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Genetics of Disorders of Sex Development:

The DSD-TRN Experience

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Abstract

The Disorders of Sex Development (DSD) Consensus Conference, held in Chicago in 2005, identified several domains of care where improvement was needed.[1] In particular, it called for the establishment of an infrastructure for collaborative interdisciplinary clinical practice and research, with the goal of integrating scientific understanding of DSD with real-time standardization and improvement in clinical practice. The DSD-Translational Research Network (DSD-TRN) was created in response, the first such North American infrastructure, a network of 4 (now expanded to 10) research and clinical sites and a central registry, with the collaboration of Accord Alliance, a nonprofit convener of diverse DSD stakeholders. To address the variability within and across medical, surgical, and behavioral health aspects of care, the DSD-TRN is dedicated to the standardization of diagnostic and treatment protocols in order to enhance clinical and scientific discovery, as well as quality of life outcomes for patients and their families. A critical aspect of this standardization of practice is a commitment to an early and comprehensive diagnostic process (including genetic), associated with extensive standardized phenotyping and psychosocial screening and support of patients and families. A recent review of the state of clinical, biochemical, genetic, and psychosocial evaluations of the newborn or adolescent with DSD, 10 years after the consensus statement, continued to highlight the need for a thorough diagnostic

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process that sets in motion informed discussions with parents (and newly diagnosed adolescent patients) regarding treatment options.[2] Developmental pathways of sex determination and differentiation impacted in isolated and syndromic DSD conditions were recently reviewed.[3–5] This article will briefly review the main categories of genetic causes of DSD and the diagnostic revolution promised by the advent of new genomic technology, and will present the DSD-TRN guidelines for genetic diagnosis, features of the registry for future research, and a peek into some early registry data.

Keywords

Disorders of sex development; Genotype; Phenotype; Genomic sequencing

Disorders Of Sex Development Phenotypic And Genotypic Spectrum

The term DSD encompasses a wide phenotypic spectrum, and, while DSDs associated with uncertain gender assignment are relatively rare, the prevalence of DSDs as a whole might have been underestimated. Depending on what conditions are included, combined incidences from 1 case per 200 patients to 1% to 2% are routinely quoted, with great differences between conditions.[2] Hypospadias (atypical location of the urethral meatus) is reported to have increased to approximately 1 case per 125 newborn boys, and cryptorchidism (failure of testicular descent) is seen in as many as 3% of full-term newborn boys.[6] Evidence is emerging that these conditions may represent part of a phenotypic spectrum, sharing a genetic etiology with more complex forms of DSDs.[7]

DSDs have been historically classified according to overlapping categories:

Sex chromosome complement—46,XX, 46,XY, other, mosaic

Gonadal structure—testicular DSD, ovotesticular DSD, gonadal dysgenesis

Gonadal functional status—gonadal dysgenesis, disorders of androgen biosynthesis

When presenting as developmental disorders, DSDs may be isolated, or part of a syndrome, typically of unknown etiology:

- Isolated hypospadias (46,XY)
- Cloacal exstrophy/OEIS spectrum (46,XX or 46,XY)
- Müllerian structures, developmental anomalies (MRKH, vaginal atresia, Müllerian agenesis; 46,XX)

In addition, DSDs can be found as part of complex multiorgan developmental syndromes.[5]

Known genetic causes of DSDs range from chromosomal aneuploidies, such as Turner syndrome or Klinefelter syndrome, to small copy number variants (CNVs) of open reading frames or promoter regions, to discrete variants in single genes. Major single gene etiologies of isolated or syndromic DSD are listed in Table 1. Broad categories include

Sex chromosome complement variants—Turner syndrome (45, X, typically mosaic), Klinefelter syndrome (47,XXY, possibly mosaic), variants of higher chromosomal

count,[8] mosaic 45,X/46,XY mixed gonadal dysgenesis, and 46,XX/46,XY ovotesticular DSD

46,XX disorders of ovarian development—These include 46,XX testicular and ovotesticular DSDs, as well as 46,XX gonadal dysgenesis. Known etiologies for isolated testicular and ovotesticular DSDs overlap and include (typically *de novo*) *SRY* translocation (~85% of 46,XX testicular and ~ 10% of ovotesticular DSDs, respectively), and *SOX9* or *SOX3* gene CNVs. Variants in *RSPO1* cause a rare form of syndromic 46,XX testicular DSD.[9] 46,XX gonadal dysgenesis leads to premature ovarian failure (POF) caused by failure of ovarian development or resistance to gonadotropins. Rare mutations in the FSH receptor (*FSHR*, autosomal recessive), *BMP15* (X-linked), *NR5A1* (autosomal dominant), and *STAG3* explain a few of the cases.[10–12]

46,XY disorders of testicular development (including complete and partial gonadal dysgenesis, Swyer syndrome). The main cause is mutations or deletions of *SRY*. Rarer causes are variants in *DHH*, *NR5A1 (SF1)*, *MAP3K1*, *CBX2*, duplication of *NROB1* (*DAX1*) or WNT4, and 9p24.3 (including *DMRT1*) deletion.[13] Patterns of inheritance include sex limited autosomal recessive and dominant, X-linked and Y-linked. It is therefore critical to assess genetic etiology for genetic counseling of these conditions.

