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CASE REPORT

A novel variant luteinizing hormone receptor in the first transmembrane helix of two homozygous Iranian patients: case report

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Abstract

Background: Leydig cell hypoplasia (LCH) is a rare autosomal recessive endocrine syndrome that affects the normal development of male external genitalia in 46, XY individuals and is one of the causes of disorder of sexual differentiation (DSD) in males. The responsible gene of LCH is *LHCGR* which is located on the chromosome 2 and its various mutations lead to different degrees of the disease ranging from micropenis to complete XY DSD.

Case presentation: In this study, we have investigated the clinical presentation and molecular findings of two siblings with complete male LCH and XY DSD. This is the first detailed report of individuals with LCH from Iran. It aimed to study the molecular and clinical characteristics of two sisters with type 1 LCH. Whole exome sequencing was used for these patients to find the underlying genetic cause of the disease. Our Iranian DSD patients had external genitalia (normal labia major and minor, the external opening of the urethra beneath the clitoris) and bilateral testicular tissues in the inguinal region, which were removed by surgical exploration.

Conclusions: Genetic sequencing showed the homozygous variants of the *LHCGR* gene in the patients, a novel duplication variant in exon 11, c.1091dupT -or pLeu365Profs*5. This mutation is described as likely pathogenic. We think that this case report can widen the genotypic spectrum of the *LHCGR* variants. Moreover, this study emphasizes the significant rule of Whole Exome Sequencing in differentiating various causes of disorder of sexual differentiation.

Keywords: Leydig cell hypoplasia, Disorder of sexual differentiation, LHCGR gene, Novel variants

Background

Male sexual differentiation basically depends on gonadotropic hormones, luteinizing hormone (LH), and placental human chorionic gonadotropin (hCG). The impact of both glycoprotein hormones is mediated by luteinizing hormone/chorionic gonadotropin receptor (LHCGR), whose impairment may lead to diminished or lack of virilization in utero and reproductive dysfunction. During embryogenesis, hCG binds to LHCGR receptors in utero and produces testosterone and dihydrotestosterone, which are necessary for male sexual differentiation [1]. In pubertal maturation, male sexual fertility and function depend on the interaction between LH and LHCGR. The *LHCGR* gene consists of 11 exons and 10 introns, spans > 80 kb on chromosome 2p21, and is mainly gonadal. It is highly expressed in testicular Leydig cells and ovarian theca cells. Exons 1–10 and a part of exon 11 encode for the extracellular domain, which accounts for ligand binding. The remaining parts of exon 11 encode the seven transmembranes and intracellular domains involved in signal transduction [2]. Both activating and inactivating variants of the *LHCGR* gene may lead to diseases. In 46, XY individuals, constitutive activation of

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LHCGR results in male precocious puberty and Leydig cell adenomas, while inactivating mutations chiefly cause LCH and hypergonadotropic hypogonadism. In 46, XX individuals, loss-of-function mutations usually present with normal external genital and breast development. However, they may have absent or delayed menarche. The 46, XX individuals who have LHCGR mutations with decreased function of receptors are resistant to LH and although the follicle maturation may be normal, ovulation is rarely observed in them [3].

Leydig cell hypoplasia (LCH) is an autosomal recessive disorder which founds in two major types. Type I is a less frequent and sevier form of 46, XY disorder of sex development with total dysfunction of receptor that results in a complete female phenotype and male disorder of sexual differentiation (DSD). It may present with a blind vagina, amenorrhea, and absence of breast development. Type II LCH, which is more common, is associated with 46, XY male DSD with micropenis and/or hypospadias and/or cryptorchidism [4]. The varying degrees of receptor gene mutations are responsible for different phenotypes in patients as there is a phenotype-genotype correlation in this disorder. Heretofore, more than 83 *LHCGR* mutation variants have been reported in male genital and gonadal development disorders. Herein, we report the first detailed cases of LCH in Iran due to a novel homozygous duplication mutation (c.1091dupT) in two siblings with 46, XY to widen the genotype–phenotype correlation spectrum of the *LHCGR* variants.

