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Identification of a Rare Mutation in the *SRD5A2* Gene in an Iranian Family with Sex Development Disorder

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Abstract

5 α-Reductase type 2 deficiency, caused by mutations in the *SRD5A2* gene, leads to an autosomal recessive disorder of sex differentiation (DSD) in 46, XY persons. A 2 years old female with ambiguous genitalia was referred to Genetic Foundation of Khorasan Razavi (GFKR), IRAN. Her secondary sex characteristics, level of sex hormones, the development of reproductive system and karyotype were assessed. Whole-exome sequencing (WES) was preformed to find the pathogenic genetic variations associated with ambiguous genitalia. Also, Sanger sequencing was used to verify the WES results in the patient. Segregation analysis was performed to confirm mutation in the parents and other relatives. Physical examination and ultrasonography data demonstrated that the patient have testis in the left labium majus and right groin but does not have a uterus or ovaries. Sex hormone examination revealed that hormone therapy was successful and the level of FSH, LH, testosterone, and dihydrotestostron (DHT) was 2.8 mlu/ml, 2.4 mlu/ml, 15ng/dl, 21pg/ml, respectively. Cytogenetic study showed 46XY compatible to normal male karyotype. According to WES result and Sanger sequencing a homozygote loss of function mutation (c.16C>T; p.Gln6Ter) in *SRD5A2* has been detected. Segregation analysis confirmed the mutation in the family. Homozygote mutation in *SRD5A2* is the main cause of disorders of sex development in this family.

Keywords: *SRD5A2*; Testosterone; Dihydrotestostron; Sex Development Disorder.

Introduction

46,XY disorder of sex development (DSD) is determined by a female phenotype despite normal 46,XY karyotype. The clinical phenotype of these patients is distinguished by a female appearance, containing female external genitalia, breasts, and other secondary sex characteristics, but the presence of bilateral undescended testicles and the absence of a uterus and ovaries (1).

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Table 1. PCR primer pair used for amplification of SRD5A2 coding sequences

Exon	Primers	Sequence (5'-3')	Annealing temperature (℃)	PCR product
1	Forward	CCGCGCTCTCTTCTGGGA	57	314
	Reverse	GCAGAAGAGGCCCAGAAGTA	56	

SRD5A2 gene, that encodes the steroid 5α reductase 2, loss of function mutations are one of the most common cause of 46,XY DSD. This gene is placed at chromosome 2p23 including 5 exons, encoding a 254 amino acid protein containing at least four putative transmembrane regions and an androgen-binding domain at its N-terminal end (2-4).

The 5a-reductase deficiency (OMIM number #264600) is an autosomal recessive disorder which reduces or blocks the function of this enzyme (*SRD5A*). 5a-reductase enzyme plays a key role to convert testosterone into dihydrotestosterone (DHT), the main hormone responsible for developmental of male appearance and male sex secondary characteristics (5).

SRD5A2 gene mutations play also the main role in various disease susceptibilities including prostate cancer, hypospadias, micropenis, breast cancer, etc. (3).

At least 174 different disease-causing mutations associated with this gene have been characterized (http:// www.hgmd.cf.ac.uk, accessed May 2021). These mutations include missense/nonsense, splice junction alteration and small or whole gene deletion.

In this experiment, we found a rare homozygote mutation in *SRD5A2* gene, in a family with sex disorder, which had been previously reported in Japanese population (6), but it has not been reported in Iran. The introduction of this rare mutation due to the high coefficient of relationship in IRAN can be considered as the Founder mutation and the first selection to investigate the cause of the disease in patients with symptoms of sex development disorders.

Materials and Methods

A newborn patient, presumably girl of consanguineous parents (first cousin), was referred to Genetic Foundation of Khorasan Razavi (GFKR) with ambiguous genitalia. Because of numerous history of consanguineous marriages, there were four affected individuals with similar problem in the family.