46,XX and 46,XY disorders of steroid hormone biosynthesis—Congenital adrenal hyperplasia[14] results from mutations in the biosynthetic pathways of adrenal hormones, including the androgen biosynthesis pathway. Frequency is the same in males and females; however, the resulting DSD is different. The most frequent form (>90%) is associated with recessive mutations in the *CYP21A2* gene. The main DSD concern is that of virilization of women, but effect is poorly described in 46,XY individuals.

- Much rarer causes include: *STAR* and *3-βHSD* deficiencies (which result in androgen deficiency and male hypovirilization), and 11-hydroxylase (*CYP11B1*) deficiency (causing excess androgen and virilization of affected women).
- *POR* deficiency causes a distinct form of autosomal recessive CAH that can result in DSD in both 46,XX and 46,XY individuals, including infertility, PCOS, primary amenorrhea, with Antley Bixler syndrome at the most severe end of the phenotypic spectrum.[15]
- Aromatase deficiency (*CYP19A1* gene, recessive inheritance) causes elevated levels of androgens *in utero* and deficit of estrogen later, and may present as virilized genitalia in a 46,XX newborn or primary amenorrhea in adolescence.
- Androgen biosynthesis defects, such as *5ARD* or *17βHSD3* deficiency (both autosomal recessive), result in DSD in 46,XY individuals.[16,17]

46,XY disorders of hormonal action include

- Androgen insensitivity syndrome (AIS)—Variants in the X-linked androgen receptor (*AR*) cause complete or partial AIS, frequent forms of 46,XY DSD.

- Persistent Müllerian duct syndrome is caused by mutations in the genes coding for the anti-Müllerian hormone or its receptors. Inheritance is autosomal recessive, but the phenotype is expressed only in 46,XY individuals.[18]
- LH receptor—Inactivating mutations cause Leydig cell agenesis/hypoplasia, an extremely rare autosomal (sex-limited) recessive form of DSD. Conversely, constitutively active variants cause an autosomal-dominant familial form of precocious puberty in boys.[19]

Improve and Accelerate The Path to an Accurate Diagnosis for Disorders of Sex Development

In spite of this long list of known genetic etiologies, currently known genes explain about only half of the cases. Many patients with DSD have historically experienced long diagnostic odysseys, in part because of uncoordinated diagnostic approaches, and many more never receive a definitive diagnosis. This, of course, is a common concern for patients with rare disorders.[20] About 10 years ago, a European survey of 6000 patients with rare, but well-known genetic entities (not including DSDs) showed that time between first symptoms and definitive diagnostic extended from 5 to 30 years for 1 in 4 patients. Of those who received an early diagnosis, this diagnosis proved to be wrong in 40% of the cases, leading to inappropriate treatments.[21]

In DSD care, an accurate diagnosis is critical to predicting the occurrence of life-threatening crises (such as in salt-wasting forms of CAH), response to hormone replacement therapy (eg, androgen in/sensitivity), eventual gender, fertility, recurrence risk, and cancer risk. In addition, the well-documented empowerment of patients with rare chronic disorders after a diagnosis is reached allows them to plan for health-related and psychological effects of their condition for an optimal quality of life.[3,22]

Many DSDs are caused by enzymatic defects in synthesis of steroid hormones. Testing for those is rapid and relatively inexpensive, and may be critical, as in CAH. However, because of phenotypic overlap between the various forms of DSDs, endocrine testing alone frequently yields an ambiguous diagnosis.[23,24] A genetic diagnosis is indispensable in these cases, as well as for the enzymes for which no diagnostic endocrine test exists, for variants affecting proteins other than enzymes, as well as to counsel families for further pregnancies and prenatal diagnosis. Also because of phenotypic overlap, serial single candidate gene testing, which has been the traditional approach for DSD genetic testing, is highly inefficient and can become prohibitively expensive.[25,26] Over the past decade, chromosomal microarrays have proven indispensable tools for diagnosis of DSD caused by CNVs, and should be prioritized in cases of syndromic DSD.[5,27–30] Genome-wide maps of CNVs of known pathogenicity as well as of uncertain clinical significance will continue to support clinical diagnosis and drive research for new etiologies.[31] The advent of next-generation sequencing in the realm of clinical diagnosis in the past 4 to 5 years is now allowing providers to rethink the diagnostic process.

Genomic Sequencing as A Primary Diagnostic Tool for Disorders of Sex Development

The University of California Los Angeles (UCLA) Clinical Genomic Center has been at the forefront of this effort, being one of the original two academic centers to offer clinical exome testing in the United States, starting at the beginning of 2012, and has reported high diagnostic success for rare disorders.[32] This diagnostic rate, around a third for trio cases, has proven remarkably similar across platforms, types of disorders, or countries.[33–38] In spite of high costs and poor insurance coverage, exome sequencing is cost-effective in specific clinical scenarios, such as when multiple genetic etiologies result in overlapping phenotypes,[36,39] as is the case in DSDs. The early results of large-scale studies of clinical utility and cost-effectiveness of genomic sequencing such as the MedSeq Project are encouraging.[40] Finally, turn-around time has rapidly gone down—now routinely 4 weeks at the UCLA center, including sequencing and interpretation, and down to 1 week for exceptional urgent cases—making the use of next-generation sequencing as a first-tier diagnostic test a realistic possibility in DSD care.