Case presentation

The target patients were two sisters. The first patient (referred to as patient 1) was 19 years old and the second one (referred to as patient 2) was 13 as of the time of writing. The social gender of both patients was female. The two patients were born full-term by vaginal delivery, and their parents were healthy with consanguineous marriage (Fig. 1).

Patient 1 had referred to Imam Reza hospital with bilateral inguinal masses when she was 6 years old. The patient had no other symptoms at the time of presentation. At her first presentation, observations were stable and physical examination revealed that the patient had female external genitalia, with normal bilateral labia majora and minora, and external opening of urethra beneath the clitoris. However, bilateral palpable masses were found in inguinal regions, initially assumed to be inguinal herniation.

The patient underwent abdominopelvic ultrasonography, which revealed testicular tissues in the inguinal region of both sides, sized 17×8 mm^2. The ultrasound did not show any sign of the uterus or other Mullerian structures in the pelvic cavity. Based on the ultrasound

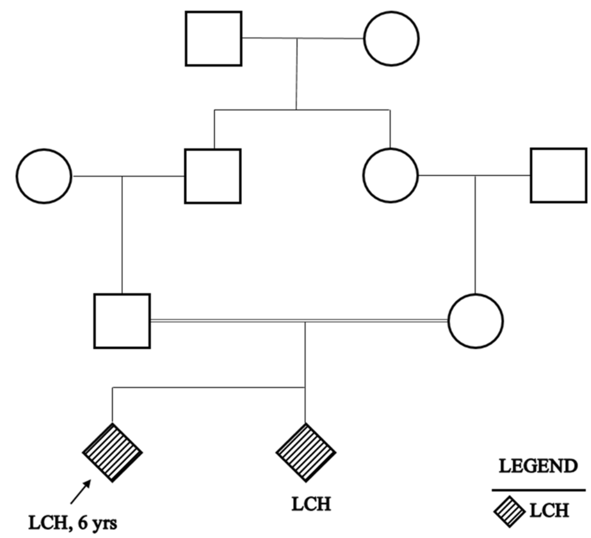

Fig. 1 The pedigree of the patients' family. Circles indicate females; squares indicate males, and diamonds indicate no specific sex

findings, the patient was diagnosed with androgen insensitivity syndrome (AIS).

Genetic analysis showed the patient's karyotype as 46, XY; however, no pathogenic variant was found in her *SRY* gene. Moreover, cystourethroscopy was performed for the patient and revealed a blind vaginal pouch, 1–1.5 cm in depth. However, the urethra and the bladder were normal.

Surgical removal of the inguinal masses was performed for the patient based on recommendations from pediatrics surgeons and endocrinologists. Histopathological study of the excised sample showed seminiferous tubules lined by Sertoli cells without germ cells, which confirmed the presence of testicular tissue. The patient underwent treatment with ethinyl estradiol at 14 years of age to induce the presence of secondary sexual characteristics in her.

The younger sibling, patient 2, was born with female external genitalia and had female social gender. However, physical examinations and abdominopelvic ultrasonography revealed that this sibling was suffering from the same

Table 1 Laboratory results of the patient 2

No.	Test	Result	Normal range
1	Testosterone	1.0 ng/dl	8–86 ng/dl
2	D.H.E.A SO_4	3.3 ng/ml	100–600 ng/ml
4	17OH Progesterone	0.3 ng/ml	0.8–5 ng/ml
5	Cortisol (8AM)	16.6 µg/dl	9.4–26 µg/dl
6	Androstenedione	0.1 ng/dl	0.3–2.4 ng/dl
7	Dihydro Testosterone	0.5 pg/ml	24–368 pg/ml

