Case Report

Identification of a Novel Mutation in an Iranian Family With 17-β Hydroxysteroid Dehydrogenase Type 3 Deficiency: A Case Series

Abolfazl Heidari[1], **Ali Homaei**[2], **Fatemeh Saffari**[3*]

1. Reference Laboratory of Qazvin Medical University, Iran Sana Medical Genetics Laboratory, Qazvin, Iran.
2. Student of Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Children Growth Research Center, Research Institute for Prevention of Non-Communicable Diseases, Qazvin University of Medical Sciences, Qazvin, Iran.

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ABSTRACT

Background: We presented the clinical and genetic features of a male ambiguity due to 17-beta-hydroxysteroid dehydrogenase 3 (17B-HSD3) deficiency.

Methods: The proposita was an 11-year-old girl and the first child of a consanguineous family. The external genitalia were completely female and had a short vaginal pouch. She had palpable gonads in her inguinal area and underwent bilateral gonadectomy at the age of two. At the age of 10, she was referred to our clinic for more evaluation. In pelvic sonography, uterine and ovarian were not seen. Her karyotype was 46, XY, and her LH and FSH levels were elevated, and three of the patient's aunts and one of the mother's aunts had similar signs.

Conclusions: We identified a novel homozygous missense variation (c.731T>A, p. Ile244Lys) in the HSD17B3 gene. This alteration changes Isoleucine to Lysine in exon 10.

1. Introduction

The 17- beta-hydroxysteroid dehydrogenase 3 (17-β-HSD-3) deficiencies affect testosterone biosynthesis and are one of the rare causes of 46, XY Disorders of Sex Development (DSD) (1). The HSD17B3 gene is located on human chromosome 9q22 and includes 11 exons (Figure 1-A). This gene encodes an enzyme, 17-beta hydroxysteroid dehydrogenase 3, which is the last and key isozymes in the control of male steroid synthesis and exclusively acts on the testes. This enzyme converts androstenedione to testosterone, which has more biological power. This change is necessary for the normal development of the internal and external genitalia of the male fetus (2, 3). Affected individuals have female external genitalia with male normal wolffian ducts at birth (4). The incidence of this disorder is estimated at 1

* *Corresponding Author:*
Fatemeh Saffari, PhD.
Address: *Children Growth Research Center, Research Institute for Prevention of Non-communicable Diseases, Qazvin University of Medical Sciences, Qazvin, Iran.*
Tel: *+98 (283) 3328709*
E-mail: *drfa_saffari@yahoo.com*

in 147,000 newborns (5) and is higher in the Arab population of Gaza, and its prevalence is approximately 1 in every 200 to 300 people, which is due to the high rate of consanguineous marriages (6). Pathogenic mutations in the HSD17B3 gene (MIM# 264300) are associated with the impaired sexual development of the 46, XY fetuses. The mutations may be homozygous or compound heterozygous, leading to 17βHSD-3 deficiency with a wide range of sexual ambiguity in the 46, XY fetuses (7).

It is difficult to diagnose asymptomatic 46, XX cases with 17-β-HSD-3 because the reproductive system and female gender roles are normal. The diagnosis of 17-βHSD-3deficiency is made by hormonal testing and confirmed by molecular genetic evaluation.

Here, we describe the clinical and genetic findings of a large family with several 46, XY cases with a new mutation in the HSD17B3 gene. Proposita was an 11-year-old girl who was referred for examination due to the infertility of her aunts. Three of the patient's aunts and the patient's mother's aunt also had the disorder (Figure 1-B).

2. Case Presentation

The proposita was an 11-year-old girl and the first child of a consanguineous family. She was born by cesarean section with a height of 52 cm and a weight of 4200 grams. The external genitalia were completely female and had a short vaginal pouch. She had palpable gonads in her inguinal area and underwent bilateral gonadectomy at the age of two. Other physical examinations were normal. At the age of 10, she was referred to our clinic for more evaluation at the Hospital for the Sick Children in Qazvin, Iran In pelvic sonography, uterine and ovarian were not seen. Her peripheral blood karyotype was 46, XY. The laboratory tests at the time of referral are shown in Table 1.

