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Recent Findings on the Genetics of Disorders of Sex Development

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Abstract

Purpose of review—Disorders of sex development (DSD) are a diverse group of conditions affecting gonadal development, sexual differentiation or chromosomal sex. In this review, we will discuss recent literature on the genetic causes of DSD, with a focus on novel genetic sequencing technologies, new phenotypes associated with known DSD genes, and increasing recognition of the role of genetic regulatory elements in DSD.

Methods—We performed a comprehensive search of PubMed through August 2016 to identify important peer-reviewed publications from 2015–2016 on the topic of DSD genetics.

Summary of Recent Findings—Whole-exome sequencing was used to successfully identify genetic causes of DSD in 35% of a cohort of 46,XY subjects who had not previously received a genetic diagnosis.

A novel mutation in *NR5A1* has been identified as a cause of 46,XX testicular and ovotesticular DSD, demonstrating a previously unappreciated role of *NR5A1* in preventing testicular differentiation in 46,XX individuals.

Genetic regulatory elements of *SOX9* have been identified as causes of 46,XX and 46,XY DSD.

Key terms

Gene; genetics; exome; disorder of sex development

Introduction

Disorders of sex development (DSD) represent a heterogeneous group of "congenital conditions in which development of chromosomal, gonadal, or anatomic sex is atypical" [1]. Over the past 30 years, we have learned much about the genetics of these disorders. By the late 1980s the human androgen receptor gene (*AR*) had been characterized [2] and by the early 1990s the critical testis-determining region on the Y chromosome, *SRY,* was described [3]. In the past two decades, the pace of genetic discovery has increased rapidly, with the

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identification of many new genetic causes of DSD. More than 50 genes have now been implicated in the pathogenesis of these disorders [4]. These genes encode essential factors in gonadal development or in sex-hormone synthesis or responsiveness (fig 1).

This review focuses on recent insights into the genetic causes of disorders of sex development as well as the use of innovative sequencing technologies to facilitate understanding of the pathogenesis and phenotypic spectrum of DSD. Recent reports in the DSD field have been in three areas: 1) application of whole-exome sequencing to the identification of genetic causes of DSD, 2) expansion of the observed phenotypes associated with variants in known DSD-associated genes and 3) an examination of the role of genomic regulation in human disease.

Whole-exome sequencing in genetic diagnosis of DSD

In the last decade, whole exome-sequencing (WES) has greatly improved the ability to identify disease-causing genetic variants. WES is a method for sequencing all of the protein-coding regions (exons) of an individual's genome, which can then be compared to databases containing large numbers of control exomes to identify possible disease-causing variants [5]. Recently, this approach has been applied to individuals with DSD [6]. Baxter et al. performed whole-exome sequencing in 40 subjects with 46,XY DSD who had not previously received a genetic diagnosis and identified pathogenic or likely pathogenic variants in known DSD genes in 35% of these patients (fig 2).

NR5A1

Baxter et al. identified a pathogenic, heterozygous *NR5A1* variant in one individual in the 46, XY DSD cohort who previously carried a clinical diagnosis of androgen insensitivity syndrome [7]*. *NR5A1* (also known as steroidogenic factor 1, or *SF-1*), encodes a nuclear receptor expressed in the gonadal ridge as well as in Leydig cells later in gonadal development [8]. *NR5A1* acts as a transcription factor involved in the formation of the bipotential gonad and is also essential for the subsequent differentiation of the testis via several downstream factors including *SOX9* (see discussion of this gene below) [8,9]. Homozygous *NR5A1* mutations are rare and cause complete gonadal dysgenesis as well as adrenal insufficiency, as *NR5A1* also has a critical role in development of the adrenal cortex. In humans, heterozygous *NR5A1* mutations are found in 10–15% of individuals with 46,XY DSD, but are not associated with adrenal insufficiency [10,11]. 46,XY individuals with pathogenic heterozygous *NR5A1* mutations may have a broad range of phenotypes ranging from isolated male-factor infertility [12], to mild undervirilization with hypospadias and/or cryptorchidism, to severe undervirilization. Gonadal phenotypes range from normal to dysgenesis and anorchia [11]. Previous work has established that DSD gene mutations play a role in milder forms of undervirilization not typically classified as DSD, such as isolated hypospadias [10,13]. Our group recently used whole-exome sequencing to identify novel pathogenic *NR5A1* mutations in two unrelated 46,XY individuals with bifid scrotum and penoscrotal hypospadias but not cryptorchidism [14]*. Additional novel phenotypes associated with *NR5A1* mutations are discussed below.

