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CASE REPORT

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A novel pathogenic variant of *SRD5A2* in an Iranian psuedohermaphrodite male

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Abstract

Deficiency of the 5-alpha-reductase may have an important role in 46,XY DSD in some cohorts. The prenatal ultrasonography and karyotyping can trigger the attention toward the presence of a DSD in fetus.

KEYWORDS

disorders of sex development, pseudohermaphroditism, *SRD5A2*, variant

1 | INTRODUCTION

SRD5A2 mutations have been reported to cause an autosomal recessive form of male pseudohermaphroditism (disorders of sex development: SDS). Here, a novel mutation in *SRD5A2* gene is reported in an Iranian patient with pseudohermaphroditism. A 5-month-old infant with female genitalia referred to endocrinology clinic at Pediatric Center of Excellence. Existence of testes was confirmed by ultrasonography. Karyotype showed a 46,XY DSD. Molecular analysis including PCR and sequencing of *SRD5A2* showed a novel variant in this gene. In silico analyses were also performed to determine the potential pathogenecity of this mutation. Sequencing of *SRD5A2* gene showed a novel variant, c.476T > G (p.Ile159Arg). The variant was heterozygous in the patient's parents. Prediction analysis using available software tools such as PROVEAN and Phyre2 was consistent with pathogenicity of the variant. Considering that the

5-alpha-reductase deficiency unlike other diseases can be treated at pubertal age, early diagnosis could be helpful in treatment strategies in addition to prevention programs. In silico analysis is helpful to predict the pathogenicity of novel variants.

Disorders of sex development (DSDs) are congenital anomalies, in which there is atypical development of sexual tissues.[1,2] DSDs are categorized into several etiological groups; congenital adrenal hyperplasia (CAH), complete androgen insensitivity syndrome (CAIS), partial androgen insensitivity syndrome (PAIS), and 5-alpha-reductase deficiency (5ARD) are the most common causes of DSDs.[3]

SRD5A2 mutations have been reported to cause an autosomal recessive form of DSD. This gene, located on 2p23.1, encodes the 5-alpha-reductase which converts testosterone to dihydrotestosterone (DHT). DHT is required for development of normal male external genitalia.[4] Patients with the *SRD5A2* mutations may have variable clinical features from complete female genitalia, genital ambiguity to undervirilized male genitalia.[5,6] Signs such as bifid scrotum, clitoral-like phallus, cryptorchidism, and pseudovaginal perineoscrotal hypospadias may be observed in patients.[3,7]

More than one hundred *SRD5A2* mutations have been reported at Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). Some mutations are frequent in specific populations, and ethnic-specific prevalence of mutations has been described.[5] In this study, a novel variant in *SRD5A2* gene is described in an Iranian patient. The pathogenicity of this variant is also confirmed.

2 | METHODS

2.1 | Case presentation and workup

A 5-month-old infant with female genitalia was referred to the endocrinology clinic (Figure 1A). Prenatal screening (amniocentesis) had showed a 46,XY karyotype. However, repeated ultrasonogrphic findings reported female genitalia. The initial karyotype 46,XY and the prenatal ultrasonography which showed female external genitalia triggered the attention toward the probable presence of a DSD in this child. At 5th month after birth, an ultrasound was performed and reported no testicles, but second ultrasound confirmed the existence of both testicles in the inguinal canals.

There were no report of ovaries or uterus in the ultrasounds, and there was a pseudovagina. Regarding the lack of electrolyte imbalance and definite female genitalia during first 5 months, it was unlikely that the patient had congenital adrenal hyperplasia. Therefore, 46,XY DSD was noted for this