Her maternal family consisted of two brothers and eight sisters who were living in the same village. We realized that three of the patient's aunts and one of the mother's aunts had the same phenotype (Figure 1-A). All affected cases had similar clinical findings, including increased voice deepening, hirsutism, and primary amenorrhea. On their physical examination, breasts were in Tanner stage 1, but pubic hairs were in Tanner stage 5. Their labioscrotal skins were rugged and pigmented and their clitorises were large about 7 cm. Two of them had a short vaginal pouch, but the other two did not have a vaginal pouch and underwent genitoplasty in their adulthood. The palpable gonads were located in labioscrotal folds or in the inguinal area, which later underwent gonadectomy. On pelvic ultrasound, no internal female structures were seen. Their karyotype was 46, XY and they were not genetically tested until the case index was diagnosed. The data regarding their laboratory tests were not available.

To determine the molecular etiology, Whole-Exome Sequencing (WES) was performed and DNAs were extracted. The exon kit v. 7.0 (Agilent Technologies, Santa Clara, CA, USA) was prepared according to the manufac-

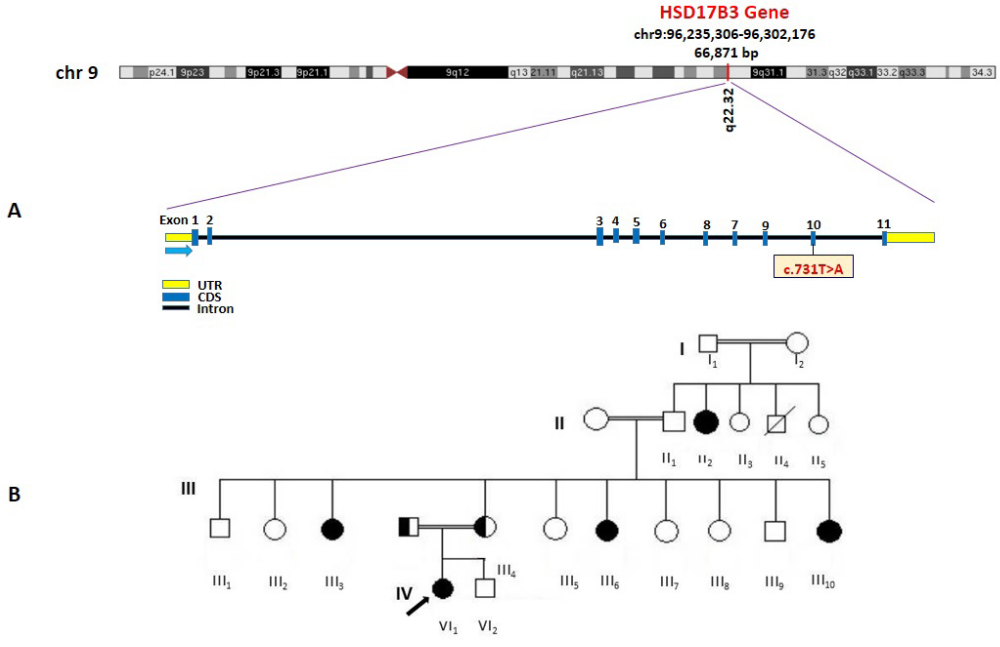

Figure 1. HSD17B3 gene structure and patient's pedigree

A: HSD17B3 gene structure and the mutation; B: Family pedigree of the patient.