WT1

Two subjects in the Baxter study had variants in *WT1,* which encodes a transcription factor involved in embryonic renal and gonadal development. [7] Alternative splicing of *WT1* RNA transcripts can result in more than 20 isoforms of the protein [15].

Several phenotypes are associated with mutations in the *WT1* gene. The most severe phenotype, “WAGR” syndrome, is characterized by Wilms tumor, aniridia, genitourinary malformations (specifically undervirilization in 46,XY children), and intellectual disability. WAGR syndrome is caused by a deletion of the *WT1* gene. Frasier syndrome in 46,XY children is caused by splice-site mutations in the *WT1* gene, which alter the ratio of protein isoforms. Frasier syndrome is characterized by varying degrees of gonadal dysgenesis (partial to complete), renal failure later in childhood due to focal segmental glomerulosclerosis, and increased risk for gonadoblastoma. Denys-Drash syndrome is caused by missense mutations affecting the zinc-finger DNA binding region of the *WT1* gene, and is characterized by varying degrees of gonadal dysgenesis (partial to complete), renal failure early in childhood due to diffuse mesangial sclerosis, and increased risk for Wilms tumor and gonadoblastoma [16]. Isolated diffuse mesangial sclerosis has been attributed to *WT1* mutations, in some cases the same missense mutations that have been described in association with Denys-Drash in other individuals [15,17–20].

In the Baxter study, one subject with *WT1* variant presented with Müllerian structures and dysgenetic gonads bilaterally. On histology, the left gonad was consistent with an immature testis, and the right gonad contained fibro-fatty connective tissue with a possible vas deferens detected. This individual had end-stage renal disease and a previous history of bilateral nephrectomy, though no history of Wilms tumor. The second subject had cryptorchidism and a urogenital sinus, with bilateral testes, one with normal histology and one dysgenetic. There was no known renal disease in this patient, though medical records were incomplete [7].

A recent report investigating genotype-phenotype relationships in a cohort of pediatric patients with steroid-resistant nephrotic syndrome identified *WT1* mutations in 21 of the 354 subjects (5.4%). Of these subjects, 12 had a 46,XY karyotype, and all of these subjects had a disorder of sex development, with eight having complete undervirilization of the external genitalia. The remaining four 46,XY subjects had partial undervirilization leading to hypospadias, with or without chordee and cryptorchidism. One 46,XY individual underwent gonadectomy at the age of nine years and was found to have gonadoblastoma. Of the nine 46,XX individuals with *WT1* mutations, one individual was found to have absence of ovaries, but DSD was not observed in the remaining eight individuals; later ovarian function was not reported [21]*.

Baxter et al. also identified genetic variants in several additional DSD-associated genes, including *DHH, MAP3K1,* and *MAMLD1* (which are involved in testicular development), *LHCGR* (which encodes the LH/hCG receptor), *AMHR*2 (which encodes the anti-mullerian hormone receptor) *STAR* and *HSD17B3* (which are involved in testosterone synthesis), *AR* (which encodes the androgen receptor), and *CHD7* (which is mutated in CHARGE syndrome, which includes genital anomalies among other features). These results highlight

the utility of comprehensive genetic testing in the clinical setting to establish genetic diagnosis and in the research setting to expand our understanding of the frequency and phenotypic variability for specific genetic causes of DSD [7].

A Role for *NR5A1* in ovarian fate specification

As discussed above, *NR5A1* has been thought to be chiefly involved in early gonadal development and testis determination. The majority of *NR5A1* mutations associated with DSD to date have been identified in 46,XY individuals with varying degrees of undervirilization. Phenotypic manifestations of *NR5A1* mutations in 46,XX individuals can include ovarian hypoplasia, premature ovarian insufficiency, and/or primary or secondary amenorrhea with otherwise normal urogenital structures. In some cases, fertility is preserved [22].

Recently, two groups have described another phenotype associated with an *NR5A1* variant in 46,XX individuals. Bashamboo et al. identified five cases of 46,XX testicular or ovotesticular DSD from four families due to identical mutations in *NR5A1* resulting in an arginine-to-tryptophan change at position 92 (p.Arg92Trp), which lies in the DNA-binding domain of the NR5A1 protein. Testicular DSD is characterized by the presence of testicular tissue and virilized external genitalia in a 46,XX individual. Ovotesticular DSD is characterized by the coexistence of ovarian and testicular tissue in the gonads. Phenotypes ranged from ambiguous genitalia, to penoscrotal hypospadias, to micropenis noted at birth, to small testes and low testosterone noted in adolescence. Of note, one proband had a sibling with labial fusion, clitoral enlargement, and dysgenetic testes in the inguinal region who was found to have a 46,XY karyotype and the same *NR5A1* mutation [23]*.

