>
<td>12q13.1</td>
<td>46,XY PGD and polynephropathy</td>
<td>Single homozygous mutation p.Met1Thr—phenotype resembles mouse knockout.</td>
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<td>46,XY CGD or PGD</td>
<td>4 homozygous mutations associated with CGD (Leu162Pro,1086delG, c.271_273delGAG, c.57_60dupAGCC); a single case of PGD associated with heterozygous mutation reported (1086delG).</td>
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<td>Chromobox homolog 2, Drosophila polycomb class (CBX2)</td>
<td>602 770</td>
<td>17q25</td>
<td>46,XY girl with apparently normal ovaries (FSH levels elevated)</td>
<td>One case reported with two heterozygous mutations p.Pro98Leu and p.Arg443Pro inherited from the father and mother, respectively.</td>
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<td>Alpha thalassemia/mental retardation syndrome X-linked (ATRX)</td>
<td>300 032</td>
<td>Xq13</td>
<td>Alpha thalassaemia, severe psychomotor retardation, characteristic facial features, microcephaly, short stature, cardiac, skeletal and urogenital abnormalities</td>
<td>Variable gonadal phenotype in 80% of XY individuals from CGD to hypospadias; 20% have normal male external genitalia.</td>
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<td>Mitogen-activated protein kinase kinase 1 (MAP3K1)</td>
<td>600 982</td>
<td>5q11.2</td>
<td>46,XY CGD and 46,XY PGD</td>
<td>No somatic anomalies reported; Splice site, missense or in-frame deletions that can appear to act as gain-of-function alleles reported. Mutations in the related Map3k4 gene are associated with 46,XY DSD in the mouse.</td>
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<tr>
<td>609 625</td>
<td>300 018</td>
<td>154 230</td>
<td>300 322</td>
<td>592 3</td>
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In one family, a 46,XY male had foetal testicular dysgenesis and ambiguous genitalia. Affected infants appeared normal at birth, but developed signs of visceral and autonomic dysfunction early in life, followed by death before age 12 months due to abrupt cardiorespiratory distress.  

Aristaless-related homeobox (ARX) gene is involved in the development of the central nervous system and is responsible for severe and milder phenotypes of X-linked developmental disorders, including severe congenital or postnatal microcephaly, lissencephaly, agenesis of the corpus callosum, neonatal-onset intractable epilepsy, poor temperature regulation, chronic diarrhea, and abnormalities of male and female genitalia. Female carriers may be unaffected or have a milder phenotype.

Arx homozygous knockout mice exhibit a block in foetal Leydig cell differentiation and regression of the Mullerian duct development and gonadal dysgenesis.

DMRT1, known to be involved in sex determination in birds and in some species of fish, is located within the deletion interval. No pathogenic mutations have been described within the coding sequence of the gene.

DMRT1 is encoded by the DMRT1 gene. DMRT1 is a candidate gene for the development of the Mullerian duct development and gonadal dysgenesis.

46,XY CGD and 46,XY PGD associated with various degrees of gonadal dysgenesis whereas 46,XX females with 9p deletions show apparently normal ovarian development and function.

Deletion 10q26.1 609 625 10q26.1

Multiple congenital anomalies including 46,XY male with cryptorchidism to 46,XY CGD and 46,XY PGD associated with 46,XY CGD and 46,XY PGD. 46,XY CGD and 46,XY PGD are not located within the minimal duplicated region.

Arx deficiency results in a block in foetal Leydig cell differentiation and regression of the Mullerian duct development and gonadal dysgenesis.

DAX1, encoded by the NR0B1 gene, is the candidate gene. DAX1 can negatively regulate NR5A1 target genes and may, in some cellular contexts, act as a co-activator of NR5A1.
in these cases as an investigational procedure with the aim of restoring fertility.

Other syndromic forms of 46,XY DSD include those associated with mutations in the X-chromosome located genes ARX and ATRX and the autosomal genes WT1, GATA4, FOG2 and TSPYL1 gene (Table 1). With the exception of SRY and NR5A1, single gene mutations in non-syndromic forms of 46,XY gonadal dysgenesis are rare (Table 1). Overall, the aetiology of 46,XY CGD or PGD can be determined in ~50% of cases who present with completely female external genitalia or clitoromegaly.