As a consequence, DSD-TRN best practice guidelines recommend early, comprehensive genetic testing as a means to improve the path to an accurate diagnosis and optimized management for DSD patients.[25] An early success of the DSD-TRN using exome sequencing resulted from a collaboration of 3 DSD-TRN teams, UCLA, Seattle Children's Hospital, and University of Michigan.[41] For all of these patients, many of whom had previously undergone extensive, unsuccessful genetic and endocrine testing, exome sequencing streamlined the diagnostic process. It yielded a diagnosis in cases where endocrine testing had been ambiguous, in genes for which clinical testing as single-gene testing was not available in the United States, and, in several cases, it critically modified clinical management, compared with the working diagnosis the patient had previously been carrying, or even oriented gender identity.[25]

Disorders of Sex Development Translational Research Network Recommendations for Disorders of Sex Development Genomic Testing

The primary gene list used at the UCLA Clinical Genomic Center currently has 78 genes (see Table 1), allowing testing at the same time for many DSD genes that may not be available for individual clinical testing. The adoption in March 2015 of an enhanced capture protocol (http://pathology.ucla.edu/cgc-resources) has greatly increased the coverage for all DSD genes. This resolved a few limitations of the test, such as the poor coverage of *AMH* (under 60%) or *SOX3* (78%) in the previous capture process. Sixty-five of the 78 genes are now covered at 100% (vs only half with the previous protocol), and another 7 genes are covered at 97% or above.

In addition, there is a secondary gene list, including genes that cause urogenital conditions in animal models or are involved in molecular pathways with genes known to cause DSD in people. Variants in such preclinical genes are reported with the hope of providing the clinical

team with avenues to orient further exploration (endocrine, imaging) of the patient's phenotype toward a definitive diagnosis.

Samples should be submitted as trios of patient plus biological parents, as this enhances diagnostic yield by at least 50%.[32,34]

In addition to the UCLA Clinical Genomic Center, DSD-TRN network sites have used other sequencing resources for a variety of reasons, such as the State of New York's mandate that priority be given to in-state companies, development of in-house facilities, or the ease of pricing out-of-pocket expenses for patients offered by companies such as GeneDx. However, a unique strength of the UCLA facility is the weekly Genomic Data Board, a group of approximately 20 clinical and molecular geneticists, laboratory directors, genetic counselors, bench researchers, and referring clinicians who participate in the interpretation of exome results. Bioinformatic work is performed in advance of each meeting, and a data file organized through in-house-developed tools,[32] reports patient information, clinical keywords, regions of homozygosity, variants, and information on variants such as minor allele frequency (an indicator of pathogenic likelihood), protein damage prediction, and tissue expression patterns. The multidisciplinary expertise of the team, including multiple DSD specialists, as well as having all the sequences interpreted by the same team, is expected to enhance variant calling accuracy and reliability.

Regardless of origin of the sequence, for patients who agree to participate in the DSD-TRN database, an annotated set of variants for each exome will be made accessible to network investigators through secure electronic access to the registry. Future research using these data will allow comparison of variant calling across platforms, research into new etiologies, and call reassessment as new genetic causative genes are discovered.

Standardized Deep Phenotyping In Disorders of Sex Development Translational Research Network Practice

Accurately predicting natural history and consequences of intervention is predicated on understanding risk (ie, accurate phenotype/genotype correlation). It is also critical to the ability to interpret the variants identified by exome sequencing. In parallel with extensive genotyping, the DSD-TRN therefore undertook an effort to collect comprehensive, standardized phenotyping data, with the goals of informing clinical care and uncovering fine, cryptic phenotype/genotype correlations not currently apparent.

The aim is to describe precisely, reliably, and quantitatively, the traits involved in the phenotype of sex development, including genito-urinary anatomy, comprehensive endocrine profile, mental health, social environment, family and pregnancy history, environmental exposures, and genetic profile. To collect this information, the network developed standardized clinical forms for all specialties involved in the interdisciplinary care of families living with DSD. Almost 2000 discrete data points per patient are collected:

- Endocrine profile—A main form plus 3 forms for the most common stimulation tests used to diagnose DSDs (hCG, ACTH, GnRH) document the patient's history of endocrine testing. For each analyte, a standard set of features is

collected: laboratory where testing was performed, normal range of value for this laboratory for this patient's age/sex if available, measured value of the analyte, and a call (normal, abnormal, cannot judge). Most importantly, this is completed by an “opinion of the endocrinologist” comment, to ensure accuracy of complex interpretation for patients whose sex may be incongruent with their chromosomes, who may present atypical anatomy and function, or who may be under hormone replacement therapy.

- Urogenital Anatomy Forms Urogenital Anatomy Forms to be completed at each patient encounter and for each imaging, to document anatomy longitudinally across development, endocrine interventions, and/or surgeries.
- A schedule of psychosocial questionnaires document risk and resilience factors in the family, the patient's psychosocial and educational adaptation, self- and body-image, and gender development over time.
- A physical examination form, filled at each encounter, tracks the evolution of all systems, including genital anatomy (Supplementary Fig. 1).

Standardization of Diagnostic Process and Genetic Practice Reporting

The genetic diagnostic process by clinical teams and for individual patients is captured by multiple different documents.

