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► **To cite this version:**

Maëva Elzaiat, Ken Mcelreavey, Anu Bashamboo. Genetics of 46,XY gonadal dysgenesis. Best Practice and Research: Clinical Endocrinology and Metabolism, 2022, 36 (1), pp.101633. 10.1016/j.beem.2022.101633 . hal-04150390

HAL Id: hal-04150390

<https://hal.science/hal-04150390v1>

Submitted on 22 Jul 2024

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Genetics of 46,XY Gonadal Dysgenesis

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Abstract

In 46,XY men, testis is determined by a genetic network(s) that both promotes testis formation and represses ovarian development. Disruption of this process results in a lack of testis-determination and affected individuals present with 46,XY gonadal dysgenesis (GD), a part of the spectrum of Disorders/Differences of Sex Development/Determination (DSD). A minority of all cases of GD are associated with pathogenic variants in key players of testis-determination, SRY, SOX9, MAP3K1 and NR5A1. However, most of the cases remain unexplained. Recently, unbiased exome sequencing approaches have revealed new genes and loci that may cause 46,XY GD. We critically evaluate the evidence to support causality of these factors and describe how functional studies are continuing to improve our understanding of genotype-phenotype relationships in genes that are established causes of GD. As genomic data continues to be generated from DSD cohorts, we propose several recommendations to help interpret the data and establish causality.

Keywords

46,XY DSD; testis development; gonadal dysgenesis; high throughput sequencing; genetic etiology.

Introduction

Mammalian sex determination is a tightly controlled and highly complex process where the bipotential gonad anlage develops to form either a testis or an ovary (1). In mammals, the function of the Y-linked sex-determining gene *SRY* is to activate the expression of the downstream effector *SOX9*, which in turn induces Sertoli cells formation, the first somatic cell lineage to differentiate during testis formation. *Sry* also initiates a positive feedback loop between *SOX9* and *FGF9*, which results in up-regulation of *FGF9* and repression of ovarian gene (and *Sox9* repressor) *Wnt4* in the male gonad (1,2). Although *Sox9* is a downstream target for *SRY*, it is possible that *SRY* could be involved in direct modulation of other, as yet, unrecognised factors. Another key factor in mammalian sex-determination is the evolutionarily conserved DM-domain transcription factor *DMRT1*. The *DMRT* gene family is involved in sex determination in mammals, birds, frogs, flies and worms (3). Although *Dmrt1* is not required for primary testis-determination in mice, we established its requirement for humans (4). Genetic and molecular analysis suggest that *DMRT1* functionally interacts with *SOX9* to control testis development (5, 6). *DMRT1* and *SOX9* may collaborate to control gene expression since they often bind closely together on target genes in the foetal testis, juvenile Sertoli cells, and when expressed ectopically in the ovary (5,6). *DMRT1* binding to DNA enhances *SOX9*-binding to target DNA elements (5). Ovarian somatic sex determination requires the *Rspo1/Wnt4/b-catenin* signalling pathway, as well as a *Foxl2/Runx1* gene regulatory network (1, 7-9). How these pathways interact and cooperate to control ovary development is still unclear. During mouse ovarian development *Rspo1* cooperates with *Wnt4* to stabilize β -CATENIN, to counteract the establishment of the pro-testis *SOX9/FGF9* positive feedback loop (1, 8). Mice that carry a constitutive active form of β -CATENIN are XY and female (1). *Foxl2* is required to maintain the ovarian phenotype at the postnatal stage. Targeted ablation of *Foxl2* in the adult ovary leads to cellular reprogramming where granulosa cells trans-differentiate into Sertoli cells (10). *FOXL2*, together with the *Rspo1/Wnt4/ β -catenin* signalling pathway negatively regulates *Sox9* expression and, in co-operation with *RUNX1* negatively regulates the expression of *Dmrt1* (9, 10). Trans-differentiation also occurs in the adult mouse testis, following the loss of *Dmrt1* expression in Sertoli cells which trans-differentiate into granulosa and theca cells (11). The absence of *Foxl2* suppresses the trans-differentiation of

XY *Dmrt1* mutants suggesting that at least in mouse *Dmrt1* and *Foxl2* direct antagonistic regulatory networks that maintain somatic sex identity in the adult gonadal tissues (12). The processes of transdifferentiation of gonadal tissues has not been explored in human and other primate species. The exact mechanism whereby adult gene ablation results in reprogramming to another cell fate remains to be defined but may involve retinoic acid (RA) signalling (12).

Mammalian sex-determination pathways have been elucidated from data from single gene loss-of-function models, however recent studies using combinatorial loss-of-function alleles have revealed a surprising complexity and redundancy in the system. For example, the absence of both *Sox9* (pro-testis) and *Rspo1* (pro-ovary) in XX fetuses results in the development of ovotestes (13). *Sox10* and *Sox8* that are normally repressed by *Rspo1* are activated in the double knockout gonads and presumably compensate for the absence of *Sox9* (13). Furthermore, when XX and XY *Rspo1*^{KO}+*Sox9*^{KO} gonads are compared at the same stage, the XY gonads appear more masculinised, suggesting that *Sry* may be inducing the expression of testis-specific genes other than *Sox9* (13). Similar data have been observed for *β-catenin*^{KO}+*Sox9*^{KO} (14). These data highlight the complexity of genetic interactions and the difficulty in unravelling either an instructive or a permissive role (or both) for these signalling pathways. Given all its complexity, sex determination requires precise synergistic temporal-spatial action of multiple factors involved in activation and repression of the testis or ovarian pathways. In the human, disruption of these processes can result in a wide spectrum of reproductive phenotypes referred to as the Disorders/Differences of Sex Development/determination (DSD).

DSDs are a group heterogeneous rare congenital conditions in which the chromosomal, phenotypic, and anatomical sex are discordant (15, 16). DSD are often detected at birth with newborn presenting with ambiguous genitalia (estimated incidence of 1 in 4,500-5,500) or in adolescence with atypical pubertal development (15, 16). The etiology of 46,XY DSD is defined as either gonadal dysgenesis (GD), disorders of androgens synthesis or action, or disorders of AMH synthesis or action. 46,XY gonadal DSD includes 46,XY complete or partial gonadal dysgenesis (CGD or PGD), undervirilisation or undermasculinisation of an XY male. 46,XX DSD includes GD and overvirilisation or masculinisation of an XX female. Approximately

15% of all cases of 46,XY complete gonadal dysgenesis result from mutations involving the *SRY* gene. Along with *SRY*, mutation in *NR5A1* (encoding steroidogenic factor-1, SF1) and *MAP3K1* are the most prevalent causes of 46,XY gonadal dysgenesis, but together they explain less than 40% of all non-syndromic forms of 46,XY gonadal dysgenesis. Mutations in other genes including *SOX9*, *SOX8*, *GATA4*, *DMRT1*, *FOG2*, *WT1*, *DHH*, *CBX2*, *PBX1*, *ATRX*, *FGF9* and *ZNRF3* are reported in rare individuals as a cause of both syndromic and non-syndromic forms of 46,XY GD as well as some 46,XY boys with severe penoscrotal hypospadias (17-19). Overviews on the genetics of other forms of 46,XY DSD including reviews on AR, AMH signalling and disorders of androgen synthesis have been published recently (20-23). In this review we will discuss genes that have been recently recognized to cause GD as well as new insights into the mechanisms by which well-established genes cause DSD.

46,XY GD involving transcription factors and their co-factors

Testis-determination is governed by gene regulatory networks with transcription factors at their core. A large proportion of DSD is caused by variants involving key transcription factors and their co-factors.

Table 1 lists the transcription factors that are currently associated with GD. Recent progress in either the identification of new transcription factors/co-factors causing DSD or how known genes cause DSD, are discussed below.