Disorders of androgen synthesis

The human LH/CG receptor, a member of the G protein-coupled receptor family, has two natural ligands, luteinizing hormone (LH) and human chorionic gonadotropin (hCG). hCG exerts its effect during early embryogenesis, inducing foetal Leydig cell differentiation and testosterone production. LH promotes testosterone production by Leydig cells. Inactivating mutations of the LHCGR gene encoding the LH receptor are a rare cause of Leydig cell hypoplasia (LCH) in 46,XY individuals. The phenotype ranges from completely female external genitalia, lack of breast development and primary amenorrhoea (completely inactive receptor) to normal male sex differentiation with hypospadias or a micropenis (partial receptor activity). Mutations of the LHCGR may explain only around 50% of cases LCH, suggesting a contribution of other genetic factors to the phenotype.

Mutations in genes at each step of the classical pathway of androgen biosynthesis and conversion of testosterone to DHT have been described (Table 2). These are rare autosomal recessive disorders and in several instances distinct genetic mutations can results in phenocopies with very similar biochemical profiles. As well as the classical pathway of androgen biosynthesis, 17-hydroxyprogesterone (17-OHP) can be converted to DHT via an alternative ‘backdoor’ route that bypasses the conventional intermediates androstenedione and testosterone that was first identified in foetal male marsupials who produce DHT without the intermediacy of testosterone. Pioneering work by Flück et al. identified mutations involving enzymes in this pathway in a Swiss family of 46,XY DSD and a sporadic case of 46,XY DSD. The AKR1C1–4 genes encode four enzymes belonging to a family of 15 aldo-ketoreductases that act as ketosteroid reductases and hydroxysteroid oxidases. Only the AKR1C2 and AKR1C4 enzymes can oxidize 3aDiol to DHT in vitro. Genetic analyses of a family with two 46,XY cousins with cryptorchidism and undervirilized external genitalia and a
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<tr>
<td>7-Dehydrocholesterol reductase (<em>DHCR7</em>)</td>
<td>602 858</td>
<td>11q13.4</td>
<td>Smith-Lemli-Opitz Syndrome; variable phenotype including typical facial appearance, growth and mental retardation, hypotonia, anomalies of the heart, lungs, brain, limbs, genitalia and kidneys</td>
<td>DHCR7 is required for cholesterol synthesis from 7-dehydrocholesterol. Cholesterol is required for testosterone biosynthesis. Rare autosomal recessive mutations³³</td>
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<td>Steroidogenic acute regulatory protein (StAR)</td>
<td>600 617</td>
<td>8p11.23</td>
<td>Lipoid CAH. Usually phenotypic female or slightly virilized. Adrenal failure. Rare cases with late primary adrenal failure and male external genitalia reported**</td>
<td>StAR is required for transport of cholesterol to mitochondria where biosynthesis of steroids is initiated. Homozygotes or compound heterozygotes mutations. Milder phenotype due to mutated proteins retaining some biological activity³⁴,³⁵ CYP11A1 encodes the cholesterol side-chain cleavage enzyme (P450scc) that initiates steroidogenesis by converting cholesterol to pregnenolone. Rare heterozygous, and compound heterozygous and homozygous mutations have been reported³⁶,³⁷</td>
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<td>Cytochrome P450, subfamily XIA, polypeptide 1 (<em>CYP11A1</em>)</td>
<td>118 485</td>
<td>15q21.3</td>
<td>The phenotypic spectrum in 46,XY individuals ranges from prematurity, female external genitalia and severe early-onset adrenal failure to term birth with ambiguous genitalia and later-onset adrenal failure</td>
<td>CYP11A1 encodes the cholesterol side-chain cleavage enzyme (P450scc) that initiates steroidogenesis by converting cholesterol to pregnenolone. Recessive homozygous and compound heterozygous mutations have been reported³⁶,³⁷ HSD3B2 mutations affect all three adrenal steroidogenic pathways (mineralocorticoids, glucocorticoids, sex steroids). Rare autosomal recessive mutations³⁸,³⁹ CYP17A1 mutations usually result in glucocorticoid deficiency and sex steroid deficiency since it catalyses 17α-hydroxylation of pregnenolone and progesterone as well as the conversion of 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA). Recessive homozygous and compound heterozygous mutations identified. Evidence of founder and recurrent mutations. Four mutations associated with isolated 17, 20 lyase deficiency⁰⁰–⁰²</td>