Documentation of Family History

Obtaining extensive family background information is a hallmark of DSD-TRN practice, toward an optimal diagnostic process. This information is collected mostly in the intake form, and includes extensive data about parental health and reproductive history, pregnancy exposures, prenatal testing, birth circumstances, and congenital defects (Supplementary Fig. 2).

Documentation of Genetic Testing and Diagnosis

A cumulative genetics form collects results of all genetic tests performed on patients and family members over time (Supplementary Fig. 3). Results reporting for all tests (karyotype, chromosomal microarray, SRY status, variants from exome sequencing, or single gene testing) is standardized to ensure data accuracy and comparison across providers. Negative results are also recorded, to document the diagnostic process. Year of testing is recorded to help interpretation of result if techniques have evolved and to quantify time to diagnosis. The front page of the form highlights genetic diagnosis as well as interpretation by the geneticist. For patients participating in the registry, annotated sequencing variants are uploaded to the registry to support further research into etiology and fine genotype/phenotype correlations.

A definitive genetic diagnosis is considered reached when the phenotype of the patient can be explained by any of the following:

- Aneuploid or mosaic complement of sex chromosomes

- CNV that has previously been described in DSDs
- Likely pathogenic or pathogenic variant identified in known DSD gene

A normal karyotype discordant with genital phenotype (eg, 46,XY karyotype in a phenotypic female) is not considered a diagnosis.

Documentation of Genetic Practice

The physical examination form contains a series of questions documenting what genetic counseling was provided to patients at each encounter. This is eventually to be completed by a mirror form filled by patients after the encounter, to document the family's understanding and support practice improvement. Pursuit of a genetic diagnosis is documented in the teams' monthly clinic reports, where each team details what genetic tests have been ordered and whether a genetic diagnosis has been pursued/achieved for each patient in clinic (Supplementary Fig. 4).

In parallel, the physical examination form tracks the evolution of the working diagnosis until a definitive genetic diagnosis has been achieved. For example, a working diagnostic could evolve from ambiguous genitalia at the first encounter with a newborn, to 46,XX DSD with ambiguous genitalia once a karyotype has been performed, then 46,XX ovotesticular DSD once pathology of the gonad has been ascertained and, perhaps, finally 46,XX ovotesticular DSD with *SOX9* duplication when a definitive genetic diagnosis has been achieved. Analysis of working diagnosis registry data should allow one to determine evolution of time to diagnosis over time, as well as condition-to-condition and site-to-site variability. In association with psychosocial data, it should allow assessment of the influence of an accurate diagnosis on clinical management and quality-of-life outcomes for various DSD conditions.

Collectively, clinical specialty forms serve multiple purposes:

- Supporting the clinical team's adherence to network best practice guidelines by providing the list of data points that needs to be documented for each patient
- Ensuring phenotypic description and genetic variant reporting is standardized
- Clarifying electronic medical records, by grouping in a single set of standard documents all historical and longitudinal patient information
- Supporting interdisciplinary team function and decision-making by showcasing interpretation of results by each specialist for the information of other providers
- For patients who agree to participate in the study, the forms serve as registry data-collecting tools

Preliminary Disorders of Sex Development Translational Research Network Registry Findings

Diagnostic Effort by the Disorders of Sex Development Translational Research Network Team Increased the Percentage of Patients with a Firm Diagnosis from 24% to 46%

A survey of the database in August 2016 showed that a genetics form was entered into the registry for 144 out of the 303 probands enrolled (a form was also entered for 7 affected siblings) (Table 2). Data entry varied greatly between sites, from just under 6% (2 sites) to 100% of completion (3 sites). Out of the 144 probands, 35 had a diagnosis prior to their first visit to the interdisciplinary DSD-TRN team. Clinical care by the DSD-TRN team resulted in establishing a firm diagnosis in 30% of the remaining patients (30 of 101 patients), for a total of 66 of 144 (46%) patients currently with a firm diagnosis. Diagnostic success too was variable from site to site, with 3 sites reporting zero diagnosis and 4 sites more than 50% of patients with a firm genetic diagnosis.

The accuracy of this percentage must be viewed cautiously, as no genetic data are available in the registry for half of the enrolled probands. When the number of diagnoses reported achieved was compared with total number of enrolled patients, the ratios fell around a quarter to a third of patients with a firm genetic diagnosis, more in line with typical diagnostic success for DSD. One suspects there might be a bias in favor of prioritizing form completion and registry data entry for patients in whom a genetic diagnosis has been pursued, if not achieved, and that teams may more rarely create mostly blank registry forms for patients when no genetic testing has been pursued. Two sites, where reporting had been completed, maintained an exceptional diagnostic rate of 64% and 67% in their patients.

The Conditions of 6% of Probands Reported in the Disorders of Sex Development Translational Research Network Registry are Familial

Over 40% of probands had a reported call for “Is proband condition familial?” (17 yes, 44 no de novo) (Table 3). However, data QC through search of other variables showed that testing of other family members had been pursued in a limited number of cases. A *SOX9* duplication and a nondiagnostic rearrangement involving 2 autosomes were confirmed to be *de novo* by karyotype of the parents. Two chromosomal microarrays, identifying diagnoses of Klinefelter and deletion of the entire *AR* gene, were performed in trios confirming the *de novo* status. In 2 patients, the parental origin of compound heterozygous variants was identified by trio exome, but the condition was not familial. In contrast, 9 cases were proven familial (8 reported, 1 not reported) through the existence of affected family members (2 Swyer syndromes without genetic diagnosis, 2 *MAP3K1*, 1 *CYP21A2* CAH, and 4 AIS with *AR* variants).