Baetens et al. reported the same mutation in three members of a cohort of eleven unrelated individuals with 46,XX testicular or ovotesticular DSD. Phenotypes ranged from mild clitoromegaly, to ambiguous genitalia, to micropenis and hypospadias [24]*.

Baetens et al. demonstrated expression of *NR5A1* in the developing fetal human ovary and testis, in contrast to mouse studies that have shown expression in the early testis but minimal expression in the ovary [25], consistent with a role for *NR5A1* in ovarian differentiation. The authors hypothesized that the variant identified in these cases disrupted *NR5A1* activity in testis-opposing pathways in the developing ovary. Indeed, they specifically demonstrate that the Arg92Trp mutant NR5A1 protein exhibited less robust ability to synergize with β-catenin, a component of the Wnt signaling pathway, in promoting the expression of genes that inhibit testicular differentiation [24].

An additional phenotype for *NR5A1* mutations in 46,XY individuals was explored by Ferlin et al. They found a prevalence of 1.8% for heterozygous missense *NR5A1* mutations in infertile males with severe impairment in spermatogenesis, with a higher rate of mutations (2.7%) in men with a prior history of cryptorchidism, as compared to 1.4% of men without cryptorchidism [26]. This report was comparable to previous reports that identified an *NR5A1* mutation prevalence of 4% in a cohort with idiopathic male infertility due to failure of spermatogenesis [27].

Mutations in the Regulatory Region of *SOX9*

Recent work in the field of DSD genetics has included an examination of the role of genetic regulatory elements in the pathogenesis of DSD, particularly involving the *SOX9* gene. *SOX9* is a target of *SRY* and is essential for testicular differentiation [28]. Loss-of-function mutations in *SOX9* are a cause of 46,XY DSD, and large duplications near *SOX9*, hypothesized to be gain-of-function mutations, have been identified in patients with 46,XX DSD [29–31]. Studies in mice have identified specific enhancer regions required for the expression of *SOX9* in early testis development [32]. Benko et al. had previously described five DSD cases associated with disruption of *SOX9* regulatory regions. Three 46,XX individuals with virilization and ovotesticular DSD were found to have duplications in a regulatory region upstream of the *SOX9* gene. Furthermore, two 46,XY individuals with gonadal dysgenesis and severe undervirilization were found to have deletions in the same region. Based on the location of the duplications and deletions discussed above, the authors hypothesize the existence of a 517–595 kilobase (kb) regulatory region upstream of *SOX9* that when duplicated may drive a testicular fate for the bipotential gonad in 46,XX individuals, possibly through modification/relaxation of epigenetic repressors. When deleted in 46,XY individuals, *SOX9* expression is restricted, leading to impaired testicular development [33].

Hyon et al. describe three males with phenotypically normal male genitalia who presented with infertility and were found to have low testicular volume and azospermia. On further evaluation, all three cases had 46,XX karyotypes and duplications upstream of the *SOX9* gene overlapping with the region described by Benko et al. The authors were able to identify a smaller minimal critical regulatory region of 40–41.9 kb located ~600kb upstream of *SOX9*, which, when duplicated, may be sufficient to induce testicular differentiation in 46,XX individuals [34]*.

More recently, Kim et al. were able to further narrow the critical regulatory regions upstream of the *SOX9* gene associated with 46,XX and 46,XY DSD. This group identified duplications that all shared a 68-kb region—located within the region upstream of *SOX9* described by Benko et al.—in 3 unrelated individuals with 46,XX ovotesticular DSD. They similarly identified deletions upstream of *SOX9* in one member of a six-generation kindred with autosomal dominant DSD in five 46,XY members, characterized by gonadal dysgenesis and severe undervirilization. Three additional families were described with undervirilization and gonadal dysgenesis in 46,XY family members and deletions in a region upstream of *SOX9*. All described deletions share a single 32.5 kb region which is not located within the region upstream of *SOX9* described by Benko et al. It is near, though does not overlap, the 68kb region that they found to be associated with 46,XX DSD and overlaps with previously described deletions upstream of *SOX9* causing 46,XY DSD [30,35]*.

The authors hypothesize the existence of two separate regulatory regions. The region that is deleted in cases of 46,XY DSD may serve as a *SOX9* enhancer, and its absence may contribute to insufficient expression of the transcription factor, preventing normal testicular differentiation. Conversely, a separate *SOX9* enhancer duplication may increase gene and

protein product associated with Sertoli cell differentiation, which may “push” the undifferentiated gonad into a testicular fate [35].