Physical examinations including height, weight, and female external genitalia were done. Diagnosis based on a color doppler ultrasonography was employed for internal genital organs survey. Cytogenetic analysis was carried out to investigate the proband's karyotype. Patient was treated with DHT hormone and topical DHT gel to return serum hormone levels within physiologic range to stimulate sexual development. After hormone therapy, serum levels of follicle‑stimulating hormone (FSH), luteinizing hormone (LH), Testosterone (T), and dehydrotestosterone (DHT) were measured for the proband. Subsequently the patient underwent gender reassignment surgery from female to male at the age of 2 years.

Based on the clinical features and biochemical test results, WES was also applied to detection of the main cause of disorder. Genomic DNA was extracted from blood samples using DNA extraction kit (ROCHE, Germany). Whole-exome capture was carried out using the Agilent SureSelect Target Enrichment Kit (G7530-90000). The libraries were sequenced by Illumina HiSeq 2500 sequencer. Sequencing reads were aligned to human reference genome hg19 and bioinformatics analysis was performed. Sequence analysis was done using the Sanger sequencing method by specific primers for confirmation of identified mutation. Primers sequences are presented in Table 1.

Results

The physical examination revealed female external genitalia (genital image not available), but a blind‑ending vagina. Ultrasonography survey showed that the patient doesn't have a uterus or ovaries, but has Undescended testicles.

Chromosomal analysis revealed a 46, XY chromosomal constitution with no anomalies in the number or structure of chromosomes based on G-banding high resolution technique (Fig. 1).

Data from hormonal test, following hormone therapy, demonstrated that FSH (2.8 micIU/ml), LH (2.4 mIU/ ml), T (15 ng ml−1) and DHT (21 pg/ml) levels of the proband were normal. These data revealed that hormone therapy was successful.

Analysis of the *SRD5A2* gene by WES sequencing showed this patient harbored a single base transition mutation (c.16C>T) (HGMD: CM033029) leading to an amber stop codon (p.Q6X). Furthermore, there was history of DSD in the family. Patient's aunt showed symptoms associated with ambiguous genitalia (Fig. 2). So segregation analysis using specific primers and Sanger sequencing was done for the family. Sequencing results confirmed patient's parents and aunt to be heterozygous and homozygous for this mutation