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<tr>
<td>3-Beta-hydroxysteroid dehydrogenase 2 (<em>HSD3B2</em>)</td>
<td>109 715</td>
<td>1p12</td>
<td>Wide spectrum of phenotypes—severe salt-wasting and non-salt-wasting forms, with or without ambiguous genitalia in affected males. Gynaecomastia at puberty</td>
<td>HSD3B2 mutations affect all three adrenal steroidogenic pathways (mineralocorticoids, glucocorticoids, sex steroids). Rare autosomal recessive mutations³⁸,³⁹ CYP17A1 mutations usually result in glucocorticoid deficiency and sex steroid deficiency since it catalyses 17α-hydroxylation of pregnenolone and progesterone as well as the conversion of 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA). Recessive homozygous and compound heterozygous mutations identified. Evidence of founder and recurrent mutations. Four mutations associated with isolated 17, 20 lyase deficiency⁰⁰–⁰²</td>
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<tr>
<td>Cytochrome P450, family 17, subfamily A, polypeptide 1 (<em>CYP17A1</em>)</td>
<td>609 300</td>
<td>10q24.33</td>
<td>XY patients with 17α-hydroxylase and 17–20 lyase deficiency: 46,XY female or partially virilized external genitalia with cryptorchidism. Male internal genitalia hypoplastic with gynaecomastia at puberty. High blood pressure. XY patients with isolated 17–20 lyase deficiency: ambiguous genitalia, micropenis, perineal hypospadias and cryptorchidism</td>
<td>CYP17A1 mutations usually result in glucocorticoid deficiency and sex steroid deficiency since it catalyses 17α-hydroxylation of pregnenolone and progesterone as well as the conversion of 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA). Recessive homozygous and compound heterozygous mutations identified. Evidence of founder and recurrent mutations. Four mutations associated with isolated 17, 20 lyase deficiency⁰⁰–⁰²</td>
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<td>17-Beta hydroxysteroid dehydrogenase III (<em>HSD17B3</em>)</td>
<td>605 573</td>
<td>9q22.32</td>
<td>XY with female or ambiguous genitalia with intra-abdominal or inguinal testis</td>
<td>Recessive homozygous or compound heterozygous mutations¹⁰³</td>
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<td>Cytochrome P450 Oxidoreductase (POR)</td>
<td>124 015</td>
<td>7q21.11</td>
<td>P450 oxidoreductase deficiency. 46,XY boys under-virilized, from borderline micropenis to severe perineoscrotal hypospadias. Patients may have skeletal—predominantly craniofacial—malformations similar to those observed in Antley–Bixler syndrome</td>
<td>Recessive compound heterozygous and homozygous mutations with evidence for founder mutations</td>
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<td>Cytochrome b5, Type A (CYB5A)</td>
<td>613 218</td>
<td>18q22.3</td>
<td>Variable level of virilization from hypospadias to female genitalia and type IV hereditary methaemoglobinaemia has been reported. Isolated 17, 20 lyase deficiency</td>
<td>Cytochrome b5 participates in 17α-hydroxylation in adrenal steroidogenesis. Homozygous splice site mutation associated with 46,XY female genitalia and type IV hereditary methaemoglobinaemia. Homozygous nonsense and missense mutations associated with isolated 17, 20 lyase deficiency</td>
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maternal aunt with female external genitalia, all showing the evidence of 17, 20 lyase deficiency, revealed a compound heterozygote missense mutation in AKR1C2 and the absence of exon 2 of AKR1C4. A sporadic case carrying, on one allele a complex rearrangement between AKR1C1 and AKR1C2, and on the second allele a maternally inherited missense mutation in AKR1C2 was also identified. These rare cases serve to underline the need for both the classical and alternative route for DHT synthesis during normal male sexual differentiation.