Variants in CBX2 are a very rare cause of 46,XYGD

The Chromobox homolog 2 (*CBX2*) gene, encodes a component of the polycomb (PcG) multiprotein complex that act as gatekeepers of transcription. Mice lacking *Cbx2* show male-to-female gonadal sex reversal (24). In mice genomic data indicate that *Cbx2* indirectly controls testis formation, by blocking the upregulation ovarian pathway genes and this results in the stabilization of the testicular fate (25). In the human, only a single case of 46,XY DSD has been reported to carry a pathogenic variant in *CBX2* (26). The 46,XY phenotypic female had bilateral ovaries and carried the biallelic variants, p.P98L (paternally inherited) and p.R443P (maternally inherited). The pathogenicity of these variants was

supported by functional analysis of the mutated proteins, which showed altered biological activity. Pathogenic variants in CBX2 associated with XY DSD are rare as other large-scale genomic studies have failed to identify any pathogenic variants in the gene (e.g. 27-29).

An isoform of CBX2, termed CBX2.2 - a truncated form of the main reference sequence CBX2.1 - has been suggested to cause 46,XY DSD (30). Two heterozygous variants (p.C132R and p.C154fs) were identified and proposed as pathogenic. However, analysis of population genetics data (<https://gnomad.broadinstitute.org>) indicates that p.C154fs is present in 1/125 of African-American populations including several 46,XY men who are homozygous for the variant. The CBX2.2 isoform has 23 reported loss-of-function (LOF) variants in the gnomAD database, several of which are present in the general populations at frequencies that are inconsistent with causing GD. These data exclude CBX2.2 as a cause of 46,XY DSD.

Variants located in the DMRT1-DM domain cause 46,XY GD

DMRT1 plays a key role in sex-determination in many vertebrates (3). In the human, deletions of chromosome 9p24 that contains several genes together with *DMRT1* are characterized by intellectual disability and a range of somatic anomalies. Approximately 70% of 46,XY individuals have anomalies of sex development ranging from GD to men with hypospadias and/or cryptorchidism (31). 9p24 deletions are a relatively common cause of syndromic 46,XY GD, pathogenic variants within the *DMRT1* coding sequences are however rare. We identified a *de novo* missense variant (p.R111G) in the functional DM-DNA-binding domain in a patient with non-syndromic 46,XY CGD (4). Functional assays indicated that the variant may cause GD by a combination of both haploinsufficiency and dominant negative activity on the wild-type DMRT1 protein. A second *DMRT1* variant, also associated with 46,XY CGD has been reported (29). This p.R80S variant is also located in the DM-domain and is predicted to disrupt DNA-binding. Large-scale exome sequencing studies of DSD cohorts (28) have not reported other *DMRT1* pathogenic variants. This suggest that pathogenic variants may be clustered within the functional DM-domain and disrupt the biological activity of the protein perhaps acting in a dominant negative manner.

GATA4 and co-factor ZFPM2 (FOG2) cause syndromic and non-syndromic 46,XY DSD

GATA4, a zinc finger transcription factor with two conserved type IV zinc finger domains plays a key role in testis development (32, 26). XY mice lacking *Gata4* are infertile and have PGD (33). Mice carrying a missense variant (p.V217G) in the N-terminal zinc finger (N-ZF) have testicular dysgenesis. This is due to a lack of physical interaction of the mutant protein with the cofactor ZFPM2 (FOG2; 33-35). Although pathogenic variants in GATA4 were first reported in association with congenital heart disease (CHD) and not DSD, a fraction of XY individuals with 8p23.1 deletions that includes the *GATA4* locus, have hypospadias and bilateral cryptorchidism suggesting a link to XY DSD (36). We established that GATA4 causes XY GD by identification a familial case of 46,XY DSD and CHD carrying heterozygous missense variant (p.G221R; 37). This variant is also located in the N-ZF that is essential for testis formation in the mouse. This mutant protein lacked DNA-binding activity, was unable to transactivate the AMH promoter and did not bind to the ZFPM2 protein (37). Other GATA4 variants that are established to cause 46,XY GD are also located within the N-ZF motif (38-40). Functional studies have indicated that variants located in sequences flanking the N-ZF are not responsible for GD (40).

The GATA4 cofactor, ZFPM2 (FOG2), acts as a context-dependent co-repressor or co-activator through binding to the GATA N-ZF (41). ZFPM2 also plays a key role in testis-determination since XY *Zfpm2*^{-/-} mice fail to develop testis (42). We established that variants in ZFPM2 cause 46,XY DSD by identification of a familial case of 46,XY GD, where a heterozygous missense variant (p.S402R) segregated with the phenotype and abolishes the interaction of the ZFPM2 with the GATA4 protein (43). A second independent individual had a *de novo* missense variant (p.R260Q) in the N-ZF of the protein as well as homozygosity for a rare missense variant p.M544I that altered the biological activity of the protein (43). Large scale exome sequencing studies havenot identified pathogenic variants in *ZFPM2* suggesting that ZFPM2 is a very rare cause of DSD (28, 29 and unpublished data).

MYRF: A new player in 46,XY DSD

Myelin regulatory factor (MYRF) is a widely expressed, large membrane-associated homo-trimeric protein that self-cleaves to release an N-terminal immunoglobulin-type Ndt80 that binds to DNA and regulates gene transcription (44-46). Deleterious, LOF and often *de novo* variants in MYRF are associated with congenital diaphragmatic hernia (CDH),

cardiac anomalies including Scimitar syndrome, encephalopathy and urogenital anomalies (ambiguous external genitalia, hypospadias, horseshoe kidney, chordee or cryptorchidism; 47-51). Most MYRF variants causing syndromic DSD are *de novo* and LOF. Two 46,XY boys have been reported with DSD (micropenis, hypospadias, small testis and cryptorchidism with low levels of testosterone and AMH) and no evidence of somatic anomalies (51). These data suggest MYRF variants may cause PGD but this needs to be confirmed by gonadal histology. We have observed syndromic forms of 46,XYGD associated with MYRF LOF variants (unpublished data). In 46,XX individuals, small ovaries and a lack of internal genitalia have been reported (51). Due to small number of reported cases of DSD linked to MYRF, neither the genotype-phenotype correlation nor the full range of phenotypes associated with variants in the gene has been established.

New Genomic Variants involving SOX8 & SOX9 are associated with DSD

Over the past 27 years pathogenic variants in the *SOX9* gene have been reported in association with Campomelic dysplasia together with varying degrees of testicular anomalies in around 75% of affected 46,XY individuals (52, 53). However, the recent discoveries of structural changes involving multiple regions both upstream and downstream of the *SOX9* gene has rekindled the interest in *SOX9* variants as a cause of non-syndromic XY and XX DSD.

The *SOX9* locus is large (approximately 2 Mb) and contains multiple enhancer elements that drive *SOX9* expression in different tissues during sex-determination and chondrocyte differentiation (54). The genomic analysis of the *SOX9* locus in individuals with DSD identified a key regulatory element termed *RevSex*, located approximately 600kb upstream from *SOX9*. *RevSex* is duplicated in individuals with 46,XX (ovo)testicular DSD and deleted in individuals with 46,XY GD (55-58). Further analysis of individuals with 46,XX DSD has narrowed *RevSex* to a 24 kb minimal region that contains a core enhancer motif termed eSR-B (59). Another non-overlapping 5.2 kb core enhancer element, termed XYSR, lies adjacent to eSR-B (60). The XYSR region contains the enhancer motif eSR-A. Deletions of eSR-A are associated with XY GD, whereas duplications are associated with XX DSD (61). The structural changes within *RevSex* are an important cause of DSD. Together they can explain 10% of all patients with either 46,XY GD or *SRY*-negative 46,XX (ovo)testicular DSD.

In mice, a 1.4 kb region, termed *Tesco*, located 13 kb upstream of *Sox9* positively regulates *Sox9* expression by binding to sex-determining factors NR5A1, SRY and SOX9 (62). However, biological role of *Tesco* is unclear since deletions of *Tesco* in mice does not cause male-to-female sex-reversal and rearrangements involving *TESCO* have not been identified in DSD (63). A third potential *SOX9* enhancer of 1.2kb, termed eALDI, was identified by a bioinformatics screen (61). eALDI is located immediately upstream of *TESCO*, but no rearrangements or variants of eALDI are reported in DSD (61). These regulatory elements are not routinely screened in a diagnostic setting and thus the incidence of DSD due to these rearrangements may be underestimated. It is also possible that other regions at the *SOX9* locus may contribute to DSD.

