Molecular and Cellular Endocrinology 444 (2017) 19–25

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Diagnostic yield of targeted gene panel sequencing to identify the genetic etiology of disorders of sex development

Ja Hye Kim [a], Eungu Kang [b], Sun Hee Heo [c], Gu-Hwan Kim [d], Ja-Hyun Jang [e], Eun-Hae Cho [e], Beom Hee Lee [b], Han-Wook Yoo [b], Jin-Ho Choi [b, *]

[a] Department of Pediatrics, Hanyang University Guri Hospital, Hanyang University College of Medicine, Guri, Republic of Korea
[b] Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Republic of Korea
[c] Asan Institute for Life Sciences, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Republic of Korea
[d] Medical Genetics Center, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Republic of Korea
[e] Green Cross Genome, Yongin, Republic of Korea

ARTICLE INFO

Article history:
Received 3 December 2016
Received in revised form
21 January 2017
Accepted 23 January 2017
Available online 24 January 2017

Keywords:
Androgen receptor
CYP17A1
Disorders of sex development
Exome sequencing
SRD5A2

ABSTRACT

Disorders of sex development (DSD) vary phenotypically and are caused by a number of genetic etiologies. This study investigated the genetic etiology of DSD patients using targeted exome sequencing of 67 known DSD-associated genes in humans. This study included 37 patients with 46, XY DSD and seven patients with 46, XX DSD. We identified known pathogenic mutations or deletion in nine (20.5%) patients in the *AR*, *CYP17A1*, *SRD5A1*, and *DMRT1/2* genes. Novel variants were identified in nine patients (20.5%) in the *AR*, *ATRX*, *CYP17A1*, *CHD7*, *MAP3K1*, *NR5A1*, and *WWOX* genes. Among them, four patients harbored pathogenic or likely pathogenic variants, while the remaining five patients (11.4%) had variants of uncertain significance. We were able to make a genetic diagnosis in 29.5% of patients with pathogenic or likely pathogenic mutations. Targeted exome sequencing is an efficient tool to improve the diagnostic yield of DSD, despite its phenotypic and genetic heterogeneity.

1. Introduction

Disorders of sex development (DSD) are defined as a congenital condition in which the development of chromosomal, gonadal, and anatomical sex is atypical (Lee et al., 2006). Although there are limited data on the prevalence of DSD, ambiguous genitalia have been reported to occur in approximately 1/4500–5500 live births (Sax, 2002; Thyen et al., 2006).

The correct etiological diagnosis of DSD is challenging because of gender assignment and corrective surgery for external genitalia. Traditionally, the diagnosis of DSD is based on endocrine tests, imaging studies, chromosome analysis, and Sanger sequencing of the candidate genes. Conventional Sanger sequencing is useful when the clinical features and biochemical tests suggest disruption of a specific pathway (Achermann et al., 2015). However, where the potential cause of the DSD is not clear, or not indicated by the endocrine profiles, a specific molecular diagnosis can be made in only ~20% of patients with DSD (Houk et al., 2006), making the Sanger sequencing inefficient, costly, and time-consuming. Therefore, the etiological diagnosis of DSD is difficult to establish because of a lack of standardization of anatomical and endocrine phenotyping and poor genotype-phenotype correlation (Barseghyan et al., 2015).

Recently, next-generation sequencing (NGS) technologies have been developed that allow high-throughput analyses. NGS has revolutionized the identification of causative genes of diseases with genetic heterogeneity using massive parallel sequencing of multiple samples simultaneously. However, whole genome or exome sequencing is expensive and laborious, and the results are difficult to interpret. Thus, targeted enrichment techniques have been developed to reduce sequencing cost and time using targeted exome capture.

A pilot study using a targeted capture approach for 35 known DSD genes identified the genetic causes in two of five previously undiagnosed DSD patients (Arboleda et al., 2013). The authors

Abbreviations: DSD, disorders of sex development; NGS, next-generation sequencing; VUS, variants of uncertain clinical significance.

* Corresponding author. Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, 88, Olympic-Ro 43-Gil, Songpa-Gu, Seoul 05505, Republic of Korea.
E-mail address: jhc@amc.seoul.kr (J.-H. Choi).

http://dx.doi.org/10.1016/j.mce.2017.01.037
0303-7207/

expanded this approach to 40 patients with 46, XY DSD, and identified a likely genetic diagnosis in more than one third of the cases (Baxter et al., 2015). In addition, targeted exome sequencing of 219 genes previously reported to be associated with DSD, sex determination, sex differentiation, and hypogonadism enabled a genetic diagnosis in eight of 21 (38.1%) patients with DSD (Dong et al., 2016). Although these studies included a relatively small number of patients, they suggested that targeted NGS could be considered as a first-tier diagnostic tool for DSD and represents a comprehensive and efficient method to diagnose DSD. A recent report of targeted gene sequencing of a large international patient cohort identified likely genetic diagnosis in 43% of patients with 46, XY DSD (Eggers et al., 2016). Consequently, we designed a targeted DSD panel focusing on previously reported DSD-associated genes in humans to reduce the cost, time, and complexity of interpreting the results, making this technique applicable to the molecular diagnosis of DSD. Therefore, the present study was performed to investigate the genetic etiology of patients with DSD using targeted exome sequencing of 67 known human DSD-associated genes.

