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Commentary

Next-Generation Sequencing as First-Line Diagnostic Test in Patients With Disorders of Sex Development?

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Abbreviations: AMH, anti-Müllerian hormone; DSD, disorders of sexual development.

Disorders of sex development (DSD) are a group of highly heterogeneous disorders presenting mainly at birth or at pubertal age, characterized by discordance between chromosomal, gonadal, and/or genital sex. Newborns usually present with ambiguous genitalia while adolescents present with atypical or downright absent pubertal development (1). The underlying pathogenesis may rely on gonadal differentiation or on sex hormone biosynthesis or action. Indeed, the pioneering experiments performed by Alfred Jost in the 1940s demonstrated that the actions of 2 testicular hormones—androgens and the subsequently characterized anti-Müllerian hormone (AMH)—are essential for the virilization of the fetus, while in the absence of testicular hormones the fetal genitalia undertake the female pathway (2). The primordia of the external genitalia are identical in all embryos before gonadal differentiation, and their degree of virilization during fetal life depends on the extent of androgen action. This can be clinically assessed using the external genitalia score, based on a standardized description of the external genitalia, which applies a graded scale from female (score 0) to male (score 10.5-12), with intermediate values in patients with ambiguous genitalia (3). Disorders of gonadal differentiation occur in individuals with atypical sex chromosomes but may also present as a consequence of single gene variants, particularly in 46,XY patients. Isolated impaired testosterone or dihydrotestosterone (DHT) biosynthesis and target-organ insensitivity to androgens are other endocrine etiologies of DSD, usually resulting from gene mutations in 46,XY individuals (4). Atypical, rather than ambiguous, genitalia are characteristic of complex dysmorphic syndromes that are not endocrine-related and prompt a different diagnostic approach (5).

Although the approach to investigating patients with a suspected 46,XY DSD varies according to the age at presentation, it generally includes a pelvic ultrasonography to identify uterus and Fallopian tubes and the determination of testicular hormones in serum (1, 6). When both testosterone and AMH are in the female range and a uterus is present, a disorder of testicular differentiation (testicular dysgenesis) is suspected. When serum testosterone is in the female range but AMH is in the male range, occurring with the absence of a uterus, a disorder of androgen synthesis is highly likely. Finally, in the absence of a uterus but with serum testosterone and AMH in the male range, androgen insensitivity and 5α-reductase type 2 deficiency are the main differential diagnoses. This clinical assessment pathway was used from the 1980s until the first decade of the present century to drive genetic studies using the "candidate gene" approach. It was time-consuming and led to a genetic diagnosis in only ~20% of the 46,XY cases (7).

DSD represent a major healthcare burden because of their difficult clinical management. The uncertainty about the newborn's sex and the potential long-term outcomes, such as gonadal cancer, infertility, or associated comorbidities, are usually distressing. Providing an etiologic diagnosis based on molecular genetic results is of utmost importance: putting a name to the underlying condition improves its acceptance in the patient and family and contributes to a personalized healthcare approach based on a precise diagnosis. In that sense, massive parallel sequencing techniques have introduced major advancement in the past few years. From targeted gene panels to whole exome and whole genome sequencing (4), molecular genetic assessments have progressively improved the rate of etiologic diagnosis in patients with DSD (4).

The recent study by Gomes and colleagues (8) provides a major contribution to clarify the extent to which the combination of clinical and hormonal studies with molecular genetic results obtained by massive parallel sequencing improved the precision diagnosis in 46,XY patients with DSD. In a uniquely large study for a rare condition like DSD, the authors analyzed 263 patients with 46,XY DSD attending a single center in São Paulo, Brazil. All participants were thoroughly studied, including the anatomical description of the genitalia, an

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extended serum hormone profile, imaging assessment, and histologic findings, in order to be classified into 3 groups: 64 had gonadal dysgenesis, 121 showed disorders of testicular hormone (testosterone or AMH) secretion or action, and 70 were DSD of unknown etiology. Using Sanger sequencing, a DSD target panel or whole exome sequencing, the authors were able to establish a genetic etiology in ~60% of the cases, with variations according to the subgroups: the rate of positive results was 36% in patients with gonadal dysgenesis, 97% in those with disorders of testicular hormone secretion or action, and 32% in those with a previously unidentified cause for the DSD. Thus, they show that the combination of clinical/biochemical and genetic approaches significantly improve the rate of etiologic diagnosis in 46,XY patients with DSD. Furthermore, they provide evidence that, when applied as a first-line test, massive parallel sequencing effectively identifies patients with disorders of testicular hormone production or action, simplifying the subsequent hormonal workup.

Can we foresee genetic testing completely replacing the traditional diagnostic approach in DSD patients in the near future? Massive parallel sequencing techniques have certain constraints. The technology is not widely available, costs are not universally affordable, and the interpretation of results requires an expert multidisciplinary team. Panels target a limited number of genes and need to be reformulated as new genes are discovered. They are progressively being replaced by a straight whole exome sequencing approach, as sample processing capacity increases and costs decrease. Whole exome and genome sequencing may result in the detection of secondary or incidental findings, requiring that the pretest counseling process clearly establish which results the patient wishes or not to be returned. Although these issues might be progressively solved, the deep phenotyping of patients at the pretest stage remains a keystone for the analysis of the massive results ("big data") yielded by most of these techniques, especially when gene variants found need to be prioritized in order to assign a "pathogenic" or "likely pathogenic" label. Therefore, the combination of phenotypic data, obtained by thorough anatomical, hormonal, and histological data, together with genotyping will probably remain irreplaceable.

Disclosure

The author has nothing to disclose.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References

1. Grinspon RP, Castro S, Rey RA. Up-to-date clinical and biochemical workup of the child and the adolescent with a suspected disorder of sex development. *Horm Res Paediatr.* Published online ahead of print November 15, 2021. doi:10.1159/000519895
2. Jost A. Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Prog Horm Res*. 1953;8379:418.
3. van der Straaten S, Springer A, Zecic A, *et al*. The External Genitalia Score (EGS): a European multicenter validation study. *J Clin Endocrinol Metab*. 2020;105(3):e222-e230.
4. Delot EC, Vilain E. Towards improved genetic diagnosis of human differences of sex development. *Nat Rev Genet*. 2021;22(9):588-602.
5. Grinspon RP, Rey RA. When hormone defects cannot explain it: malformative disorders of sex development. *Birth Defects Res C Embryo Today*. 2014;102(4):359-373.
6. Ahmed SF, Achermann J, Alderson J, *et al*. Society for Endocrinology UK Guidance on the initial evaluation of a suspected difference or disorder of sex development (Revised 2021). *Clin Endocrinol*. 2021;95(6):818-840.
7. Achermann JC, Domenice S, Bachega TA, Nishi MY, Mendonça BB. Disorders of sex development: effect of molecular diagnostics. *Nat Rev Endocrinol*. 2015;11(8):478-488.
8. Gomes NL, Batista RL, Nishi MY, *et al*. Contribution of clinical and genetic approaches for diagnosing 209 index cases with 46,XY differences of sex development. *J Clin Endocrinol Metab*. 2022. doi:10.1210/clinem/dgac064