Frequency of Specific Genetic Diagnoses in the Disorders of Sex Development Translational Research Network Registry

Karyotype was reported in almost all (92%) patients (Table 4). Of those reported, approximately 15% had sex chromosome complement anomalies, 16 of 18 in mosaic form. Normal 46,XX and 46,XY karyotypes were found in 36% and 50%, respectively. Of the 15

with no reported karyotypes, most were conditions where genetic diagnosis is rarely attained or may be viewed as unnecessary by some: 6 CAH, 2 cloaca, 4 MRKH, 1 SRD5A2 deficiency, and 2 without a clear working diagnosis.

Mosaic Turner syndrome accounted for the vast majority of sex chromosome complement anomalies. A majority of those had a marker Y chromosome (isodicentric Y). Such marker chromosomes, which are rare in the general population, are found with elevated frequency in people with infertility (45 times more frequent) or developmental delay (60 times).[42] Their frequency in Turner syndrome, as well as the wide associated phenotypic range, became rapidly apparent during the clinical case videoconferences held by the DSD-TRN.[43]

CAH was the predominant diagnosis, with 14 cases of genetically documented *CYP21A2* deficiency in 46,XX individuals and 4 cases of *17βHSD* deficiency in 46,XY individuals. No other etiologies of CAH were reported. Search on the working diagnosis of the physical examination form identified one more *17βHSD* deficiency, one *CYP11A2* deficiency, and another 19 potential CAH cases for which no genetics form has been filed or genetic testing was not pursued.

In 46,XY individuals, the most frequent diagnosis was likely pathogenic or pathogenic variants in the *AR* receptor, leading to complete or partial AIS. No mutations or deletions of SRY were reported. The next most common diagnosis was 5α-reduc-tase deficiency, with 5 cases.

Efficacy and Completion of the Diagnostic Process

Chromosomal microarrays—CMA was performed in 43 (30%) of patients for whom a genetics form was entered in the registry, whether they had syndromic or isolated DSDs (Table 5). Six diagnoses were made: 2 loss and 1 gain of portion of Y chromosome, 1 Klinefelter syndrome, 1 deletion of entire *AR* gene, and 1 WAGR syndrome. In addition, CNVs of unknown clinical significance were identified in another 5 patients, 4 of whom had a firm diagnosis obtained by another method. These may represent avenues of research to identify modifier genes.

Chromosomal microarrays have clear demonstrated diagnostic value, especially in syndromic cases and for isolated DSDs due to typically submicroscopic CNVs (eg, *SOX3*, *SOX9*, *NR0B1*, *WNT4).*[7,27] In the DSD-TRN registry, CMA identified absence or excess of whole chromosomes, as the diagnosis of Klinefelter, or of an entire gene (*AR*).

Single gene testing—Thirty-five patients had molecular diagnoses after single-gene testing. This included 14 cases of *CYP21A2*-deficiency CAH, which were likely tested to confirm a suspected endocrine diagnosis. Serial single-gene testing was reported in 47 patients. In 12 patients, it yielded no diagnosis, with an average of 1.6 genes tested per patient. In 8 patients with a diagnosis and multiple testing, 2.7 genes were tested on average. Genes tested that turned out to be wrong guesses were: *AR* (11), *5ARD2* (6), *SRY* (3), *WT1* (3), *LHCGR* (2), *HSD17B3* (2), and 1 each for *AKR1C4*, *AMH*, *AMHR2*, *CYP11B1*, *DHH*, *FGFR1*, *KAL1*, *MAMLD1*, *MAPK8*, *NR0B1*, *PTEN*, *SOX9*, *WNT4*, and *ZFPM2*. Thus,

while *AR* and *5ARD2* were the most frequently diagnostic genes in 46,XY individuals (15 and 5, respectively), they were wrongly suspected equally frequently (11 and 6).

Exome sequencing—Although few exomes have yet been reported in the registry, diagnosis success was high, with a definitive diagnosis identified in 44%. As previously reported, trio exome was more efficient than singleton exome; 3 of 5 trio exomes versus only 1 of 4 singleton exomes reached a diagnosis.

Completion of genetic diagnostic process—Among the 77 patients for whom no diagnosis has been reached, 2 patients have exhausted the genetic diagnostic options currently available on a clinical basis (karyo-type, FISH, CMA, trio exome). Three have undergone singleton exome (and CMA) and might benefit from reassessment of their variants using parental controls, given the higher diagnostic rate of trio exome. Another 14 patients had CMA but no exome performed, and 58 patients had neither CMA nor exome. Therefore, available genetic diagnostic options have not been exhausted in the vast majority (97%) of patients who remain without a diagnosis.