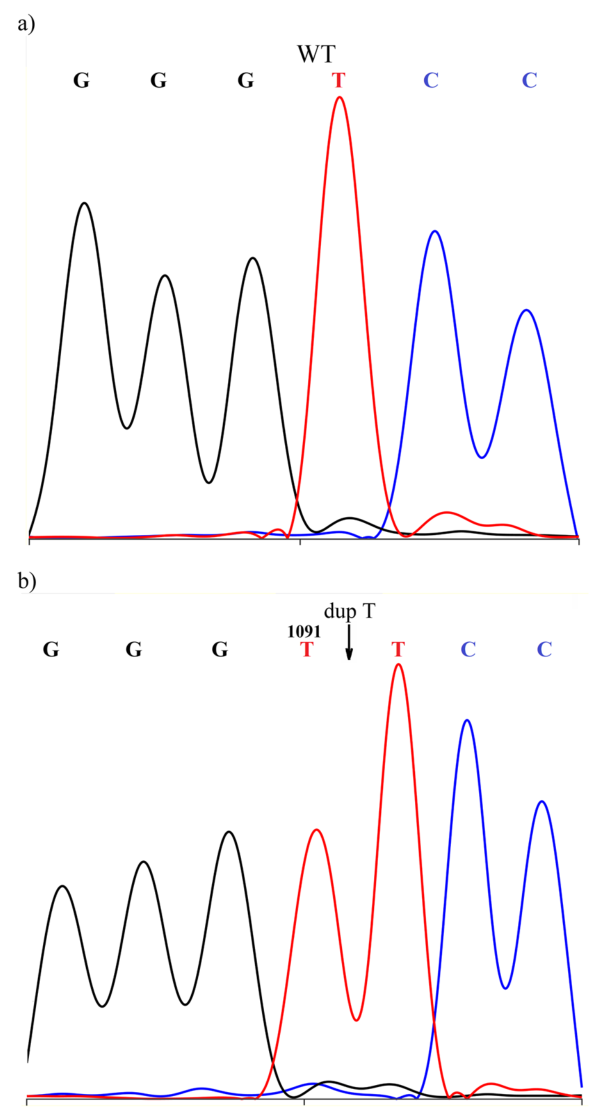

Fig. 2 Genetic sequencing of the LHCGR gene. **a** Sanger sequencing of DNA control individuals (Wild Type; WT) and **b** homozygous duplication mutation c.1091dupT in patient

condition and had testicular tissues in inguinal regions. Genetic analysis also showed the patient's karyotype as 46, XY without any pathogenic variant in the SRY gene. Surgical removal of inguinal masses was also done for patient 2.

Laboratory results of patient 2 before surgery, summarized in Table 1, indicated deficient levels of male sex hormones. This finding was not consistent with the typical biochemical findings of AIS. Due to the occurrence of a similar case in the family, genetic sequencing was performed for the patient to reach a definite diagnosis.

Genetic sequencing

To reach a more precise genetic diagnosis, the whole-exome sequencing (WES) analysis was performed to screen for causal variants. To this end, a total amount of more than > 1 µg of genomic DNA from proband was subjected to WES at Macrogen (Seoul, South Korea) on the Genome Analyzer HiSeq 4000 (Illumina, San Diego, CA, USA). Briefly, genomic DNA was sheared to an apparent size range of 150–200 bp of DNA fragments. The library had been prepared using SureSelect V6 Library Pre-Kit and guidelines (Agilent Technologies, CA, USA). An output file with VCF format was generated that included all single nucleotide variants (SNVs) and indels annotated with the reference genome using the existing databases to indicate the information of each variant. The obtained variants were annotated with ANNOVAR software. Homozygous missense, splice site, start codon change, stop loss, and indel variants with minor allele frequency < 1% were further filtered in dbSNP (version 138), 1000 Genomes Project, Exome Aggregation Consortium (ExAC), and gnomAD. The process was followed by an analysis of the potential pathogenicity of the novel variant, based on ACMG standards and guidelines for the interpretation of sequencing variants and using bioinformatics tools, including SIFT (sift.jcvi.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), CADD, Mutation Taster (http://www.mutationtaster.org), FATHMM (fathmm.biocompute.org.uk), and MutationAssessor) http://www.MutationAssessor.org). Conservation scores of variation sites were predicted using GERP + + and PhyloP. Allele frequency in the normal Iranian population was analyzed by Iranome (http://www.iranome.ir).