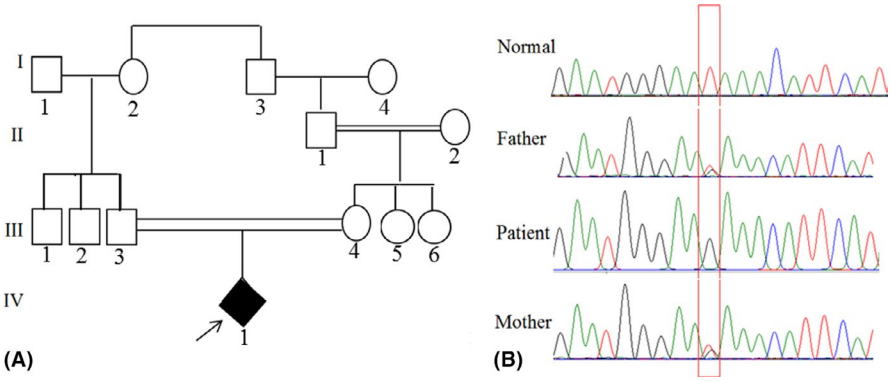

FIGURE 1 Pedigree of the family (A) and electropherograms (B) of normal, the patient and his parents; c.476T > G substitution causes Ile to Arg at position 159 of protein. The parents are heterozygotes for this variant, and the patient is homozygote

TABLE 1 The result of biochemical test

Test	Result	Normal range		Unit
		Male	Female	
LH	1.02	1-9	2-10	IU/L
FSH	0.25	1-10	20-50	IU/L
17OHP	66.2	20-100	20-100	ng/dL
Testosterone/DHT	25	275-875	23-75	ng/dL
Free testosterone	0.0	0.4-0.9	0.15-0.6	pg/mL
Na (Sodium)	130	135-145	135-145	mEq/L
K (Potassium)	3.5	3.7-5.9	3.7-5.9	mEq/L

Abbreviations: 17OHP, 17-hydroxyprogesterone; DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; LH, Luteinizing hormone.

TABLE 2 Primer sequences for coding regions of *SRD5A2* in this study

Exon no.	Primer sequences 5′ to 3′ direction
1 Forward	AGAAAGGGGTATTGCTGCGA
Reverse	CTTGTCAACTCTCTAGCGTCCAA
2 Forward	CTTAAGAAAGAGGTGGGGATGAGA
Reverse	ATTGCAGTAGGGAGAGGCCAT
3 Forward	GCCACGTCTTAGGACCATTCTTA
Reverse	GTATCATTCGTGCCCTCACTGT
4 Forward	GATTCCACCAAACTCCTATGACT
Reverse	CTTCGGTTTCTCAATCTTCCTC
5 Forward	CATCGAAATAGTCAGGCCCAA
Reverse	CAATAGCTAAGAAGCAACTGTCGC