2. Material and methods

2.1. Subjects

Patients who did not reach a specific diagnosis despite genetic, hormonal, and imaging studies were included in the study. They comprised 37 patients (84.1%) with 46, XY DSD and seven patients (15.9%) with 46, XX DSD. Patients with sex chromosome DSD and 46, XX DSD who were positive for the *SRY* gene were excluded from this study. Four patients with known genetic diagnoses were included as positive controls, including two patients with androgen insensitivity syndrome (AIS) (p.L446Ffs*56 and p.M743I in *AR*, respectively) (Subjects 4 and 12 in Supplemental Table 1), one with 5-reductase deficiency (p.R240Q homozygote in *SRD5A2*) (Subject 10), and one with a *DMRT1*/2 deletion (Subject 11). The detailed clinical phenotypes of the patients are summarized in Tables 1 and 2, and Supplemental Table 1. This study was approved by the Institutional Review Board at the Asan Medical Center, and informed consent was obtained from all the patients or their parents.

2.2. Design of a targeted DSD panel and exome sequencing

Genomic DNA was extracted from peripheral blood leukocytes using a Gentra Puregene blood kit (Qiagen, Hilden, Germany). A targeted DSD panel was designed to include 67 genes that have been previously reported to be associated with DSD in humans from online databases, including PubMed (http://www.ncbi.nim.nih.gov/pubmed), Online Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/omim), and GeneTests (http://www.genetests.org/) (Supplemental Tables 2–4). We designed oligonucleotide bait tiles against exons and exon-intron boundary regions of 67 genes spanning a 152.953 kbp region with 2914 probes. Exomes were captured using customized a SureSelect Target Enrichment System Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on the Miseq platform (Illumina Inc., San Diego, CA, USA). The average sequencing depth cover was over 150 × on the target regions and approximately 99.46% of targeted bases were read more than 1 × . Reads were aligned to the reference genome using the Burrows-Wheeler alignment program (BWA version 0.7.5) and the aligned reads were processed using SAM tools (http://samtools.sourceforge.net/) and PICARD (http://picard.souceforge.net/) to remove potential PCR duplicates. Local realignment and base quality recalibration was performed by the Genome Analysis Toolkit (GATK version 3.30). Variant calling was performed by the GATK. Annotation was done by Variant Effect Predictor.

To minimize the risk of false-positive findings, only the variants with Phred scores ≥15 were analyzed. Variants with minor allele frequencies over 1% in the 1000genomes browser (http://browser.1000genomes.org/), NHLBI ESP Exome Variant Server (http://evs.gs.washington.edu/EVS/), and genome Aggregation Database

Table 1
Phenotype and genetic findings in patients with pathogenic or likely pathogenic variants.

Subject	Clinical features	Anatomy and gonads	Sex of rearing	Identified deleterious sequence variants		
				Gene	Nucleotide	Protein
1	Primary amenorrhea	No uterus, blind vagina, inguinal testes	F	*AR*	c.2257c > T	p.R753*
2	Bilateral inguinal hernia	No uterus, blind vagina, inguinal testes	F	*AR*	c.1768 + 1G > A	
3	Primary amenorrhea	No uterus, blind vagina, intra-abdominal testes	F	*AR*	c.2296G > A	p.A766T
4	Genital hypoplasia, partial spontaneous puberty with gynecomastia	Micropenis, hypospadias, descended testes	M	*AR*	c.2229G > A	p.M743I
12	Primary amenorrhea	No uterus, blind vagina, inguinal testes	F	*AR*	c.1337dupT	p.L446Ffs*56[a]
5	Primary amenorrhea, hypertension	Streaky gonads in pelvic cavity	F	*CYP17A1*	c.1118A > T, homozygous	p.H373L/p.H373L
6	Inguinal hernia, prehypertension	No uterus, blind vagina, inguinal testes	F	*CYP17A1*	c.1118A > T, homozygous	p.H373L/p.H373L
7	Primary amenorrhea, prehypertension	No uterus, blind vagina, inguinal testes	F	*CYP17A1*	c.1117C > A/ c.1118A > T	p.H373N/p.H373L
8	Inguinal hernia, adrenal insufficiency	No uterus, blind vagina, inguinal testes	F	*CYP17A1*	c.1053_1055delCCT/ c.987delC	p.L353del/ p.Y329Kfs*90[a]
10	Clitoromegaly	No uterus, inguinal testes	F	*SRD5A2*	c.736G > A, homozygous	p.R246Q/p.R246Q
11	Delayed puberty, epilepsy, mental retardation, SNHL	No uterus, inguinal testes	F	*DMRT1/2*	DMRT1/2 deletion	
15	Clitoromegaly	Atrophic testes in pelvic cavity	F	*NR5A1*	c.205C > G	p.R69G
38	Genital hypoplasia, coloboma, microtia, hearing loss	Micropenis, cryptorchidism,	M	*CHD7*	c.5656delC	p.H1886Mfs*13[a]

AIS, androgen insensitivity syndrome; F, female; M, male; SNHL, sensorineural hearing loss; * termination codon.
[a] Novel variant.