Testosterone metabolism to DHT is a key step in androgen action during male genital differentiation. There are two steroid 5α-reductase enzymes that catalyse the 5α-reductase reaction. 46,XY DSD has been reported in association with mutations of one of these enzymes, steroid 5α-reductase type 2 enzyme, which is encoded by SRD5A2. SRD5A2 is predominantly expressed in external genital tissues and prostate gland. Over 70 homozygous or compound heterozygous pathogenic mutations have been reported in the SRD5A2 gene (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/). SRD5A2 deficiency is an autosomal recessive disorder that leads to impairment of the conversion of T to DHT and it is classically defined by a predominantly female phenotype at birth and significant virilization without gynaecomastia at puberty. However, many cases with SRD5A2 deficiency have been described with undervirilization of male external genitalia at birth as well as in adult 46,XY DSD individuals with a female phenotype.

**Other causes of XY DSD**

Mutations in the DNA-binding and ligand-binding domains AR are identified in around 90% of 46,XY with a clinical diagnosis of complete androgen insensitivity syndrome. However, in those with a phenotype consistent with partial androgen insensitivity syndrome (PAIS) the percentage of cases presenting with pathogenic AR mutations is much lower at ~20%. Some of these cases may be explained by mutations in the NR5A1 gene but others may be due to defects in levels of AR expression/translation/post-translation modifications or possibly by mutations in one of the >200 AR coregulators.

*MAML1* (mastermind-like domain-containing protein 1), located on Xq28, is expressed in foetal Sertoli and Leydig cells during critical period for sex development. The precise biological function(s) of *MAML1* in testicular development is unknown but there is evidence to suggest that it may contribute to Leydig cell function. 46,XY patients carrying deletions of X chromosome region including *MAMLD1* have severe undervirilization of the external genitalia. Excluding patients with gonadal dysgenesis or isolated cryptorchidism, a total of 239 46,XY DSD patients have been
screened for mutations in MAMLD1 and 11 mutations (5%) were identified in patients with affected external genitalia.8,9,42,43

Persistent Müllerian duct syndrome (PMDS) is a rare disorder in 46,XY individuals who usually present with unilateral cryptorchidism with a hernia containing a uterus at the contralateral side. In 85% of cases a mutation can be detected in either AMH or the gene coding for its receptor, AMHR2.44–46 In both cases the transmission of the phenotype follows an autosomal recessive pattern of inheritance and only XY males are affected.

The genetic factors associated with non-syndromic hypospadias have been difficult to identify. Simple hypospadias is considered multifactorial with the contribution of several genetic and environmental factors. Familial clustering without a clear inheritance pattern has been reported and some families show an autosomal dominant or autosomal recessive mode of inheritance.47 Other factors that may have a genetic or epigenetic component, such as low birth weight are also linked to the formation of hypospadias.48,49 Several genetic association studies have identified a number of loci associated with hypospadias including AR, ATF3, ESR1, ESR2, FGF8, FGF2, HSD17B3, SRD5A2, MAMLD1, DGKK, MID1, CYP1A1, GSTM1 and GSTT1. These associations have, for the most part however, not been observed in replication studies.50 Rare mutations have been identified in AR, NR5A1 and WT1 in association with hypospadias and are mainly associated with severe posterior forms rather than the distal or glandular forms. This suggests that either as yet unidentified genetic factors are responsible for most cases of hypospadias or that environmental factors may predominate in the milder glandular forms of hypospadias. Although rodent studies have demonstrated that exposure to molecules such as synthetic oestrogens or anti-androgens can lead to male reproductive anomalies, the contribution of environmental factors to human hypospadias is controversial and empirical evidence to show a causal link between the two is limited.50–53 There is epidemiological evidence to support the hypothesis that prenatal contamination by pesticides may contribute to undervirilization in newborn males,54 and that exposure of pregnant women to endocrine disruptors such as phthalate monoesters may result in newborn male external genital malformation.55

**Structured investigation of the aetiology of DSD**

As our knowledge of the genetic aetiology and its link to long-term outcomes increases and the cost of DNA analysis falls, complex high-throughput genetic technologies will become part of routine clinical care in many cases of DSD. A simple illustration of a change in practice is a
shift towards the determination of the sex chromosomes by fluorescent *in situ* hybridization rather than a karyotype. Comparative genomic hybridization (CGH)-microarray, detecting copy number variants—microdeletions and microduplications—is increasingly performed in diagnostic laboratories, especially when a condition is associated with other malformations. 