Another SOX family member, SOX8, is co-expressed with NR5A1 and SOX9 in the early stages of human testis-determination in Sertoli cells (64). Rearrangements at the *SOX8* locus are associated with 46,XY DSD. A pericentric inversion and a complex rearrangement of *SOX8* are associated with 46,XY non-syndromic GD and 46,XY syndromic GD respectively (64). A 560 kb duplication located approximately 18 kb upstream of *SOX8* was reported in a child with 46,XY GD, skeletal and cardiac anomalies and developmental delay (65). Only a single case of 46,XY GD has been described carrying a pathogenic missense variant in *SOX8* (64). This variant, located within the HMG-box, displays dominant negative activity on both wild-type *SOX8* and *SOX9* proteins. This may explain why pathogenic variants in *SOX8* causing GD are rare. Since there may be functional redundancy between *SOX8* and *SOX9* in testis-determination, variants causing GD would be predicted to interfere with both *SOX8* and *SOX9* function. Genetic studies support this with variants flanking the HMG-box associated with a milder phenotype of infertility (64).

Ribosome biogenesis and 46,XYGD: an unexpected association

A common cause of 46,XYGD and testicular regression sequence (TRS): The RNA-helicase DHX37

TRS is characterized by anomalies of genitalia and sexual duct formation and absence of gonadal tissue on one or both sides in 46,XY individuals (66-68). Families have been described with the occurrence of both TRS and 46,XY GD or agonadism, indicating a

common genetic origin for these phenotypes (69, 70). Anorchia is defined as the absence of one or two testis in an individual with typical male external genitalia. The genetic cause of TRS was unknown until recently, when we and others identified novel or very rare missense variants in an RNA-helicase termed DHX37 in a total of 36 individuals with 46,XY GD, TRS or anorchia (29, 71-73). This suggests a common genetic etiology for these phenotypes.

DHX37 is a member of the DEAH family of RNA-helicases with highly conserved functional elements including two flexibly linked RecA domains. RNA helicases are key elements of the ribosome biogenesis machinery in eukaryotic cells that are required for recruitment or dissociation of ribosomal proteins and binding factors (74-77). Although much of our knowledge on ribosome biogenesis and the role of DHX37 comes from studies in yeast, the function of DHX37 in ribosome biogenesis appears conserved (78). Thus, a subset of XY DSD, caused by DHX37 variants, define a new human ribosomopathy.

The pathogenic variants causing DSD localize to highly conserved motifs within the RecA1 and RecA2 domains of the protein (29, 71-73). There is no evidence for a genotype-phenotype correlation. Almost half of published cases are due to a single amino acid variant (p.R308Q) and the associated phenotypes range from 46,XY GD raised as female to 46,XY TRS with severe micropenis (with or without cryptorchidism and hypospadias). The mode of transmission of the phenotype is consistent with a sex-limited autosomal dominant mode of inheritance. The incidence of DHX37 pathogenic variants associated with 46,XYGD is similar to that observed with the *SRY*, *MAP3K1* or *NR5A1* genes (10-15%). DHX37 variants also account for approximately 20% of all cases of TRS (71-73).

The association of DHX37 variants with human congenital disorders is complex and includes phenotypes other than DSD. Compound heterozygous as well as *de novo* heterozygous DHX37 variants cause a complex congenital developmental syndrome termed NEDBAVC (Neurodevelopmental disorder with brain anomalies and with or without vertebral or cardiac anomalies; 79, 80) with no reported DSD. Some of these variants fall within the same functional domains that cause XY DSD and usually involve amino acids that are highly conserved. How variants in DHX37 cause either a syndromic neurodevelopmental disorder or 46,XY DSD remains to be resolved. Individuals with 46,XY DSD due to DHX37 variants may require long-term clinical monitoring, since other forms of ribosomopathies have up to a 200 fold higher risk to develop cancer (81).

46,XY GD caused by factors involved in cell signaling pathways

New perspectives on Hedgehog signaling and DSD: DHH and HHAT

The Desert Hedgehog (DHH), a product of Sertoli cells in the fetal testis, regulates the specification and development of androgen producing fetal Leydig cells (82). DHH is expressed as unprocessed preproprotein that undergoes processing and auto-catalytic cleavage. Following cleavage, the N-terminal fragment of 19 kDa (HhN) retains all Hh signaling activity (82). Biallelic variants of DHH cause 46,XY GD with or without polyneuropathy (83, 84). Recently, *in vitro* assays suggested that variants interrupting the N-terminal fragment are associated with GD and polyneuropathy, whereas those effecting the auto-processing of the protein are associated only with GD (84). Although large-scale exome sequencing studies of DSD cohorts continue to reveal heterozygous variants of DHH, methodical functional analysis indicates that these are unlikely to contribute to GD (28, 85).

Hedgehog acyltransferase (HHAT) is a member of the membrane bound-O-acyltransferase (MBOAT) family of enzymes that catalyze the attachment of specific fatty acids to secreted proteins (86). HHAT catalyzes the palmitoylation of Hedgehog family members (80). A homozygous p.G287V variant in the MBOAT domain was reported in familial case of two sibs with syndromic 46,XY and 46,XX DSD (87). The variant disrupts the ability of the HHAT protein to palmitoylate DHH (87). One sib presented with 46,XY GD and multiple congenital anomalies (Nivelon-Nivelon-Mabille syndrome), whereas the other sib was 46,XX with histologically normal ovaries and a similar complex somatic phenotype. In another consanguineous family a biallelic in-frame deletion within the MBOAT domain was associated with severe microphthalmia, microcephaly, skeletal dysplasia, facial dysmorphism and 46,XY GD (88). A further case was reported of a girl with 46,XY GD and microcephaly carried a novel homozygous missense variant within the MBOAT domain (89). Thus, homozygous variants within the MBOAT domain of HHAT cause a rare form of XY GD with variable somatic anomalies.

PPP2R3C: A new syndromic form of 46,XYGD and infertility

The gene *PPP2R3C* encodes the B''gamma subunit of protein phosphatase 2A (PP2A) that is required for dephosphorylation of serine and threonine residues in proteins (90-92). Four girls from unrelated families presented with 46,XY complete GD, facial dysmorphism, myopathy and retinal dystrophy. Each girl carried biallelic variants in *PPP2R3C*, establishing this gene as a new recessive cause of syndromic 46,XY GD (93). Male and female heterozygous carriers of *PPP2R3C* variants exhibit variable degrees of infertility (93).

ZNRF3: Disruption of WNT signaling leads to 46,XY DSD

ZNRF3 is a transmembrane E3 ubiquitin ligase that inhibits WNT signaling by targeting Frizzled receptors for degradation by ubiquitination (94, 95). R-spondins promote WNT signaling by binding to and sequestering *ZNRF3* (94, 95). The link between *ZNRF3* and 46,XY DSD was established by the identification of four variants in *ZNRF3* involving five individuals with a range of 46,XY DSD phenotypes including GD (19). Two of the four variants associated with DSD are missense and functional studies demonstrated that they disrupted the ability of *ZNRF3* to inhibit canonical WNT signals. Mice carrying only one copy of the *Znrf3* gene on a sensitized background showed a broad range of testicular dysgenesis consistent with the human DSD phenotypes. Therefore, *ZNRF3* variants may contribute to a wide spectrum of DSD phenotypes, however, with only a single published study, the incidence of *ZNRF3* variants in DSD and genotype-phenotype correlations remain to be defined.

Other genes reportedly associated 46,XYGD: Evidence for causality.

The availability of high throughput sequencing has enabled exome sequencing of large numbers of DSD cohorts, generating many possible candidate genes for GD. However, without conclusive genetic and/or supporting experimental evidence, the contribution of these gene to GD is inconclusive. These include Estrogen Receptor 2 (*ESR2*), Fibroblast Growth Factor Receptor 2 (*FGFR2*), 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (*HMGCS2*), LIM Homeobox 9 (*LHX9*) and StAR Related Lipid Transfer Domain Containing 8 (*STARD8*) genes.

