Use of Next Generation Sequencing in Clinical Practice: The Example of Disorders/Differences of Sex Development

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Disorders/Differences of sex development (DSD) are congenital conditions in which development of chromosomal, gonadal, or anatomic sex is atypical (1). DSD are chronic medical conditions collectively affecting ~1% of the population (2,3), frequently requiring life-long care by multiple specialists, and carrying a significant public health burden (1). Some are associated with life-threatening events, such as adrenal crises in congenital adrenal hyperplasia (4). DSD are also associated with increased infertility, cancer, gender dysphoria risks, psychosocial distress, and pervasive challenges to health-related quality of life for patients and families (5,6,7).

DSD are broadly classified into three categories: sex chromosome DSD, 46,XY DSD, and 46,XX DSD and are further classified according to the type of gonad found in the patient (ovary, testis, ovotestis). Currently, known etiologies include disorders of gonadal development and disorders in androgen synthesis or action, and are considered Mendelian (reviewed in Ref. 3). Sex development in humans is divided into two sequential steps: sex determination and sex differentiation. Sex determination refers to the expression of gene networks that direct the development of undifferentiated bipotential gonads into either testes or ovaries. Once developed, testes and ovaries secrete hormones that promote further sex differentiation of the body throughout embryonic development and adulthood (8). Mutations have been identified in genes that control both steps, leading to DSD (9).

Standard genetic diagnosis for DSD is typically limited to genotyping just one or two genes, chosen as likely candidates based on disease phenotype. However, because of phenotypic overlap in various DSD conditions, serial single candidate gene testing is highly inefficient: diagnostic yields of 30% for 46,XY gonadal dysgenesis, 80% for 46,XX testicular DSD, and 10% for ovotesticular DSD are widely quoted with traditional single-gene testing methods (reviewed in Ref. 8). With this approach, limited to known genes and to genes for which clinical testing is available in the country, most patients do not receive a definitive diagnosis.

We were able to increase significantly the diagnostic success for DSD using whole exome sequencing (WES), with the identification of disease-causing and likely pathogenic variants in a third of a cohort of 46,XY patients (10). We have therefore proposed a shift in the diagnostic approach to DSD to use WES as a first-line clinical test, which could lead to faster and more accurate diagnosis, and orient further clinical management, limiting unnecessary, costly, and often invasive endocrine testing and imaging (2). However, many remain unexplained (over half of the XY cases, a significant minority of XX cases, including most ovotesticular DSD, and most syndromic cases). In addition, the very large phenotypic variability in cases with known variants in the same gene is unexplained. We propose that the of use whole-genome sequencing will dramatically improve upon exome sequencing, covering both coding and non-coding parts of the genome more uniformly, as an approach to not only improve diagnostic yield, but also to identify novel genes and regulatory elements involved in DSD.

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