Identification of the causal variants and pathogenicity prediction

WES yielded a total of 22,449 reads and the mean target coverage was 106.956 reads. 95.7499% of reads had 20 × coverage, 82.3248% had 50 × coverage, and 47.4527% had 100 × coverage. In the first step, filtration of the candidate variants was carried out using these criteria: minor allele frequency (MAF) under 1% in genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/), and the benign variants, including harmless and synonymous missenses. Based on the obtained scores, the following predictions were made: the variant is probably damaging (1.00 score by PolyPhen-2), affects the protein function (0 scores by SIFT), and can be damaging (27.3 scores by CADD). Besides, this variant has not been reported in the Iranian normal population according to the Iranome database. Genomic DNA was extracted from the peripheral

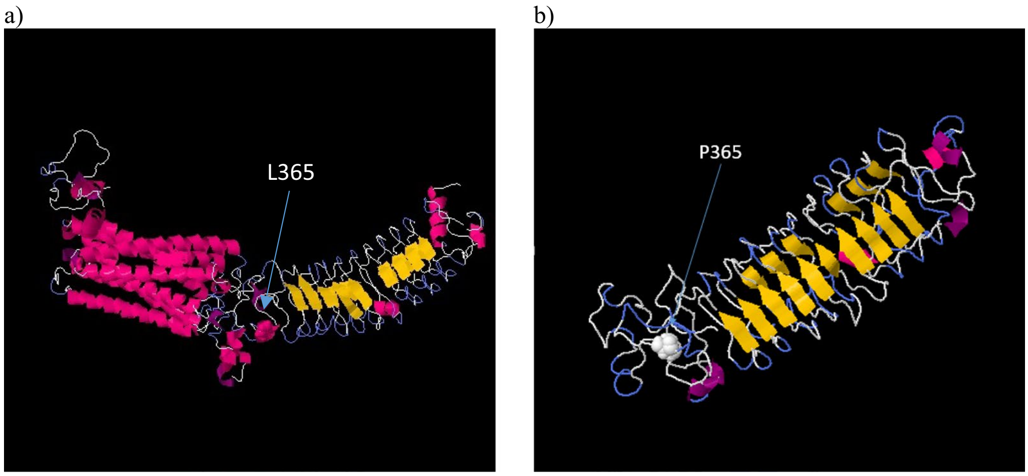

Fig. 3 The 3D structure model of the LHCGR protein **a** Wild type LHCGR, and **b** Mutant LHCGR that alters the receptor structure. The arrow indicates amino acid 365, leucine in the wild type and proline in the mutant receptor, respectively

whole blood of the two probands. Genotype analysis was performed on the extracted DNA using specific primer sets for the LHCGR gene. Sequencing of the purified amplicons was performed using an Applied Biosystems ABI 3730 XL automated DNA Sequencer. The sequences were compared to the human GenBank sequence for the LHCGR using Sequencher sequence alignment software (Version 4.10.1). The results of Sanger sequencing are shown in Fig. 2. Unfortunately, we were not able to confirm the carrier status of the parents.

The variant amino acid at codon 365 and wild type was modeled into the 3D structure of the LHCGR protein by RaptorX (publicly available at http://raptorx.uchicago.edu) to obtain more insight about the frameshift variant. The wild-type LHCGR domain data were taken from Uniprot databases (can be accessed online at http://www.uniprot.org/) and compared with this structure. It was predicted that a change of amino acid at the 365th position (p.L365P) results in a stop codon in codon 370 and produce a truncated protein without transmembrane domain (Fig. 3). In conclusion, it can be said that our analysis results show that the variant (c.1091dupT pLeu365Profs*5) is expected to have harmful effects on protein functionality.

Discussion

In this study, we report novel homozygous variants in the *LHCGR* gene of two Iranian sibling patients with 46, XY DSD and type 1 LCH. Further, a novel frameshift mutation (c.1091dupT, p.Leu365) was found in the *LHCGR* gene and related to their physiological conditions. The patients had clinical features compatible with the molecular diagnosis. Orchidectomy had been performed for the patients when they were about 6 years old. Right after the sex assignment at the age of 13, ethinyl estradiol (EE) had been prescribed for the development of secondary female characteristics.