A link between 46,XY DSD and *ESR2* was proposed by the analysis of 3 patients with monoallelic or biallelic variants in the gene (96). The phenotypes were variable, consisting of (i) a syndromic form of 46,XY gonadism, (ii) complete GD and (iii) an androgen insensitivity-like syndrome. However, the link between *ESR2* variants with these varied phenotypes is unclear. One proposed pathogenic variant is a polymorphism in South East Asian populations, which is inconsistent with pathogenicity. The supporting experimental evidence for a role of *ESR2* is weak, since expression of *ESR2* was not detected in the gonad of an 8-week-old XY human embryo and the biological activity of the mutant proteins was comparable to wild-type *ESR2* (96). The evidence supporting a contribution of *ESR2* variants to 46,XY DSD is therefore inconclusive.

The second gene proposed to cause 46,XYGD is *FGFR2*. Mice lacking *Fgfr2* fail to form testes and hence this gene is a candidate for DSD (97). A heterozygous p.C342S variant in *FGFR2* was reported in girl with 46,XY GD and craniosynostosis (98). However, heterozygous pathogenic variants in *FGFR2* are well-established to cause a range of craniosynostosis phenotypes, including Crouzon syndrome, Pfeiffer syndrome and Apert syndrome, with no evidence for DSD (99-102). The p.C342S variant is a known cause of Crouzon or Pfeiffer syndromes in 46,XY males (99, 100). This strongly suggests that the GD seen in these patients is caused by a pathogenic variant involving a DSD gene elsewhere in the genome.

The third candidate *HMGCS2* encodes a metabolic enzyme in the liver important for energy production from fatty acids (103). In a screen of patients with 46,XY GD, two heterozygous variants were identified (103), however, autosomal recessive variants in *HMGCS2* are a well-established cause of HMG-CoA synthase-2 deficiency with no evidence of DSD and heterozygous carriers are healthy (104).

The fourth candidate is *STARD8*, a Rho-GTPase, which maps to chromosome Xq13 and consists of a sterile alpha motif (SAM), GAP and START (steroidogenic acute regulatory protein [StAR]-related lipid transfer) domain (105, 106). A missense variant in, *STARD8*, has been proposed to cause 46,XY GD in two sisters (107) They carried a hemizygous missense variant (p.S993N; rs201005000), inherited from their heterozygous mother. However, this

variant, predicted to be benign, is present at a relatively high allelic frequency (1/500) in the Bulgarian population. Although a promising candidate, the evidence for pathogenicity of *STARD8* variants in DSD remains inconclusive.

The fifth candidate is *LHX9*. A single *de novo* and novel missense variant p.Q316R located within the conserved DNA-binding homeodomain, was identified in a patient with syndromic 46,XY DSD (108). Although functional studies have not been performed on this variant, *LHX9* is a good candidate for 46,XY DSD as both XY and XX mice lacking *Lhx9* are agonadal females (109).

Conclusions

Large scale high-throughput sequencing studies continue to reveal new genes and loci causing 45,XY GD. However, around 50% of all cases remain unexplained (28, 29, unpublished data). A proportion of these are likely to be due to variants in regulatory elements such as those described for *SOX9* (61). These are currently not screened in a diagnostic setting but as whole genome sequencing becomes more widely available, pathogenic variants in regulatory elements will become an important feature in the genetics of DSD.

Although new genes continue to be identified and proposed to cause GD, they raise significant challenges. The first is critical evaluation of the evidence to support causality for new genes/variants causing GD. For some factors, such as *DHX37*, pathogenicity is established by overwhelming human genetics data, even in the absence of functional studies, animal models or a biological context that is relevant for testis formation (29, 71-73). One recurrent theme is that each gene (with the likely exception of *DHX37*) explains only a very small number of cases. A single individual has been reported with a pathogenic variant in *CBX2* and only 2 individuals have been reported with pathogenic variants *DMRT1*. In this situation the evidence in support of causality may be addressed by a combination of following questions -

-Is the transmission of the variant compatible with 46,XY GD?

- Does population genetics data support a role for the gene/variant in GD?
- Is there robust experimental evidence or animal/cellular model to support pathogenicity?
- Is the gene/variant associated with other human congenital disorders that do not include DSD?
- What is the known biological role of the gene?

Where pathogenicity is established, the small number of individuals associated with pathogenic variants in each gene creates other issues. These includes establishing accurate genotype-phenotype correlations, defining the range of phenotypes associated with a single gene/variant and the lack of statistical data to accurately determinelong-term outcomes and individual cancer risk.

To overcome these issues clinical and genetic data should be made publicly available. This can be achieved by various routes including publishing data from large DSD cohorts, submitting data to international disease registries such as I-DSD (<https://home.i-dsd.org/>) and submitting data to public archives of reports on the relationship between human variations and phenotypes (e.g. ClinVar- <https://www.ncbi.nlm.nih.gov/clinvar/>).

Practice Points

- Sex determination in mammals is a complex process where the testis or an ovary develop from a common bipotential anlage. This requires precise spatio-temporal interplay between mutually exclusive and antagonistic genes and networks involved in the testicular or ovarian pathways.
- Disorders/Difference of Sex Determination/Development (DSD) are a group of heterogeneous rare congenital conditions in which the chromosomal, phenotypic, and anatomical sex are discordant. 46,XY gonadal DSD includes complete or partial gonadal dysgenesis (GD), undervirilisation or undermasculinisation of an XY male.
- A large proportion of 46,XY GD is caused by variants in key transcription factors required for establishment of the testicular fate (e.g: *SRY*, *NR5A1*, *MAP3K1* and *SOX* factors).
- Variants in the RNA-helicase *DHX37* have emerged as a common cause of 46,XYGD and testicular regression sequence, thus definingg this subset of XY DSDs as a new human ribosomopathy.
- High throughput sequencing has generatied several novel possible candidate for GD. However, rigorous genetic evidence supported by experimental data is imperative for establishing the role of these candidates in 46,XY DSD.

Research Agenda

- The identification of pathogenic variants in regulatory elements will be an important feature in the genetics of DSD.
- A critical evaluation of (genetic/experimental) evidence to support of causality for newly discovered genes/variants causing GD is still missing in some cases and needs a careful and systematic assessment.
- To enhance the diagnostic value, the clinical and genetic data should be made publicly available to overcome the lack of statistical power due to the small number of individuals associated with pathogenic variants in each gene.

Acknowledgements

This work is funded in part by a research grant from the European Society of Pediatric Endocrinology (to AB), and by the Agence Nationale de la Recherche (ANR; ANR-10-LABX-73 REVIVE to KM, ANR-17-CE14-0038-01 to KM, ANR-19-CE14-0022 to AB and ANR-19-CE14-0012 to AB).