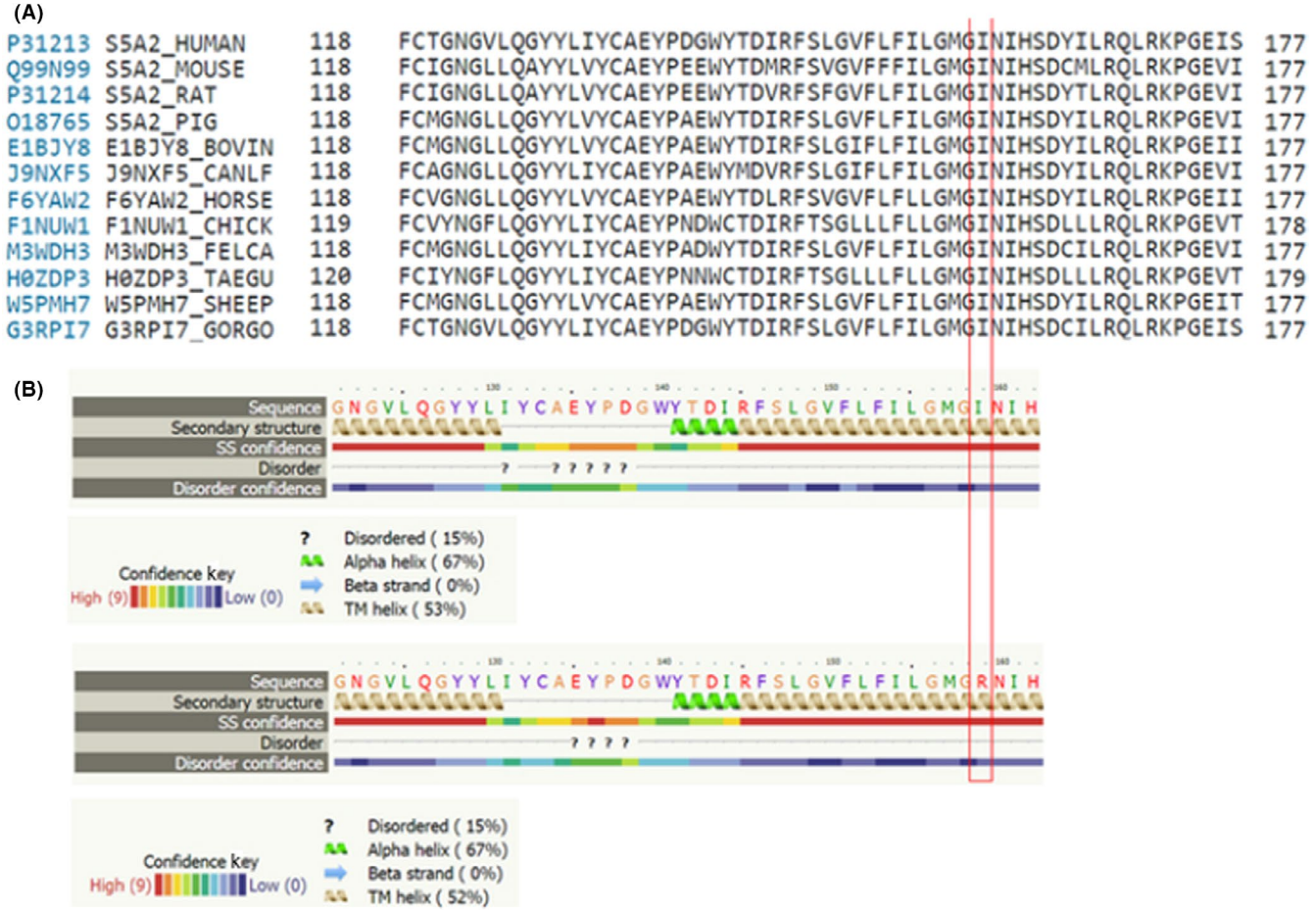

FIGURE 2 A, Amino acid alignment of SRD5A2 protein among different orthologous and paralogous members adapted from UniProt protein family members. p.Ile159 (indicated in the box) is a highly conserved residue in this protein among different species. B, Phyre 2 prediction for effect of p.Ile159Arg on secondary structure of *SRD5A2* protein

patient. Human chorionic gonadotropin test with three dosages was performed to assess the function of testosterone and its receptor and to furthermore rule out the androgen insensitivity syndrome and deficiency in 5-alpha-reductase. The results of biochemical tests for the patient showed normal gonadotropins, normal electrolytes, slightly increased testosterone, and increased testosterone/dihydrotestosterone ratio (25) (summarized in Table 1). Then, 5-alpha-reductase deficiency was suspected, for which molecular genetic testing was performed; a pathogenic homozygous mutation in *SRD5A2* gene was detected in the patient. Both parents were heterozygote for this mutation (Figure 1B).

2.2 | Genetic testing

An informed consent form was signed by the patient's parents; 5 mL of peripheral whole blood was taken from the patient. Genomic DNA was extracted using standard salting out protocol. Quantity of DNA was assessed using a spectrophotometer (NanoDrop ND2000c; Thermo Scientific).