In addition, massive parallel sequencing of the human genome is likely to become routine over the next few years. Already, exome sequencing is having a major impact in medical genetics. It is debatable whether biochemical investigations are sufficiently sensitive or specific to direct targeted genetic analysis. For instance, it would be reasonable to consider that a child with 46,XY DSD who has biochemical evidence of a disorder of testosterone synthesis should undergo further analysis of genetic abnormalities that may lead to a disorder of androgen synthesis rather than a disorder of androgen action. However, it is clear that many patients with a mutation of the AR have inadequate testosterone production on hCG stimulation. 

A low testosterone:androstenedione ratio has been used for selecting cases for further analysis of 17β-hydroxysteroid dehydrogenase (HSD) deficiency and a high testosterone: DHT ratio has been used to select cases for 5α-reductase deficiency. However, it is clear that these cut-offs are not sufficiently sensitive with genetically confirmed cases of 17β-HSD deficiency and 5α-reductase deficiency with a ‘normal’ T:A or T:DHT ratios. It is possible, therefore, that the long, arduous diagnostic process could be more effectively streamlined by the use of genetic analysis. However, the need for thorough evaluation of the physical and biochemical characteristics of the person with DSD should not be underestimated, especially as advances in imaging and biochemical analysis will continue to help the geneticist towards the correct analysis and will also ensure that the results of genetic investigations are placed in the correct clinical context. With the increased likelihood of finding additional genetic variations of unknown clinical significance, it is imperative that diagnostic genetic laboratories are closely linked to bioinformatics resources, research groups as well as biochemical and clinical experts in the field of DSD.

The genetic aetiology of most cases of 46,XY DSD is unknown and for the moment it is unclear if the phenotype in these cases is due to rare single-gene defects in a limited or large number of genes involved in male development or if the phenotype is due to cumulative rare and/or common variants in the human genome. The latter is suggested by the considerable phenotypic variability seen in familial cases of 46,XY DSD, where the major causative mutation has been identified. Therefore, it remains debatable whether every patient with 46,XY DSD with a suspected DSD should undergo parallel sequencing of a wide range of genes, mutations in which may lead to a biochemical picture, which is indistinguishable. Figure 2 shows a possible path that could...
be adopted for determining which patient should undergo genetic analysis. Based on current knowledge and the limitations of the current biochemical analyses, one could argue that analysis of a limited panel of genes is clinically justified for a routine diagnostic service as a first-line panel of genes in all cases of 46,XY DSD.

Candidate genes in DSD can be divided into the following:

(i) Androgen/steroid pathway—AR, 5ARD2, etc.
(ii) High-frequency gonadal development genes—NR5A1, SRY, MAP3K1, etc.

![Non-Urgent Genetic Evaluation Of DSD](image)

**Fig. 2** A proposed algorithm that directs genetic analysis in cases of DSD towards comparative genomic hybridization (CGH), single gene analysis and/or multiple gene analyses.
Table 3 A sample of genes broadly categorized according to their functional involvement in testosterone synthesis or gonadal development.

| Steroid hormone synthesis (steroid panel) | | |
|------------------------------------------|---------------------------------|
| CYP17A1: cytochrome P450, family 17, subfamily A, polypeptide 1 | 1 | XY |
| STAR: steroidogenic acute regulatory protein | 1 | XY |
| HSD3B2: 3beta-hydroxysteroid dehydrogenase | 1 | XY |
| CYP21A2: steroid cytochrome P450 21-hydroxylase | CAH | XX |
| CYP11B1: cytochrome P450, subfamily X, polypeptide 1 | 1 | XX |
| HSD17B3: 17-beta hydroxysteroid dehydrogenase III | 1 | XY |
| CYP19A1: cytochrome P450, subfamily XIX | 1 | XX |
| POR: cytochrome P450 oxidoreductase | 1 | XX,XY |
| CYP11A1: cytochrome P450, subfamily XIA, polypeptide 1 | 2 | XY |
| DHCR7: 7-dehydrocholesterol reductase | 2 | XY |
| LHR (LHCGR): luteinizing hormone/choriogonadotropin receptor | 1 | XY |
| DAX-1: dosage sensitive sex reversal gene on chr X gene 1 | 1 | XY | CNV |