References

- *1. Capel B. Vertebrate sex determination: evolutionary plasticity of a fundamental switch. *Nat Rev Genet.* 2017;18:675-689.
2. Bagheri-Fam S, Sim H, Bernard P, Jayakody I, Taketo MM, Scherer G, Harley VR. Loss of *Fgfr2* leads to partial XY sex reversal. *Dev Biol.* 2008 Feb 1;314(1):71-83
3. Zarkower D, Murphy MW. *DMRT1*: An Ancient Sexual Regulator Required for Human Gonadogenesis. *Sex Dev* 2021. doi: 10.1159/000518272
- *4. Murphy MW, Lee JK, Rojo S, Gearhart MD, Kurahashi K, Banerjee S, et al. An ancient protein-DNA interaction underlying metazoan sex determination. *Nat Struct Mol Biol.* 2015 Jun;22(6):442-51.
5. Rahmoun M, Lavery R, Laurent-Chaballier S, Bellora N, Philip GK, Rossitto M, et al. In mammalian foetal testes, *SOX9* regulates expression of its target genes by binding to genomic regions with conserved signatures. *Nucleic Acids Res.* 2017;45:7191-7211.
6. Lindeman RE, Murphy MW, Agrimson KS, Gewiss RL, Bardwell VJ, Gearhart MD, Zarkower D. The conserved sex regulator *DMRT1* recruits *SOX9* in sexual cell fate reprogramming. *Nucleic Acids Res.* 2021;49:6144-6164.
7. Elzaïat M, Todeschini AL, Caburet S, Veitia RA. The genetic make-up of ovarian development and function: the focus on the transcription factor *FOXL2*. *Clin Genet.* 2017;91:173-182.
- *8. Chassot AA, Gillot I, Chaboissier MC. *R-spondin1*, *WNT4*, and the *CTNNB1* signaling pathway: strict control over ovarian differentiation. *Reproduction.* 2014;148:R97-110.
9. Nicol B, Grimm SA, Chalmel F, Lecluze E, Pannetier M, Pailhoux E, Dupin-De-Beyssat E, Guiguen Y, Capel B, Yao HH. *RUNX1* maintains the identity of the fetal ovary through an interplay with *FOXL2*. *Nat Commun.* 2019;10:5116.
10. Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, et al. Somatic sex reprogramming of adult ovaries to testes by *FOXL2* ablation. *Cell.* 2009;139:1130-42.
11. Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. *DMRT1* prevents female reprogramming in the postnatal mammalian testis. *Nature.* 2011;476:101-4.
12. Minkina A, Matson CK, Lindeman RE, Ghyselinck NB, Bardwell VJ, Zarkower D. *DMRT1* protects male gonadal cells from retinoid-dependent sexual transdifferentiation. *Dev Cell.* 2014;29:511-520.

13. Lavery R, Chassot AA, Pauper E, Gregoire EP, Klopfenstein M, de Rooij DG, et al. Testicular differentiation occurs in absence of R-spondin1 and Sox9 in mouse sex reversals. *PLoS Genet.* 2012;8(12):e1003170.
14. Nicol B, Yao HH. Gonadal Identity in the Absence of Pro-Testis Factor SOX9 and Pro-Ovary Factor Beta-Catenin in Mice. *Biol Reprod.* 2015 Aug;93(2):35.
15. Hughes IA, Houk C, Ahmed SF, Lee PA; LWPES Consensus Group; ESPE Consensus Group. Consensus statement on management of intersex disorders. *Arch Dis Child* 2006, 91:554-563
- *16. Lee PA, Nordenström A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al; Global DSD Update Consortium. Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care. *Horm Res Paediatr.* 2016;85(3):158-80.
- *17. Bashamboo A, Eozenou C, Rojo S, McElreavey K. Anomalies in human sex determination provide unique insights into the complex genetic interactions of early gonad development. *Clin Genet.* 2017 Feb;91(2):143-156.
- *18. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM, Greenfield A, Bashamboo A, Hiort O, Wudy SA, McGowan R; The EU COST Action. 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.* 2018 Oct 1;179(4):R197-R206.
19. Harris A, Siggers P, Corrochano S, Warr N, Sagar D, Grimes DT, et al. ZNRF3 functions in mammalian sex determination by inhibiting canonical WNT signaling. *Proc Natl Acad Sci U S A.* 2018;115:5474-5479.
20. Josso N, Rey RA. What Does AMH Tell Us in Pediatric Disorders of Sex Development? *Front Endocrinol (Lausanne).* 2020 Sep 8;11:619.
21. Mongan NP, Tadokoro-Cuccaro R, Bunch T, Hughes IA. Androgen insensitivity syndrome. *Best Pract Res Clin Endocrinol Metab.* 2015 Aug;29(4):569-80.
22. Baronio F, Ortolano R, Menabò S, Cassio A, Baldazzi L, Di Natale V, et al. 46,XX DSD due to Androgen Excess in Monogenic Disorders of Steroidogenesis: Genetic, Biochemical, and Clinical Features. *Int J Mol Sci.* 2019;20: E4605.
23. Grinspon RP, Bergadá I, Rey RA. Male Hypogonadism and Disorders of Sex Development. *Front Endocrinol (Lausanne).* 2020 Apr 15;11:211.
24. Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Noguchi K, et al. Male-to-female sex reversal in M33 mutant mice. *Nature.* 1998 Jun 18;393(6686):688-92.

25. Garcia-Moreno SA, Lin YT, Futtner CR, Salamone IM, Capel B, Maatouk DM. CBX2 is required to stabilize the testis pathway by repressing Wnt signaling. *PLoS Genet.* 2019;15:e1007895.
26. Biason-Lauber A, Konrad D, Meyer M, DeBeaufort C, Schoenle EJ. Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the CBX2 gene. *Am J Hum Genet.* 2009;84:658-63.
27. Norling A, Hirschberg AL, Iwarsson E, Wedell A, Barbaro M. CBX2 gene analysis in patients with 46,XY and 46,XX gonadal disorders of sex development. *FertilSteril.* 2013;99:819-826.e3.
- *28. Eggers S, Sadedin S, van den Bergen JA, Robevska G, Ohnesorg T, Hewitt J, et al. Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. *Genome Biol.* 2016 Nov 29;17(1):243.
- *29. Buonocore F, Clifford-Mobley O, King TFJ, Striglioni N, Man E, Suntharalingham JP, et al. Next-Generation Sequencing Reveals Novel Genetic Variants (SRY, DMRT1, NR5A1, DHH, DHX37) in Adults With 46,XY DSD. *J Endocr Soc.* 2019;3:2341-2360
30. Sproll P, Eid W, Gomes CR, Mendonca BB, Gomes NL, Costa EM, et al. Assembling the jigsaw puzzle: CBX2 isoform 2 and its targets in disorders/differences of sex development. *Mol Genet Genomic Med.* 2018;6:785-795.
31. Ottolenghi C, McElreavey K. Deletions of 9p and the quest for a conserved mechanism of sex determination. *Mol Genet Metab.* 2000 Sep-Oct;71(1-2):397-404.
32. Robert NM, Miyamoto Y, Taniguchi H, Viger RS. LRH-1/NR5A2 cooperates with GATA factors to regulate inhibin alpha-subunit promoter activity. *Mol Cell Endocrinol.* 2006 Sep 26;257-258:65-74.
33. Manuylov NL, Zhou B, Ma Q, Fox SC, Pu WT, Tevosian SG. Conditional ablation of Gata4 and Fog2 genes in mice reveals their distinct roles in mammalian sexual differentiation. *Dev Biol.* 2011 May 15;353(2):229-41.
34. Crispino JD, Lodish MB, Thurberg BL, Litovsky SH, Collins T et al. Proper coronary vascular development and heart morphogenesis depend on interaction of GATA-4 with FOG cofactors. *Genes Dev* 2001, 15:839-44
35. Bouma GJ, Washburn LL, Albrecht KH, Eicher EM. Correct dosage of Fog2 and Gata4 transcription factors is critical for fetal testis development in mice. *Proc Natl Acad Sci U S A* 2007;104:14994-9
36. Wat MJ, Shchelochkov OA, Holder AM, Breman AM, Dagli A et al. Chromosome 8p23.1 deletions as a cause of complex congenital heart defects and diaphragmatic hernia. *Am J Med Genet A* 2009;149A:1661-77