The coding regions and exon-intron boundaries of *SRD5A2* (NG_008365.1; NM_000348.3) were amplified using the forward and reverse primers (Table 2). Briefly, PCR was performed in a final volume of 50 μL reagents; forward and reverse primers (10 pmol), template DNA (150 ng), Taq DNA polymerase (0.2 units/μL), MgCl2 (1.5 mmol/L), and dNTPs (0.4 mmol/L for each nucleotide) were used to amplify the regions by the following PCR program: initial denaturation 5 minutes at 94°C and 30 cycles for denaturation at 94°C (30 seconds), annealing at 62°C (30 seconds), extension at 72°C (30 seconds), and final extension at 72°C (7 minutes). The PCR products were directly sequenced using a sequencing analyzer ABI PRISM™ 3500 (PE Applied Biosystems) by a BigDye termination method.

Available online software tools including sorting intolerant from tolerant (SIFT), PROVEAN, combined annotation-dependent depletion (CADD), and polymorphism phenotyping (PolyPhen-2 v2.1) were applied to predict pathogenic scores of the variant.

A multiple amino acid sequence alignment was done using UniProt protein family members (UniProtKB/

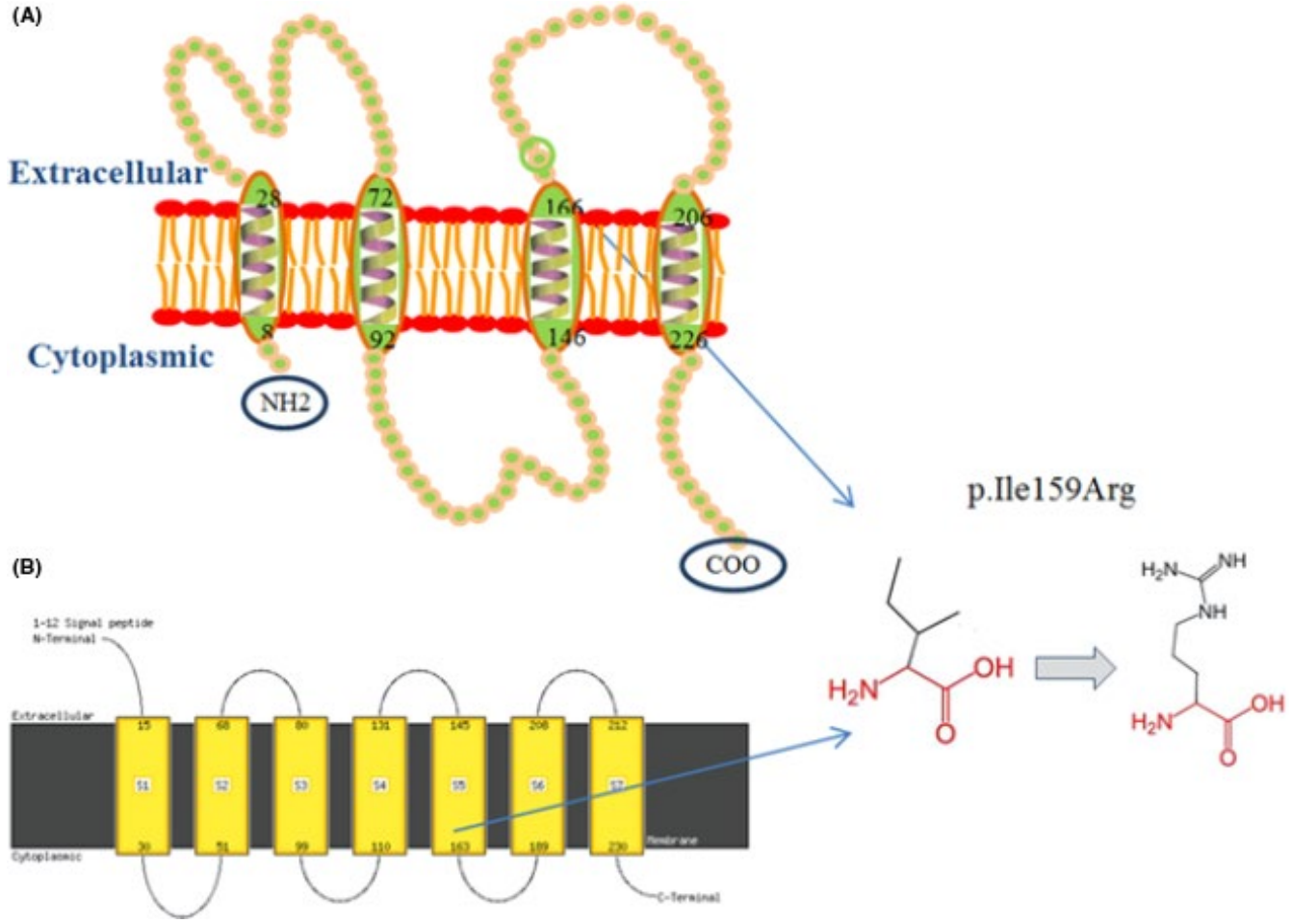

FIGURE 3 Schematic possible structure of microsomal 5-alpha-reductase domains based on (A) UniProt database annotation and (B) Phyre 2 prediction. p.Ile159Arg is located in a transmembrane domain

Swiss-Prot P31213) to check conservation of the mutated residue (Figure 2). Protein homology/analogy recognition engine V2.0 (Phyre2) and iterative threading assembly refinement (I-TASSER) server were used to predict the effects of variant on the function and structure of protein.

3 | RESULTS

One benign homozygous variant, c.265C > G (p.Leu89Val), was found in the patient as well as a novel variant of unknown significance (VUS), c.476T > G (p.Ile159Arg). Testing both parents for this mutation also showed that parents are heterozygote for this mutation. According to ClinVar, c.265C > G is a benign variant with a high frequency in normal population; thus, we did not check the benign variant in the parents. In silico analyses predicted the pathogenicity of this variant using PROVEAN (score of −6.715). Alignment analysis showed that p.Ile159 is a conserved amino acid (Figure 2). Phyre2 predicted p.Ile159Arg affects the secondary structure of the protein.

4 | DISCUSSION