| Gonadal development and androgen action (gonadal panel) | | |
|----------------------------------------------------------|---------------------------------|
| SRY: sex-determining region, Y chromosome | 1 | CNV |
| DMRT1: double sex- and MAB3-related transcription factor | 1 | |
| WT1: Wilms tumour | 1 | |
| SF1 (NR5A1): steroidogenic factor | 1 | |
| SOX9: SRY-BOX 9 | 1 | CNV |
| DHH: desert hedgehog | 1 | |
| AMH: anti-Mullerian hormone | PMDS |
| AR: androgen receptor, Xq11 | 1 | |
| SRD5A2: steroid 5-alpha-reductase 2 | 1 | |
| FOXL2: forkhead transcription factor FOXL2 | 2 | |
| RSP01: R-spondin family, member | 1 | |
| TSPYL1: TSPY-like 1 | 2 | |
| WNT4: wingless-type MMTV integration site family, member | 4 | |
| ARX: aristless-related homeobox, X-linked | 2 | |
| CXorf6 (MAMLD1): chromosome X open reading frame 6 | 1 | |
| FKBP4: FK506-binding protein 4 | 2 | |
| GATA4: GATA-binding protein 4 | 2 | |
| PBX1: pre-B-cell leukemia transcription factor 1 | 2 | |
| EMX2: empty spiracles, Drosophila, 2, homolog of | 2 | |
| CBX2: chromobox homolog 2, Drosophila polycomb class | 2 | |
| FOG2 (ZFP2): friend of GATA2 | 2 | |
| LHX9: LIM homeobox gene | 2 | |
| LHX1: LIM homeobox gene | 2 | |
| ATRX: ATR-X gene | 2 | |
| FGF9: fibroblast growth factor 9 | 2 | |
| AMHR2: anti-Mullerian hormone type II receptor | PMDS |
| HOXA13: homeobox A13 | 2 | |
| FOXF2: forkhead box F2 | 2 | |
| ESR2: estrogen receptor 2 | 2 | |
| BMP4: bone morphogenetic protein 4 | 2 | |
| BMP7: bone morphogenetic protein 7 | 2 | |

In addition, the genes are assigned a number 1 or 2 so that they can be included in the first-line analysis or second line. For some conditions such as CAH and persistent Mullerian duct syndrome (PMDS), a very focused approach and a single gene analysis may be appropriate. For some conditions, there is a need for analysis of copy number variations.
(iii) Low-frequency gonadal development genes—CBX2, GATA-4, etc.
(iv) Potential novel genes by CNV arrays—SOX3, SOX9, DMRT1, etc.
(v) Small regulatory regions of DSD genes.
(vi) DSD candidates from other animal species.

They can also be divided according to the perceived phenotype/function that may be associated with the gene and as illustrated in Fig. 1. Table 3 includes a list of genes derived from the above categories. They have been further categorized as those which could be analysed as ‘first line’ (I) and those would could be analysed as ‘second line’ (II). As whole exomic sequencing becomes more routine, the need for a first-line and second-line approach will be obsolete. However, thorough phenotypic evaluation will become even more important so that the results of the genetic analysis can be interpreted in light of the clinical findings.

Summary

Cases of 46,XY DSD are relatively rare but are very heterogeneous and can present in a variety of different ways, most commonly with ambiguous genitalia noticed in the newborn period or delayed puberty in adolescents.58 A number of genes have been identified as playing a role in these disorders; however, most cases of 46,XY DSD do not yet have a confirmed genetic diagnosis. There are limitations associated with biochemical investigations in the assessment of DSD and genetic analysis promises to be a useful adjunct in the evaluation of these patients. Combining the detailed results of advanced biochemical and genetic analysis and interpreting it with the variable phenotype that often occurs in these conditions will be the challenge for the future.

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