37. Lourenço D, Brauner R, Rybczynska M, Nihoul-Fékété C, McElreavey K, Bashamboo A. Loss-of-function mutation in GATA4 causes anomalies of human testicular development. *Proc Natl Acad Sci U S A*. 2011 Jan 25;108(4):1597-602.
38. Martinez de LaPiscina I, de Mingo C, Riedl S, Rodriguez A, Pandey AV, Fernández-Cancio M, et al. GATA4 Variants in Individuals With a 46,XY Disorder of Sex Development (DSD) May or May Not Be Associated With Cardiac Defects Depending on Second Hits in Other DSD Genes. *Front Endocrinol (Lausanne)*. 2018 Apr 4;9:142.
39. Choi JH, Lee Y, Oh A, Kim GH, Yoo HW. Molecular Characteristics of Sequence Variants in GATA4 in Patients with 46,XY Disorders of Sex Development without Cardiac Defects. *Sex Dev*. 2019;13(5-6):240-245.
40. van den Bergen JA, Robevska G, Eggers S, Riedl S, Grover SR, Bergman PB, et al. Analysis of variants in GATA4 and FOG2/ZFPM2 demonstrates benign contribution to 46,XY disorders of sex development. *Mol Genet Genomic Med*. 2020 Mar;8(3):e1095.
41. Zaytouni T, Efimenko EE, Tevosian SG. GATA transcription factors in the developing reproductive system. *Adv Genet*. 2011;76:93-134.
42. Tevosian SG, Albrecht KH, Crispino JD, Fujiwara Y, Eicher EM, Orkin SH. Gonadal differentiation, sex determination and normal Sry expression in mice require direct interaction between transcription partners GATA4 and FOG2. *Development*. 2002 Oct;129(19):4627-34
43. Bashamboo A, Brauner R, Bignon-Topalovic J, Lortat-Jacob S, Karageorgou V, Lourenco D, et al. Mutations in the FOG2/ZFPM2 gene are associated with anomalies of human testis determination. *Hum Mol Genet*. 2014 Jul 15;23(14):3657-65.
44. Kim D, Choi JO, Fan C, Shearer RS, Sharif M, Busch P, Park Y. Homo-trimerization is essential for the transcription factor function of Myrf for oligodendrocyte differentiation. *Nucleic Acids Res*. 2017;45:5112-5125.
45. Senoo H, Araki T, Fukuzawa M, Williams JG. A new kind of membrane-tethered eukaryotic transcription factor that shares an auto-proteolytic processing mechanism with bacteriophage tail-spike proteins. *J Cell Sci*. 2013;126:5247-58.
46. Li Z, Park Y, Marcotte EM. A Bacteriophage tailspike domain promotes self-cleavage of a human membrane-bound transcription factor, the myelin regulatory factor MYRF. *PLoS Biol*. 2013;11:e1001624.
47. Garnai SJ, Brinkmeier ML, Emery B, Aleman TS, Pyle LC, Veleva-Rotse B, et al. Variants in myelin regulatory factor (MYRF) cause autosomal dominant and syndromic nanophthalmos in humans and retinal degeneration in mice. *PLoS Genet*. 2019;15:e1008130.

48. Hamanaka K, Takata A, Uchiyama Y, Miyatake S, Miyake N, Mitsushashi S, et al. MYRF haploinsufficiency causes 46,XY and 46,XX disorders of sex development: bioinformatics consideration. *Hum Mol Genet.* 2019;28:2319-2329.
49. Qi H, Yu L, Zhou X, Wynn J, Zhao H, Guo Y, et al. De novo variants in congenital diaphragmatic hernia identify MYRF as a new syndrome and reveal genetic overlaps with other developmental disorders. *PLoS Genet.* 2018;14:e1007822.
50. Rossetti LZ, Glinton K, Yuan B, Liu P, Pillai N, Mizerik E, et al. Review of the phenotypic spectrum associated with haploinsufficiency of MYRF. *Am J Med Genet A.* 2019;179:1376-1382.
51. Pinz H, Pyle LC, Li D, Izumi K, Skraban C, Tarpinian J, et al. De novo variants in Myelin regulatory factor (MYRF) as candidates of a new syndrome of cardiac and urogenital anomalies. *Am J Med Genet A.* 2018;176:969-972.
52. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA et al: Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature* 1994;372:525-530.
53. Katoh-Fukui Y, Igarashi M, Nagasaki K, Horikawa R, Nagai T et al: Testicular dysgenesis/regression without campomelic dysplasia in patients carrying missense mutations and upstream deletion of SOX9. *Mol Genet Genomic Med* 2015;3:550-557.
54. Lefebvre V, Angelozzi M, Haseeb A. SOX9 in cartilage development and disease. *Curr Opin Cell Biol.* 2019 Dec;61:39-47.
55. Benko S, Gordon CT, Mallet D, Sreenivasan R, Thauvin-Robinet C et al: Disruption of a long distance regulatory region upstream of SOX9 in isolated disorders of sex development. *J Med Genet* 2011;48:825-830.
56. Cox JJ, Willatt L, Homfray T, Woods CG. A SOX9 duplication and familial 46,XX developmental testicular disorder. *N Engl J Med* 2011;364:91-93.
57. Vetro A, Dehghani MR, Kraoua L, Giorda R, Beri S et al. Testis development in the absence of SRY: chromosomal rearrangements at SOX9 and SOX3. *Eur J Hum Genet* 2015;23:1025-1032.
- *58. Hyon C, Chantot-Bastarud S, Harbuz R, Bhourri R, Perrot N et al. Refining the regulatory region upstream of SOX9 associated with 46,XX testicular disorders of Sex Development (DSD). *Am J Med Genet A* 2015;167:1851-1858.
59. Ohnesorg T, van den Bergen JA, Belluoccio D, Shankara-Narayana N, Kean AM, Vasilaras A, et al. A duplication in a patient with 46,XX ovo-testicular disorder of sex development refines the SOX9 testis-specific regulatory region to 24 kb. *Clin Genet.* 2017 Sep;92(3):347-349.

60. Kim GJ, Sock E, Buchberger A, Just W, Denzer F et al. Copy number variation of two separate regulatory regions upstream of SOX9 causes isolated 46,XY or 46,XX disorder of sex development. *J Med Genet* 2015;52:240-247.
61. Croft B, Ohnesorg T, Hewitt J, Bowles J, Quinn A, Tan J, et al. Human sex reversal is caused by duplication or deletion of core enhancers upstream of SOX9. *Nat Commun*. 2018 Dec 14;9(1):5319.
62. Sekido R, Lovell-Badge R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature*. 2008 Jun 12;453(7197):930-4.
63. Gonen N, Quinn A, O'Neill HC, Koopman P, Lovell-Badge R. Normal Levels of Sox9 Expression in the Developing Mouse Testis Depend on the TES/TESCO Enhancer, but This Does Not Act Alone. *PLoS Genet*. 2017 Jan 3;13(1):e1006520.
64. Portnoi MF, Dumargne MC, Rojo S, Witchel SF, Duncan AJ, Eozenou C, et al. Mutations involving the SRY-related gene SOX8 are associated with a spectrum of human reproductive anomalies. *Hum Mol Genet*. 2018 Apr 1;27(7):1228-1240.
65. Erickson RP, Yatsenko SA, Larson K, Cheung SW. A Case of Agonadism, Skeletal Malformations, Bicuspid Aortic Valve, and Delayed Development with a 16p13.3 Duplication Including GNG13 and SOX8 Upstream Enhancers: Are Either, Both or Neither Involved in the Phenotype? *Mol Syndromol*. 2011 Jan;1(4):185-191.
66. Edman CD, Winters AJ, Porter JC, Wilson J, MacDonald PC. Embryonic testicular regression. A clinical spectrum of XY gonadal individuals. *Obstet Gynecol*. 1977;49:208–217
67. Josso N, Briard ML. Embryonic testicular regression syndrome: variable phenotypic expression in siblings. *J Pediatr*. 1980;97:200–204
68. Pirgon Ö, Dündar BN. Vanishing testes: a literature review. *J Clin Res Pediatr Endocrinol*. 2012;4:116–120
- *69. Marcantonio SM, Fechner PY, Migeon CJ, Perlman EJ, Berkovitz GD. Embryonic testicular regression sequence: a part of the clinical spectrum of 46,XY gonadal dysgenesis. *Am J Med Genet*. 1994;49:1–5.
70. Fechner PY, Marcantonio SM, Ogata T, Rosales TO, Smith KD, Goodfellow PN, et al. Report of a kindred with X-linked (or autosomal dominant sex-limited) 46,XY partial gonadal dysgenesis. *J Clin Endocrinol Metab*. 1993;76:1248-53.
71. da Silva TE, Gomes NL, Lerário AM, Keegan CE, Nishi MY, Carvalho FM, et al. Genetic Evidence of the Association of DEAH-Box Helicase 37 Defects With 46,XY Gonadal Dysgenesis Spectrum. *J Clin Endocrinol Metab*. 2019;104:5923-5934.