Summary

Although documented evidence is scarce, a review of longitudinal quality-of-life outcomes for patients with DSD in varied settings indicates better outcomes when care is provided by a multidisciplinary team at a tertiary center.[44] This model was advocated by the Chicago Consensus[1] and is being put into place in many centers.[45] The DSD-TRN is the first network to harness the work of such teams through a common registry, expertise-sharing via clinical case videoconferences, and adherence to common best-practice recommendations. The network has undertaken a massive effort of both the standardization and the documentation of the diagnostic process. The authors and others have provided evidence for the prioritization of genetic testing, including new genomic technologies, to streamline the diagnostic process in DSD care.[23,25,33,41,46,47] With time to results of exome sequencing now in the same range as some hormonal tests, and price similar to an MRI, use of genomic technologies as a first-tier diagnostic tool should become the norm in the near future.

Even though there are still a significant number of unsolved cases even after exome sequencing, strategies to improve the interpretation and diagnostic yield have emerged. One is the reanalysis of the exome data, at least 1 year after the original analysis. As more cases become analyzed in the literature, several variants have become significant, with an increased diagnostic yield of about 10%.[48,49] Whole-genome sequencing may identify variants in known DSD genes in regions not captured by exome sequencing (promoter or deep intronic). Another promising approach to improve the diagnostic interpretation is the analysis of the transcriptome (eg, by RNA sequencing) and the combination of RNA and DNA variant exploration.[50,51]

Although many next-generation sequencing platforms are indeed being developed around the world, implementation is facing multiple hurdles, from clinicians' habits, to institutional constraints, to insurance coverage. In addition, a strong hurdle to the full adherence of clinical teams to the DSD-TRN guidelines for standardization of reporting and practice is

the current lack of integration of the standardized clinical forms into the various electronic medical records at the different sites. Time allocated to research (such as registry data entry) is also severely limited at most sites for lack of funding supporting this new effort of development and implementation of best practices. In spite of these hurdles, genetic information for half the enrolled patients is already available in the DSD-TRN registry, and early results demonstrate the value of such an infrastructure. The long-term value of an accurate diagnosis goes beyond the molecular diagnostic yield, as it supports reproductive decision making for families, identification of at-risk family members, quality of life, and general empowerment of patients. Although those outcomes (including psychosocial) may vary, they can and must be measured, in DSD practice as in the case of any other chronic condition.[52] This effort should allow production of evidence for the efficacy of various methods toward an accurate diagnosis and, most importantly, the effects of a reliable diagnosis on evolving health-related quality-of-life outcomes for patients and families living with DSDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- Although many next-generation sequencing platforms are being developed around the world, implementation is facing multiple hurdles from clinicians' habits, institutional constraints, and insurance coverage.
- A strong hurdle to the full adherence of clinical teams to the Disorders of Sex Development-Translational Research Network (DSD-TRN) guidelines for standardization of reporting and practice is the current lack of integration of the standardized clinical forms into the various electronic medical records at different sites.
- Time allocated to research (eg, registry data entry) is also severely limited at most sites for lack of funding supporting this new effort of development and implementation of best practices.
- In spite of these hurdles, genetic information for half the enrolled patients is already available in the DSD-TRN registry, and early results demonstrate the value of such an infrastructure.

Table 1
Primary gene list used at the UCLA Clinical Genome Center to call DSD variants

Gene	Alternative Names	Coverage (February 2012-February 2015) (%)	Coverage since March 2015 (%)	Reported Associated Phenotype
Sex Determination (gonadal dysgenesis, testicular and ovotesticular DSD)				
BMP15		—	**100**	46,XX premature ovarian failure
CBX2	CDCA6	99	**100**	46,XY sex reversal
DHH	HHG	85	**100**	46,XY partial or complete gonadal dysgenesis
DMRT1	DMT1	93	**100**	46,XY gonadal dysgenesis
DMRT2		76	**100**	46,XY gonadal dysgenesis
FSHR	ODG1/LGR1	—	**100**	46,XX premature ovarian failure
GATA4		64	(82)	46,XY ambiguous genitalia
HHAT		94	*99*	46,XY gonadal dysgenesis
MAP3K1	MEKK	89	*98*	46,XY sex reversal
NR0B1	DAX1/AHCH	98	**100**	46,XY sex reversal
NR5A1	SF1	97	**100**	46,XY sex reversal; 46,XX premature ovarian failure
RSPO1	RSPONDIN	**100**		46,XX sex reversal and palmoplantar hyperkeratosis
SOX3	PHP	78	**100**	46,XX sex reversal
SOX9	SRA1	**100**		46,XX sex reversal and campomelic dysplasia
SRY	TDF	**100**		46,XX (ovo)testicular DSD and 46,XY gonadal dysgenesis
STAG3	STROMALIN-3	—	(93)	46,XX premature ovarian failure
WNT4	SERKAL	92	**100**	46,XY DSD 46,XY complete gonadal dysgenesis
WT1	AWT1/WAGR	77	**100**	Wilms tumor-aniridia-genital anomalies-retardation syndrome
WWOX	SDR41C1/WOX1/FOR	95	**100**	46,XY gonadal dysgenesis
ZFPM2	FOG2	99	**100**	46,XY gonadal dysgenesis
Sex differentiation (eg, steroid synthesis/receptors)				
AKR1C2	BABP/DD/DD2/HAKRD/MCDR2	(91)	—	46,XY DSD
AKR1C4	3-a-HSD, C11/CDR/DD4/HAKRA	**100**		46,XY DSD
AMH	MIS	59	*98*	Persistent Müllerian duct syndrome (PMDS)
AMHR2	MISR2	**100**		PMDS
AR	AIS	95	*99*	Androgen insensitivity syndrome (CAIS/PAIS)
ARX	CT121/EIEE1/ISSX	50	(89)	X-linked lissencephaly with ambiguous genitalia (XLAG)
ATRX	RAD54	**100**		Alpha-thalassemia X-linked intellectual disability syndrome
CYP11A1	P450SCC	**100**		CAH, 11-hydroxylase deficiency
CYP17A1		**100**		CAH, 17-hydroxylase deficiency
CYP19A1		**100**		46,XX virilization
CYP21A2	CA21H/CAH1/CPS1	79	(90)	CAH, 21-hydroxylase deficiency