These findings narrow the “critical regions” for regulation of SOX9 in the developing gonad and demonstrate the importance of non-coding regions of the genome in normal gene expression and their role in human disease.

Conclusion

Facilitated by ongoing developments in genetic sequencing technologies, advances continue to be made in understanding the genetics of DSD and, in turn, the biology of gonadal development. Recent findings have mostly focused on previously identified DSD genes; future work will undoubtedly use whole-exome sequencing and other emerging technologies to identify new DSD genes.

Whole-exome sequencing is becoming increasingly available in the clinical setting, and the ability to provide genetic diagnoses for a large fraction of DSD promises to change the way we advise and treat patients with these conditions. For example, establishing a specific genetic diagnosis may allow for improved guidance on oncologic risk and early identification of associated issues such as renal insufficiency. As the pace of innovation in genetic diagnosis continues to accelerate, the impact of whole-exome sequencing and related methods on patient care will be an essential area of future inquiry.

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•• of outstanding interest

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Key Points

- Whole-exome sequencing has proven to be an effective modality by which to identify genetic causes of DSD, though its use has thus far been limited to a research setting.
- An expanded understanding of the genetic variants associated with DSD phenotypes has brought about a deeper understanding of the biology of gonadal development.

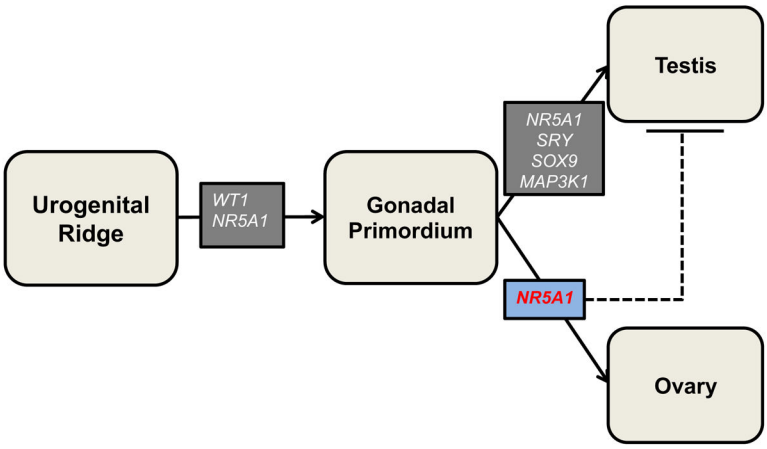

Fig 1.

Role of select DSD genes in typical genital differentiation

Dashed line: Proposed action of gene, solid line: demonstrated action of gene

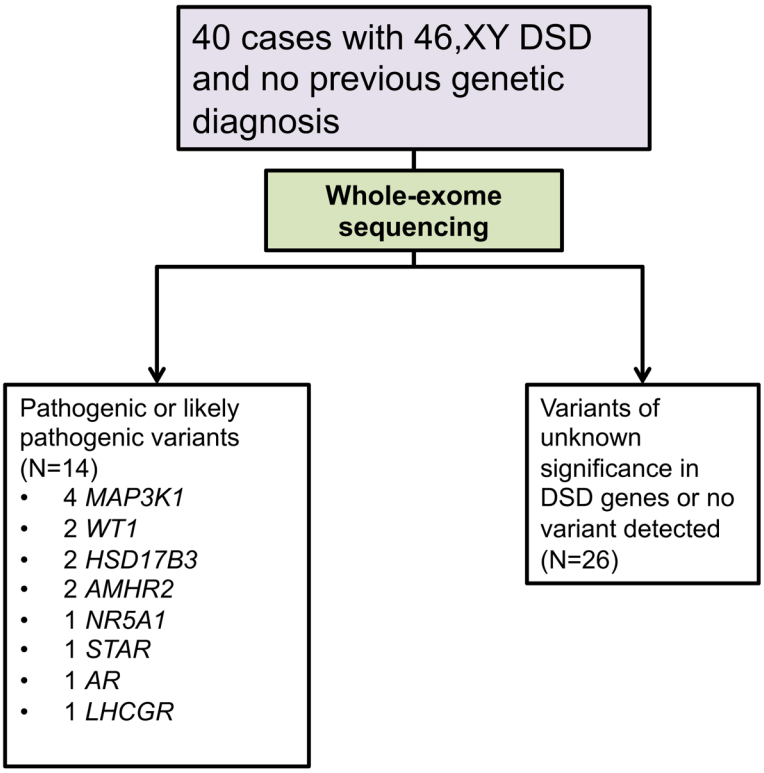

Fig 2.
Study design and genetic sequencing results in Baxter et al [7].