- *72. McElreavey K, Jorgensen A, Eozenou C, Merel T, Bignon-Topalovic J, Tan DS, et al. Pathogenic variants in the DEAH-box RNA helicase DHX37 are a frequent cause of 46,XY gonadal dysgenesis and 46,XY testicular regression syndrome. *Genet Med*. 2020;22:150-159.
73. Zidoune H, Martinerie L, Tan DS, Askari M, Rezgoune D, Ladjouze A, Boukri A, Benelmadani Y, Sifi K, Abadi N, Satta D, Rastari M, Seresht-Ahmadi M, Bignon-Topalovic J, Mazen I, Leger J, Simon D, Brauner R, Totonchi M, Jauch R, Bashamboo A, McElreavey K. Expanding DSD Phenotypes Associated with Variants in the DEAH-Box RNA Helicase DHX37. *Sex Dev*. 2021 Jul 22:1-9.
74. Phipps KR, Charette J, Baserga SJ. The small subunit processome in ribosome biogenesis—progress and prospects. *Wiley Interdiscip Rev RNA*. 2011;2:1–21.
75. Zhu J, Liu X, Anjos M, Correll CC, Johnson AW. Utp14 recruits and activates the RNA helicase Dhr1 to undock U3 snoRNA from the preribosome. *Mol Cell Biol*. 2016;36:965–978.
76. Black JJ, Wang Z, Goering LM, Johnson AW. Utp14 interaction with the small subunit processome. *RNA*. 2018;24:1214–1228.
77. Bohnsack KE, Ficner R, Bohnsack MT, Jonas S. Regulation of DEAH-box RNA helicases by G-patch proteins. *Biol Chem*. 2021 Jan 6;402(5):561-579.
78. Choudhury P, Hackert P, Memet I, Sloan KE, Bohnsack MT. The human RNA helicase DHX37 is required for release of the U3 snoRNP from pre-ribosomal particles. *RNA Biol*. 2019 Jan;16(1):54-68.
79. Karaca E, Harel T, Pehlivan D, Jhangiani SN, Gambin T, Coban Akdemir Z, et al. Genes that Affect Brain Structure and Function Identified by Rare Variant Analyses of Mendelian Neurologic Disease. *Neuron*. 2015 Nov 4;88(3):499-513.
80. Paine I, Posey JE, Grochowski CM, Jhangiani SN, Rosenheck S, Kleyner R, et al. Paralog Studies Augment Gene Discovery: DDX and DHX Genes. *Am J Hum Genet*. 2019 Aug 1;105(2):302-316.
- 81 75. Kampen KR, Sulima SO, Vereecke S, De Keersmaecker K. Hallmarks of ribosomopathies. *Nucleic Acids Res*. 2020 Feb 20;48(3):1013-1028.
- 82 76. Huang CC, Yao HH. Diverse functions of Hedgehog signaling in formation and physiology of steroidogenic organs. *Mol. Reprod. Dev*. 2010;77:489–96.
83. Umehara F, Tate G, Itoh K, Yamaguchi N, Douchi T, Mitsuya T, Osame M. A novel mutation of desert hedgehog in a patient with 46,XY partial gonadal dysgenesis accompanied by minifascicular neuropathy. *Am J Hum Genet*. 2000 Nov;67(5):1302-5
84. Tajouri A, Kharrat M, Hizem S, Zaghdoudi H, M'rad R, Simic-Schleicher G, Kaiser FJ, Hiort O, Werner R. In vitro functional characterization of the novel DHH mutations

p.(Asn337Lysfs*24) and p.(Glu212Lys) associated with gonadal dysgenesis. *Hum Mutat.* 2018;39:2097-2109.

85. Ayers K, van den Bergen J, Robevska G, Listyasari N, Raza J, Atta I, et al. Functional analysis of novel desert hedgehog gene variants improves the clinical interpretation of genomic data and provides a more accurate diagnosis for patients with 46,XY differences of sex development. *J Med Genet.* 2019 Jul;56(7):434-443.

86. Matevossian A, and Resh MD. Membrane topology of hedgehog acyltransferase. *J. Biol. Chem* 2015;290: 2235–2243.

87. Callier P, Calvel P, Matevossian A, Makrythanasis P, Bernard P, Kurosaka H, et al. Loss of function mutation in the palmitoyl-transferase HHAT leads to syndromic 46,XY disorder of sex development by impeding Hedgehog protein palmitoylation and signaling. *PLoS Genet.* 2014;10:e1004340.

88. Abdel-Salam GMH, Mazen I, Eid M, Ewida N, Shaheen R, Alkuraya FS. Biallelic novel missense HHAT variant causes syndromic microcephaly and cerebellar-vermis hypoplasia. *Am J Med Genet A.* 2019 Jun;179(6):1053-1057.

89. Mazen I, Mekkawy M, Kamel A, Essawi M, Hassan H, Abdel-Hamid M, et al. Advances in genomic diagnosis of a large cohort of Egyptian patients with disorders of sex development. *Am J Med Genet A.* 2021 Jun;185(6):1666-1677.

90. Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem J.* 2001 Feb 1;353(Pt 3):417-39.

91. Katayama K, Yamaguchi M, Noguchi K, Sugimoto Y. Protein phosphatase complex PP5/PPP2R3C dephosphorylates P-glycoprotein/ABCB1 and down-regulates the expression and function. *Cancer Lett.* 2014 Apr 1;345(1):124-31.

92. Reynhout S, Janssens V. Physiologic functions of PP2A: Lessons from genetically modified mice. *BiochimBiophys Acta Mol Cell Res.* 2019 Jan;1866(1):31-50.

93. Guran T, Yesil G, Turan S, Atay Z, Bozkurtlar E, Aghayev A, et al. PPP2R3C gene variants cause syndromic 46,XY gonadal dysgenesis and impaired spermatogenesis in humans. *Eur J Endocrinol.* 2019 May 1;180(5):291-309.

94. Hao HX, Xie Y, Zhang Y, Charlat O, Oster E, Avello M, et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature.* 2012;485:195-200.

95. Xie Y, Zamponi R, Charlat O, Ramones M, Swalley S, Jiang X, et al. Interaction with both ZNRF3 and LGR4 is required for the signalling activity of R-spondin. *EMBO Rep.* 2013;14:1120-6.