Gene	Alternative Names	Coverage (February 2012-February 2015) (%)	Coverage since March 2015 (%)	Reported Associated Phenotype
DHCR7		—	**100**	Smith Lemli Opitz syndrome
FGFR2		**100**		Apert syndrome
FOXL2	BPES	79	*97*	Blepharophimosis, ptosis, and epicanthus inversus
HSD17B3	SDR12C2	**100**		17β-hydroxysteroid dehydrogenase III deficiency (46,XY DSD)
HSD3B2	SDR11E2	**100**		CAH, 3β-hydroxysteroid dehydrogenase deficiency (46,XY DSD)
KDM5D	JARID1D/HYA	—	(60)	Y chromosome infertility
LHCGR	LCGR/LGR2/LHR/ULG5	92	**100**	Leydig cell hypoplasia
MAMLD1	CG1/F18/CXORF6	69	**100**	Hypospadias (46,XY)
POR		**100**		Cytochrome P450 oxidoreductase deficiency
SRD5A2		**100**		Steroid 5-alpha-reductase deficiency
STAR	StAR/STARD1	**100**		CAH, cholesterol desmolase deficiency
VAMP7	SYBL1/TI-VAMP	50	**100**	46,XY undervirilization
Central causes of hypogonadism				
ARL6	BBS3	**100**		Bardet Biedl syndrome
BBS1	BBS2L2	99	**100**	Bardet Biedl syndrome
BBS2	RP74	**100**		Bardet Biedl syndrome
BBS4		99	**100**	Bardet Biedl syndrome
BBS5		**100**		Bardet Biedl syndrome
BBS7	BBS2L1/FLJ10715	**100**		Bardet Biedl syndrome
BBS9	B1/PTHB1	**100**		Bardet Biedl syndrome
BBS10	FLJ23560	**100**		Bardet Biedl syndrome
BBS12	FLJ35630/FLJ41559	**100**		Bardet Biedl syndrome
CHD7	FLJ20357/FLJ20361/KIAA1416	**100**		Kallman syndrome; normosmic IGD; CHARGE syndrome
FGF8	AIGF	79	*97*	IGD with anosmia (Kallman syndrome) and normosmic IGD
FGFR1	BFGFR/CD331/CEK/FLG	98	**100**	Kallman syndrome, normosmic IGD, and Pfeiffer syndrome
FRAS1		—	**100**	Fraser syndrome
FREM2	ECM3homolog	—	**100**	Fraser syndrome
GNRH1	GNRH/GRH/LHRH	**100**		Isolated abnormality in GnRH secretion or response
GNRHR	LHRHR	**100**		Isolated abnormality in GnRH secretion or response
GRIP1		—	**100**	Fraser syndrome
HESX1	ANF/RPX	**100**		Combined pituitary hormone deficiency
HFE	HLA-H	**100**		Hemochromatosis
KAL1	anosmin-1/KALIG-1	95	**100**	IGD with anosmia (Kallman syndrome)
KISS1R	AXOR12/HOT7T175	54	**100**	Isolated abnormality in GnRH secretion or response
LEP		**100**		Morbid obesity

Gene	Alternative Names	Coverage (February 2012-February 2015) (%)	Coverage since March 2015 (%)	Reported Associated Phenotype
LEPR	CD295/OBR	95	*97*	Morbid obesity
LHX3	LIM3	87	**100**	Combined pituitary hormone deficiency
MKKS	BBS6	**100**		Bardet Biedl syndrome/McKusick-Kaufman syndrome
PCSK1	PC1/PC3/SPC3	98	**100**	Morbid obesity
PROK2	BV8/KAL4/MIT1/PK2	76	**100**	IGD with anosmia (Kallman syndrome) and normosmic IGD
PROKR2	GPR73b/GPRg2/PKR2	**100**		IGD with anosmia (Kallman syndrome) and normosmic IGD
PROP1		**100**		Combined pituitary hormone deficiency
PTPN11	NS1	99	**100**	Noonan syndrome 1
SOS1	GINGF	**100**		Noonan syndrome 4
TAC3	NKB/ZNEUROK1	**100**		Isolated abnormality in GnRH secretion or response
TACR3	neurokinin beta receptor/NK3R	**100**		Isolated abnormality in GnRH secretion or response
TRIM32	BBS11	**100**		Bardet Biedl syndrome
TTC8	BBS8	**100**		Bardet Biedl syndrome/retinitis pigmentosa, autosomal recessive

Improved coverage with the v.3 capture protocol is shown in the fourth column. Genes with 100% coverage are indicated in bold. Genes with coverage above 97% are indicated in italic. Genes with lower coverage are indicated within parenthesis. Genes not showing a coverage value in the third column were added to the list after February 2015. Coverage was not indicated in the fourth column when capture was unchanged from the previous iteration.