Table 1. Hormonal data on the proposita at the age of 10 years

Variables	Patient's Result	Reference Range Male	Reference Range Female
Estradiol, pmol/l	<33	1-288	37-258
Testosterone ng/ml	0.8	2.6-11	2.5-10
Androstenedione ng/ml	0.4	0.6-3.1	0.3-3.3
DHEA-S, nmol/ml	1.1	0.66-6.7	0.92-7.6
17 OH-Progesterone, nmol/ml	1.3	0.2-2.3	0.3-4.5
FSH, mIU/ml	140	1.4-10.9	3.5-9.7
LH mIU/ml	39.6	0.1-7.8	0.1-7.9

D HEA: Dehydroepiandrosterone; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone. *Journal of Pediatrics Review*

turer's protocols, and 75×2-bp paired-end sequencing was done on HiSeq2000 (Illumina Inc.) with an 80-120× mean coverage. The sequencing quality was confirmed using FastQC 11.5 software (8). The variant was filtered and the preliminary whole-exome data analysis was performed using Burrows-Wheeler Aligner (BWA) and the Genome Analysis Toolkit (GATK) software (9) to generate a Binary Alignment Map (BAM) and a Variant Call Format (VCF) file, respectively. Annotations of the VCF files were carried out by the WANNOVAR software, and the data were manually analyzed for the presence of candidate pathogenic variants. Variants were filtered out in different human population databases. The local NGS database, as well as public databases (the 1000 Genomes Project, the Genome Aggregation Database (gnomAD), the Genome Aggregation Consortium (ExAC), ESP 6500, and dbSNP 137) were also investigated. Pathogenicity of the variants were assayed using prediction methods (PolyPhen-2), SIFT, Mutation Taster, PROVEAN, and in silico nucleotide conservation from Genomic Evolutionary Rate Profiling (GERP) scores (10). VarSome database, a search engine for human genomic variation, was also used for classifying the candidate variant according to the criteria set by the American

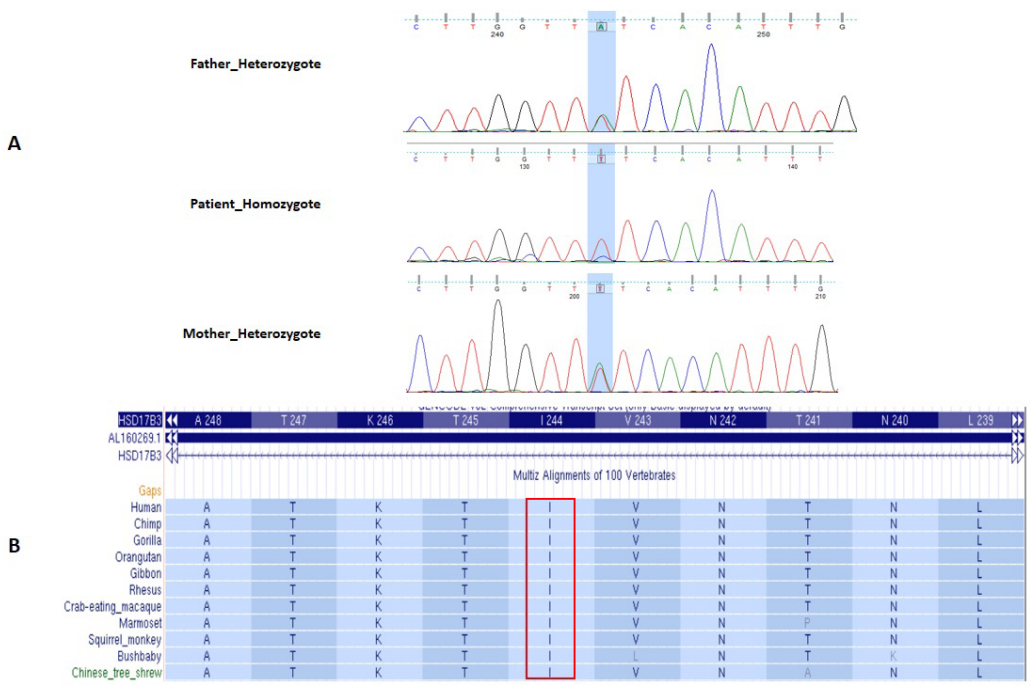

Figure 2. DNA sequencing of HSD17B3 gene *Journal of Pediatrics Review*

A: Electropherograms from Sanger confirmation in family members showing HSD17B3 (c.731T>A, p. Ile244Lys) heterozygous and homozygous mutant; B: The highly conserved state of the variant amino acid across the evolution of species.