96. Baetens D, Güran T, Mendonca BB, Gomes NL, De Cauwer L, Peelman F, et al. Biallelic and monoallelic ESR2 variants associated with 46,XY disorders of sex development. *Genet Med*. 2018;20:717-727.
97. Kim Y, Bingham N, Sekido R, Parker KL, Lovell-Badge R, Capel B. Fibroblast growth factor receptor 2 regulates proliferation and Sertoli differentiation during male sex determination. *Proc Natl Acad Sci U S A*. 2007 Oct 16;104(42):16558-63.
98. Bagheri-Fam S, Ono M, Li L, Zhao L, Ryan J, Lai R, et al. FGFR2 mutation in 46,XY sex reversal with craniosynostosis. *Hum Mol Genet*. 2015 Dec 1;24(23):6699-710.
99. Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. *Nat Genet*. 1994 Sep;8(1):98-103.
100. Rutland P, Pulleyn LJ, Reardon W, Baraitser M, Hayward R, Jones B, et al. Identical mutations in the FGFR2 gene cause both Pfeiffer and Crouzon syndrome phenotypes. *Nat Genet*. 1995 Feb;9(2):173-6.
101. Kan SH, Elanko N, Johnson D, Cornejo-Roldan L, Cook J, Reich EW, et al. Genomic screening of fibroblast growth-factor receptor 2 reveals a wide spectrum of mutations in patients with syndromic craniosynostosis. *Am J Hum Genet*. 2002 Feb;70(2):472-86
102. Tartaglia M, Di Rocco C, Lajeunie E, Valeri S, Velardi F, Battaglia PA. Jackson-Weiss syndrome: identification of two novel FGFR2 missense mutations shared with Crouzon and Pfeiffer craniosynostotic disorders. *Hum Genet*. 1997 Nov;101(1):47-50.
103. Bagheri-Fam S, Chen H, Wilson S, Ayers K, Hughes J, Sloan-Bena F, et al. The gene encoding the ketogenic enzyme HMGCS2 displays a unique expression during gonad development in mice. *PLoS One*. 2020;15:e0227411.
104. Bouchard L, Robert MF, Vinarov D, Stanley CA, Thompson GN, Morris A, et al. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency: clinical course and description of causal mutations in two patients. *Pediatr Res*. 2001 Mar;49(3):326-31
105. Durkin ME, Ullmannova V, Guan M, Popescu NC. Deleted in liver cancer 3 (DLC-3), a novel Rho GTPase-activating protein, is downregulated in cancer and inhibits tumor cell growth. *Oncogene*. 2007;26:4580-9.
106. Braun AC, Hendrick J, Eisler SA, Schmid S, Hausser A, Olayioye MA. The Rho-specific GAP protein DLC3 coordinates endocytic membrane trafficking. *J Cell Sci*. 2015;128:1386-99.
107. Ilaslan E, Calvel P, Nowak D, Szarras-Czapnik M, Slowikowska-Hilczner J, Spik A, et al. A Case of Two Sisters Suffering from 46,XY Gonadal Dysgenesis and Carrying a Mutation of a Novel Candidate Sex-Determining Gene STARD8 on the X Chromosome. *Sex Dev*. 2018;12:191-195.

108. Kunitomo M, Khokhar A, Kresge C, Edobor-Osula F, Pletcher BA. 46,XY DSD and limb abnormalities in a female with a de novo LHX9 missense mutation. *Am J Med Genet A*. 2020 Dec;182(12):2887-2890

109. Birk OS, Casiano DE, Wassif CA, Cogliati T, Zhao L, Zhao Y, et al. The LIM homeobox gene *Lhx9* is essential for mouse gonad formation. *Nature*. 2000 Feb 24;403(6772):909-13.

Gene	Location	Function	OMIM	Phenotypes observed in XY individuals	Transmission
<i>ATRX</i>	Xq13	Chromatin remodeller	300032	(i) Mental retardation-hypotonic facies syndrome, X-linked, variable testicular dysgenesis (ii) Alpha-thalassemia/mental retardation syndrome, variable testicular dysgenesis	XL XL
<i>CBX2.1</i>	17q25.3	Transcription factor	602770	(i) 46,XY female with ovaries	AR
<i>DHH</i>	12q13.12	Hedgehog signaling	650423	(i) 46,XY complete gonadal dysgenesis (ii) 46,XY complete gonadal dysgenesis with minifascicular polyneuropathy	AR AR
<i>DHX37</i>	12q24.31	ATP-binding RNA helicase	617362	(i) 46,XY complete and partial gonadal dysgenesis (ii) Testicular Regression Sequence (iii) Anorchia (iv) NEDBAVC syndrome	AD AD AD AD/AR
<i>DMRT1</i>	9p24.3	Transcription factor	602424	(i) 46,XY complete gonadal dysgenesis (point mutations) (ii) 46,XY undervirilised male + MCA (9p24 deletions) (iii) Spermatogenic failure	AD AD AD
<i>ESR2</i>	14q23.3	Nuclear hormone receptor	618187	Proposed to cause XY DSD	-
<i>FGFR2</i>	10q26.13	Tyrosine-protein kinase cell-surface receptor	176943	(i) Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis (ii) Apert syndrome (iii) Beare-Stevenson cutis gyrata syndrome (iv) Craniofacial-skeletal-dermatologic dysplasia (v) Craniosynostosis, nonspecific; Crouzon syndrome (vi) Jackson-Weiss syndrome; LADD syndrome (vii) Pfeiffer syndrome; Saethre-Chotzen syndrome (viii) Scaphocephaly and Axenfeld-Rieger anomaly. Proposed to cause XY DSD	AD AD AD AD AD AD AD AD
<i>GATA4</i>	8p23.1	Transcription factor	600576	(i) Testicular anomalies with or without congenital heart disease	AD
<i>HHAT</i>	1q32.2	Catalyzes N-terminal palmitoylation of HH proteins	605743	(i) 46,XY gonadal dysgenesis with Nivelon-Nivelon-Mabile syndrome	AR
<i>HMGCS2</i>	1p12	Mitochondrial enzyme that catalyzes the first reaction of ketogenesis	600234	(i) HMG-CoA synthase-2 deficiency. Proposed as a candidate for XY DSD.	AR
<i>LHX9</i>	1q31.3	Transcription factor	606066	(i) 46,XY complete gonadal dysgenesis with limb anomalies	AD
<i>MAP3K1</i>	5q11.2	Component of a protein kinase signal transduction cascade	600982	(i) 46,XY complete gonadal dysgenesis	AD
<i>MYRF</i>	11q12.2	Transcription factor	608329	(i) Cardiac-urogenital syndrome. Variable DSD phenotype. (ii) Encephalitis/encephalopathy, mild, with reversible myelin vacuolization. Variable DSD phenotype.	AD AD

				(iii) Probably 46,XY partial gonadal dysgenesis with no somatic anomalies	AD
<i>NR5A1</i>	9q33.3	Nuclear receptor	184757	(i) 46,XY DSD with primary adrenal failure (ii) 46,XY complete or partial gonadal dysgenesis with normal adrenal function (iii) 46,XY undervirilised male with atypical external genitalia (iv) hypospadias and micropenis (v) hypospadias (vi) bilateral anorchia (vii) Spermatogenic failure (viii) Hypospadias and asplenia	AD AD AD AD AD AD AD
<i>PPP2R3C</i>	14q13.2	Regulatory subunit of the serine/threonine phosphatase, protein phosphatase 2	615902	(i) 46,XY gonadal dysgenesis, dysmorphic facies, retinal dystrophy, and myopathy (ii) Spermatogenic failure	AR AD
<i>SOX8</i>	16p13.3	Transcription factor	605923	(i) 46,XY gonadal dysgenesis with somatic anomalies (ii) 46,XY complete gonadal dysgenesis (iii) Spermatogenic failure	AD AD AD
<i>SOX9</i>	17q24.3	Transcription factor	608106	(i) Acampomelic campomelic dysplasia (ii) Campomelic dysplasia (iii) Campomelic dysplasia with gonadal dysgenesis (iv) 46,XY complete/partial gonadal dysgenesis without Campomelic dysplasia (v) brachydactyly-anonychia (Cooks syndrome) (vi) Pierre-Robin Syndrome	AD AD AD AD AD AR
<i>STARD8</i>	Xq13.1	Rho GTPase activating protein	300689	<i>Proposed as a cause of GD</i>	XL
<i>SRY</i>	Yp11.3	Transcription factor	480000	(i) 46,XY complete gonadal dysgenesis (ii) 46,XY partial gonadal dysgenesis (iii) 46,XY premature ovarian failure	YL YL YL
<i>WT1</i>	11p.13	Transcription factor	607102	(i) Denys-Drash syndrome (ii) Frasier syndrome (iii) Meacham syndrome (iv) Nephrotic syndrome (v) Wilms tumor	AD AD AD AD AD
<i>ZFPM2</i>	8q22.3	Transcription cofactor	603693	(i) 46,XY complete or partial GD with no evidence for cardiac anomalies (ii) Congenital heart defects, diaphragmatic hernia without DSD.	AD AD
<i>ZNRF3</i>	22q12.1	E3 ubiquitin-protein ligase. Negative regulator of Wnt signaling	612062	(i) 46,XY gonadal dysgenesis (ii) 46,XY undervirilised male	AD AD

Table 1. Genes associated with 46,XY gonadal dysgenesis or proposed as candidates for the phenotype. XL X-linked, YL Y-linked, AR autosomal recessive, AD autosomal dominant