Abbreviations: CAH, congenital adrenal hyperplasia; IGD, isolated GnRH deficiency.

Table 2

Diagnostic effort by the DSD-TRN team increased the percentage of patients with a firm diagnosis from 24% to 46%

A

(Total 5 144 Forms)	**Yes**	**No**	**Not Answered**
Patient had diagnosis prior to 1st visit	35	101	8
Patient currently has diagnosis	65	71	8
Patient has diagnosis (manual assessment)	66	77	1 uncertain diagnosis

B

Site	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Dx	15	11	10	13	5	0	0	0	9
no Dx	8	21	5	12	14	1	10	4	5
% Dx	66%	37%	67%	52%	26%	0%	0%	0%	64%
# Reported	23	30	15	25	19	1	10	4	14
# Enrolled	54	31	15	39	19	17	43	72	14
% Reported	43%	97%	100%	64%	100%	6%	23%	6%	100%
% with Dx	28%	36%	67%	33%	26%	0%	0%	0%	64%

Forms entered for siblings and 2 forms mistakenly entered in duplicate were discarded prior to analysis to ensure search in unique proband data. First, an automated query for “patient has diagnosis” (true/false) and “patient had diagnosis prior to first visit to DSD-TRN team” (true/false) was run. Follow-up manual curating made use of the built-in redundancy features of the forms, designed for such data quality control. It included searching for (1) positive answers to “gene with diagnostic variant”, confirmed with entry of actual variant; (2) “CNV found” with a pathogenic or likely pathogenic call in the CMA results; (3) abnormal/mosaic karyotype, with actual data entry of mosaic percentages or sex chromosome complement. Eight forms out of 144 had no response in the “patient has/had diagnosis” fields. Answers were found using other variables for 7 of the 8 forms. Another 6 forms had erroneous reporting; 3 forms were reported as not having adiagnosis when manual validation did find one, and 3 forms were reported as having a diagnosis when infact a normal karyotype was reported, without specific gene variants. This constitutes an approximate 10% error in data entry or recording. Teams are subsequently notified of these errors for rectification as part of the practice improvement process.

In B, the ratio (%*Dx*) of number of patients with (*Dx*) or without (*no Dx*) a firm genetic diagnosis is reported per clinical site. The total number of genetic forms in the registry (# reported 5 Dx + noDx) compared with the total number of patients enrolled in the study (# Enrolled) gives an estimate of data entry completion (% reported) and of percentage of patients with a diagnosis available in the registry (% *with Dx* = Dx/Total enrolled) at a specific site.

Table 3
The conditions of 6% of probands reported in the DSD-TRN registry are familial

	Familial	De novo	Unknown	Not Answered
"Familial" reported	17	44	66	17
Accurate "familial/de novo/inherited" call	Yes: 8 Unk.: 9	Yes: 6 Unk.:38	Unk.:66	Yes: 1 Unk.: 16
Actual familial cases	9	6	129	—

Reported numbers for each answer options to the question "Is condition familial?" ("yes, familial", "no, de novo", "unknown") are shown in the "familial reported" row. Manual curating of the responses by cross-examining other data points is shown in the "accurate call" row, as Yes (accurate reporting) or Unk. (should have been reported as Unknown). Other variables examined to determine the accuracy of the call included karyotype mother/father/siblings, CMA and exome variants parent of origin, existing genetics form for an affected sibling, and phenotype/genotype shared by family member. Actual calls as they would be expected to be reported are shown in the "actual familial cases" row.

Table 4

Frequency of genetic diagnoses in the disorders of sex development translational research network registry

Karyotype		Genetic Diagnosis	
Sex Chromosome aneuploidy	19	Mosaic 45,X/46,XY	13 (10 idicY)
		46,XX Xq del	2
		Klinefelter 47,XXY	3 (2 mosaic)
		49,XXXXY	1
46,XX	46	CAH *CYP21A2*	14
		SRY+	3
		SOX9 Dup (mosaic)	1
		Kabuki syndrome	1
		No diagnosis	27
46,XY	64	PAIS/CAIS (*AR*)	15
		SRD5A2	4
		17βHSD	4
		MAP3K1	2
		WAGR (11p del)	1
		Smith Lemli Opitz	1
		No diagnosis	37
Not reported	15	*SRD5A2*	1
		Unknown	14

Table 5

The diagnostic process is not exhausted in 97% of undiagnosed cases

A			
	CMA Performed	**CMA Not Performed**	**Not Reported**
Have Dx	20	35	9
Don't have Dx	23	37	20

B				
Method	**Karotype/FISH**	**Single gene**	**CMA**	**Exome**
Diagnosis achieved	19	36	6	4
Test performed	129	47	43	9

The number of patients for whom a chromosomal microarray (CMA) was performed is indicated in A. Numbers were similar among patients who have a firm genetic diagnostic and among those who do not. B shows the method by which diagnosis was eventually achieved in comparison with the number of patients for whom the test was performed.