College of Medical Genetics (ACMG) (11). The identified mutation was validated using Sanger sequencing. The online version of Primer 3 software was used to design primers flanking candidate variants.

The region was amplified and sequenced using ABI 3500 Genetic Analyzer (Applied Biosystems Inc., 850 Lincoln Center Drive, Foster City, CA, 94401 USA). Segregation analysis using Sanger sequencing was performed, which confirmed that both parents were heterozygous at the mutation position (Figure 2-A). Amino acid alignment of the human HSD17B3 gene showed that the specific isoleucine involved in this mutation (p. Ile244Lys) is highly conserved through several species (Figure 2-B).

We identified a novel homozygous missense variation (c.731T>A, p. Ile244Lys) in the HSD17B3 gene. This alteration changes isoleucine to lysine in exon 10 (Fig. 1A). This variation, which has not been reported before is predicted to be a Variant of Unknown Significance (VUS) based on computational analysis. A local database survey in a relatively large cohort of healthy controls (1406 individuals) revealed that none of the healthy individuals exhibited the identified novel variation in the HSD17B3 gene.

3. Discussion

Here, we reported a novel homozygous missense variation in five Iranian cases diagnosed with a 17-β-HSD-3 deficiency. The 17-β-HSD-3 deficient patients may not be diagnosed at birth and it presents in adolescence with a female phenotype and primary amenorrhea, virilization, umbilical hernia, mild clitoromegaly, or urogenital sinus. In this study, proband showed no signs of masculinization because she underwent an orchiectomy when she was two years old. The other four patients were homozygous for the 17-β-HSD3 gene but had grown up as females, virilized at puberty, and were infertile. It is of note to mention that one of the girl's aunts with 46, XX karyotype, who had a homozygous mutation for identified alteration, had a normal female reproductive system and normal sexual function.

The parents were both heterozygous and the identified variant was perfectly segregated with the disease within the all affected individuals in the family. This missense variant changes isoleucine in position 244 to lysine. This novel alteration has not been reported earlier in the literature for the HSD17B3 gene. So far, more than 30 different mutations in this gene have been identified, including insertion, exonic deletion, missense, and nonsense mutations (6) with the p. Arg80Gln alteration being the most common one in the Arab population (2). In the Turkish population, c655-1;G-A, p.Ala188Val, and c.777-783del_GATAACC mutations have previously been identified (11). In a study by Ozen et al., 20 patients were followed up for 46, XY DSD, who did not have mutations in genes SRD5A2 and AR, and were analyzed using Targeted New Generation Sequence (TNGS) analysis for 56 potential genes, which may be involved in the etiology of 46, XY DSD. Mutations were identified in the 17-β-HSD-3 gene in 30% of patients. It is of note to mention that she belonged to a large family with the majority of them having consanguineous marriages, which is a common phenomenon throughout the country similar to other nations in the Middle East.

Our patients have undergone gender reassignment due to female external genitalia and possibly due to rural culture. Gender reassignment in individuals is a delicate personal and social problem and various aspects must be considered. In addition to the severity of sexual ambiguity, different cultural, social, and economic conditions can also influence decisions to change gender. Studies have shown that most of these people are raised as women due to the severity of sexual ambiguity, but cannot conceive (12, 13).

4. Conclusion

However, publishing the clinical profile of these sexually ambiguous patients along with molecular genetic diagnosis can help physicians and families make informed decisions about gender reassignment. New gene mutations reported in these patients increase known molecular mutations in the disease and aid in diagnosis. The introduced proband parents were also able to give birth to a healthy child by prenatal genetic study.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Qazvin University of Medical sciences (Code: IR.QUMS.REC.1399.474).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflicts of interest

The authors declared no conflict of interest.

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