The *SRD5A2* gene encoding steroid 5-alpha-reductase 2 is responsible for converting testosterone to dihydrotestosterone (DHT) using a double-bounded reduction. Recent studies have shown this enzyme is localized in endoplasmic reticulum (ER).[8] Schematics of the *SRD5A2* protein is shown in Figure 3. Normal plasma level of DHT is required for masculinization in male. Regarding female genitalia with male karyotype and clear increased testosterone/dihydrotestosterone ratio (25) after the HCG test, we aimed to check 5-alpha-reductase deficiency and genetic testing was performed for this patient.

A novel variant, c.476T > G (p.Ile159Arg), was found in our patient. Testing family members for this mutation showed only the patient is homozygote for the variant, and both parents are heterozygotes. This variant has not been reported in databases such as ExAc, 1000genomes, and Iranome as well as HGMD and disease-specific databases. Online software tools also predicted that it may act as a pathogenic variant. On the other hand, alignment analysis using UniProt showed p.Ile159 is conserved among other species (Figure 2); that is, it may have

an important role in this protein. p.Ile159 is located in a transmembrane domain; when it is substituted by arginine, the secondary structure of this domain may be affected. Our previous studies have shown that Iranian subpopulations have specific mutations with different frequencies in various disorders.[9–11]

Avendaño et al[5] reviewed 256 patients with 46XY DSD; clitoromegaly and hypospadias were found in 66% and 40% of patients, respectively, while our patient did not show microphalus or hypospadias. Furthermore, in the current study, there were no significant abnormal urogenital characteristics. In addition, cryptorchidism was reported in about 20% of these patients but in the current study, the existence of testes in inguinal region bilaterally was confirmed. Interestingly, as found in our patient, all cases in Avendaño's study showed virilization at puberty.

As we know patients with 5-alpha-reductase deficiency unlike other diseases can be repaired at pubertal age, thus, it is recommended to pay special attention to this disease and to determine gene mutation of patients at early stages. In silico analysis is helpful to predict the pathogenicity of novel variants.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

SD, AH, SHK, NM, and BR: performed the diagnostic steps for workup of patient along with his clinical data collection; NM: drafted the manuscript; all the authors: have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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