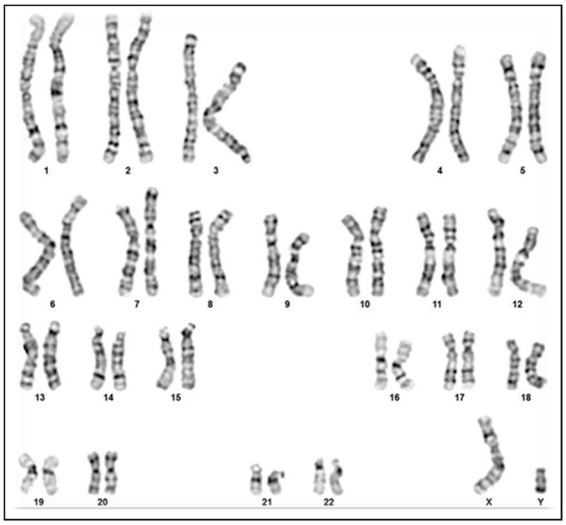

Figure 1. Chromosomal analysis of proband

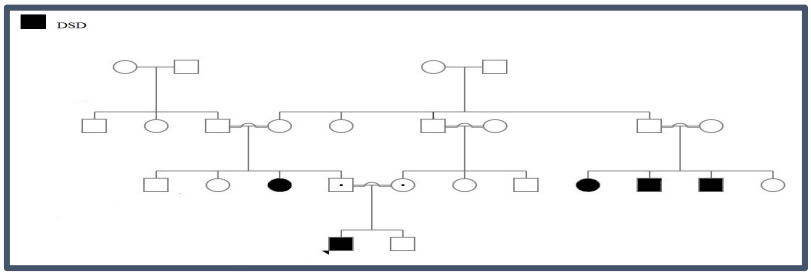

Figure 2. Pedigree of proband'sfamily with 5α-reductase deficiency

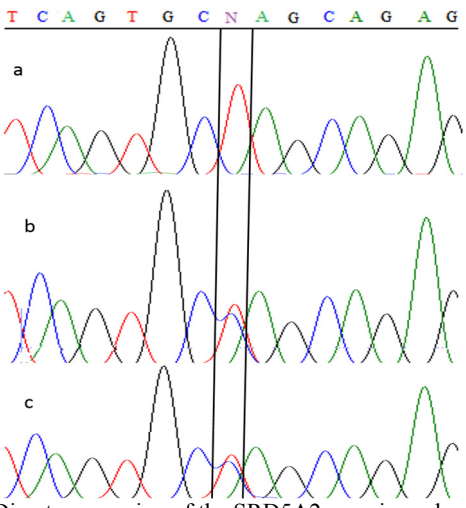

Figure 3. Direct sequencing of the SRD5A2 gene in proband's family

respectively (Fig. 3). The stop codon at the beginning of the coding sequence may leads to nonsense mediate mRNA decay which leads complete loss of protein activity. This mutation was previously reported as a pathogenic mutation correlated to 46,XY DSD in Japanese population (6) and clinvar database.

Discussion

Testosterone and its 5α-reduced metabolite, DHT, play a key role in the process of male sexual development (7). Testosterone acts to incitement the wolffian duct differentiation whereas DHT plays a fundamental role in external genital differentiation and prostate development (2). 5 α-reductase encoded by

SRD5A2 gene. Thus, patients with a mutation in this gene usually have significant ambiguities of the external genitalia, whereas wolffian duct differentiation is normal and mullerian structures are absent (2, 7). To identify the defective gene in these patients, choosing the best molecular method is based on perfect clinical examination, receiving a family history exactly, biochemical and chromosomal analysis as an initial genetic test (8, 9). In the present study, an Iranian consanguineous family was investigated for identifying the main cause of DSD disease.

According to WES results, we identified a rare homozygote mutation [c.16C>T;p.Gln6Ter; HGMD: CM033029] in *SRD5A2* gene. The first report of the p.Q6X mutation was related to a Japanese patient, who had inguinal testes presenting as a giant seminoma (6).The p.Q6X mutation, has been reported for Asian population to date (10-12). Therefore, it is clear that the mutation is relatively common in the Asian population (13). Also the result of 5α-reductase assay showed that enzymatic activity was completely blocked in this mutation due to the lack of either Testosterone or the NADPH-binding domain (13). Segregation analysis results by PCR and Sanger sequencing confirmed this mutation in the other patients in the family. So according to the data, this mutation is the main cause of genital development disorder in this family. Similarly, Zhang et al reported, a compound heterozygous mutation (p.Q6X/pG203S) in exon 1 and 4 in a Chinese patient with Hypospadias, clitoris-like phallus and palpable gonads in the inguinal canal. Also, in another study, a Thai patient with ambiguous genitalia was compound heterozygous for the same mutations (p.Q6X/p.G203S) (10). According to literature, exons 1 and 4 might be hotspots for *SRD5A2* mutations in Asian hypospadias patients. Rahimi and colleagues indicated correlation between V89L and A49T polymorphisms in exon 1 andG196S in exon 4 and the risk of hypospadias in 109 Iranian hypospadias patients (14).

In conclusion, reporting of this case report, as a rare genetic disease, makes genetic counselors of this geographical area (IRAN) more aware of this disease and its symptoms. Therefore, due to the high coefficient of relationship in IRAN, this result helps them to choose this mutation as the founder mutation and the first selection for disease investigation about patients with sex disorder to avoid wasting time and additional costs.

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