DNA Copy Number Variations in Patients with 46,XY Disorders of Sex Development

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Purpose: Less than 50% of cases of 46,XY disorders of sex development are genetically defined after karyotyping and/or sequencing of known causal genes. Since copy number variations are often missed by karyotyping and sequencing, we assessed patients with unexplained 46,XY disorders of sex development using array comparative genomic hybridization for possible disease causing genomic variants.

Materials and Methods: DNA from unexplained cases of 46,XY disorders of sex development were tested by whole genome array comparative genomic hybridization. In cases where novel copy number variations were detected parental testing was performed to identify whether copy number variations were de novo or inherited.

Results: Of the 12 patients who underwent array comparative genomic hybridization testing 2 had possible copy number variations causing disorders of sex development, both maternally inherited microdeletions. One case, with a maternal history of premature ovarian failure, had a cosegregating microdeletion on 9q33.3 involving *NR5A1*. The other case, with a maternal family history of congenital heart disease, had a cosegregating microdeletion on 8p23.1 upstream of *GATA4*.

Conclusions: In this cohort copy number variations involving or adjacent to known causal genes led to 46,XY disorders of sex development in 2 of 12 previously unexplained cases (17%). Copy number variation testing is clinically indicated for unexplained cases of 46,XY disorders of sex development to aid in genetic counseling for family planning.

Key Words: comparative genomic hybridization, disorders of sex development, DNA copy number variations

Abbreviations and Acronyms

aCGH = array comparative genomic hybridization
CHD = congenital heart disease
CNV = copy number variation
DSD = disorders of sex development
FISH = follicle-stimulating hormone
GD = gonadal dysgenesis
POF = premature ovarian failure
SRY = sex-determining region Y

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Determination of a sexual phenotype in humans depends on the presence and activity of regulatory networks that stimulate the bipotential gonad to develop into a testis or ovary. In males sex-determining region Y initiates sex determination by stimulating Sertoli cell differentiation through a genetically controlled network. Deregulation of this network can result in partial or complete failure of testis determination. Male sex differentiation involves müllerian duct regression, wolffian duct development and external genitalia virilization, which rely on Sertoli cell derived anti-müllerian hormone and Leydig cell derived testosterone. Disruption of the sex determination or differentiation networks causes

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disorders of sex development, defined as congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical.[1]

DSD cases with a 46,XY karyotype are clinically and genetically heterogeneous but can be classified as gonadal (testicular) dysgenesis, decreased fetal androgen biosynthesis, decreased fetal anti-müllerian hormone production/action or androgen action defects. While the genetic basis of the last 3 categories is better understood, the 46,XY DSD/GD group is incompletely characterized. Phenotypically speaking, 46,XY DSD/GD can be either complete GD (previously called "sex reversal"), yielding female external genitalia, streak gonads and müllerian structures, or partial GD, yielding ambiguous external genitalia, dysgenetic testes, and partial development of müllerian and wolffian structures.

Many genes have been identified that are crucial for proper testis determination and thus mediate testicular dysgenesis when genetically disrupted (see Appendix). These genomic variations can be detected by gene sequencing. Larger genomic rearrangements of 46,XY DSD/GD causal genes can cause a copy number variation, yielding deletions or duplications of the genes or their regulatory regions. For example CNVs of *SRY* (deletion), *SOX9* (deletion) and *DAX1* (duplication) are known to cause 46,XY DSD/GD. Unfortunately these genomic variations can be missed on karyotyping. Array comparative genomic hybridization, also known as chromosome microarray analysis or cytogenomic microarray analysis, is a microarray based genomic copy number analysis method capable of assessing for CNVs throughout the genome.

Mutations in known sex-determining genes account for almost 50% of cases of 46,XY DSD, and thus the genetic basis for the remaining 50% is unknown.[2] However, these data are suspect, since screening for mutations in known sex-determining genes typically identifies only point mutations, while genomic rearrangements such as CNVs as well as deep intronic, upstream and downstream regulatory mutations are often missed by sequencing and undetectable by karyotyping. We hypothesized that identification of novel CNVs may uncover the underlying genetic cause of cases of 46,XY DSD of unknown etiology. We screened patients with genetically unexplained 46,XY DSD by aCGH to assess for variations in novel gonadal genes, in the regulatory elements of known gonadal genes or in known gonadal genes that were not detected by karyotyping or candidate gene sequencing.

MATERIALS AND METHODS

Study Population

Patients with 46,XY DSD/GD were recruited for standard of care and research genetic testing from 2010 to 2012. Informed consent was obtained according to a University of Texas Southwestern Medical Center review board approved protocol. Study inclusion criteria consisted of presentation with sporadic, nonfamilial, ambiguous genitalia (hypospadias with cryptorchidism, age adjusted phallic size more than 2 SD above normal for a clitoris or more than 2 SD below normal for a penis, normal phallus with or without hypospadias and bilateral nonpalpable testes, virilized female, and/or discordance between genital appearance and karyotype), which after phenotyping and genotyping were deemed 46,XY DSD of unknown etiology (with GD in some cases). Many of the patients had undergone targeted gene sequencing to evaluate for mutations that fit the clinical phenotype, although this testing had provided no cause. Therefore, Clinical Laboratory Improvement Amendments certified CNV testing was offered to assess for CNVs in genes known to cause 46,XY DSD and unknown novel 46,XY DSD genes as standard of care. Patients with known chromosomal abnormalities or genetic causes of the disease were excluded. Genomic DNA was extracted from peripheral blood lymphocytes of the subjects via the Puregene® DNA isolation kit according to manufacturer protocols.

Array Comparative Genomic Hybridization/FISH

Array comparative genomic hybridization was performed using the Baylor College of Medicine CGH Microarray, version 8.1 (180,000 oligonucleotides) or 8.3 (400,000 oligonucleotides, Agilent®). Digestion, labeling and hybridization were completed following manufacturer protocols. Baylor College of Medicine Web based software was used for genomic copy number analysis. The computational methods have been described previously.[3] When possible, in cases where novel genomic CNVs of potential significance were detected parental testing was performed to identify whether CNVs were de novo or inherited. Confirmatory and parental FISH analyses were performed using standard procedures developed by the Medical Genetics Laboratories at Baylor College of Medicine.

Multiplex Ligation-Dependent Probe Amplification Analysis

DNA was screened for CNVs using the SALSA® P234-A1 GATA4 MLPA® kit according to manufacturer directions. Amplified products were separated by size on an ABI Prism® 3100 genetic analyzer and data were analyzed using GeneMarker®, version 2.2.0.

RESULTS

Eight white, 3 Hispanic and 1 black patient with 46,XY DSD/GD were screened via aCGH, urogenital phenotypes and genetic testing results (supplementary Appendix, http://jurology.com/). Four previously unreported genomic rearrangements were identified. Two of these patients had 0.076-0.263 Mb microduplications of Xp21.1 and 15q12q13.1, which contained the genes *DMD* and *OCA2*, respectively. Since both of these microduplications are remote from any genes associated with sexual development, they are of unclear clinical significance regarding DSD/GD