Based on the variant interpretation guidelines from the American College of Medical Genetics and Genomics/the Association for Molecular Pathology (ACMG-AMP) [5], the found missense variant is taken together and by considering the clinical and molecular aspects, the patients were diagnosed with type 1 LCH.

After developing testicular structures, both fetal LH and maternal hCG act on the testicular LHCGR to elevate the synthesis and secretion of testosterone, which is critical for normal reproductive development. Especially, the functional differentiation of Leydig cells by the onset of fetal life seems to be independent of LH/hCG, while in contrast, differentiation and proliferation in the subsequent development stages are LH/hCG-dependent [6]. So far, 83 variants have been reported for the *LHCGR* gene in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/). Among these, 53 are nonsenses and missenses, 5 are gross deletions, 6 are small deletions, 5 are small insertions, 5 are splicing variants, and one of them is gross insertion. The number of inactivating compound heterozygous and homozygous variants, which have the capability of altering the structure of the LHCGR protein and its functionality, is more than the number of activating variants. In Table 2, the variants of the exon 11 of the *LHCGR* gene are listed, which are extracted from the literature.

Table 2 The variants of the exon 11 of the LHCGR gene

No.	Base change	Amino acid change	Location	Phenotype	References
1	c.1027 T > A (het)	p.C343S (het)	EC Domain	Pseudohermaphroditism (46, XY)	[7]
2	c.1060G > A(hom)	p.E345K	EC Domain	Pseudohermaphroditism (46, XY)	[8]
3	c.1103 T > C(het)	p.L368P	TM1	Male precocious puberty	[9]
4	c. 1126C > T(het)	p.A373V	TM1	Male precocious puberty	[10]
5	c.1121 T > C (hom)	p.I374T	TM1	Pseudohermaphroditism (46, XY)	[11]
6	c.1175C > T(hom)	p.T392I	IL1	Leydig cell hypoplasia & male pseudo-hermaphroditism	[11]
7	c.1192 T > C	p.T398M	TM2	Male precocious puberty	[12]
8	c.1199A > G (hom)	p.N400S	TM2	Empty follicle syndrome	[13]
9	c.1244 T > C (hom)	p.I415T	TM2	Leydig cell hypoplasia II	[14]
10	c.1370 T > G	p.L457R	TM3	Male precocious puberty	[15]
11	c.1395G > A (hom)	p.W465Term	IL2	Primary amenorrhea	[16]
12	c.1435C > T (hom)	p.R479Term	IL2	Primary amenorrhea	[17]
13	c.1448C > A (hom)	p.A483D	TM4	Pseudohermaphroditism (46, XY)	[18]
14	c.1473G > A (hom)	p.W491Term	TM4	Pseudohermaphroditism (46, XY)	[17]
15	c.1505 T > C (hom)	p.L502P	TM4	Pseudohermaphroditism (46, XY)	[19]
16	c.1573C > T (hom)	p.Q525Term	EC Domain	Primary amenorrhea	[17]
17	c.1624A > C (hom)	p.I542L	TM5	Male precocious puberty	[20]
18	c.1627 T > C	p.C543A	TM5	Pseudohermaphroditism	[20]
19	c.1627 T > C	p.C543A	TM5	Pseudohermaphroditism	[7]
20	c.1635C > A (hom)	p.545Term	TM5	Leydig cell hypoplasia	[21]
21	c.1660C > T(hom)	p.R554Term	IL3	Luteinizing hormone resistance	[3]
22	c.1691A > G	p.G564N	IL3	Male precocious puberty	[21]
23	c.1703C > T (hom)	p.A568V	EC Domain	Male precocious puberty	[9]
24	c.1713G > A	p. M571I	TM6	Male precocious puberty	[22]
25	c.1715C > T (het)	p.572A > V	TM6	Male precocious puberty	[23]
26	c.1723A > C (het)	p.I575L	TM6	Male precocious puberty	[21]
27	c.1730C > T	p.T577I	TM6	Male precocious puberty	[24]
28	c.1733A > G	p.A578G	TM6	Male precocious puberty	[25]
29	C1741T > C	p.C581A	TM6	Male precocious puberty	[25]
30	c.1757_1758del (het)	p.S586Ffs19	TM6	Infertility	[16]
31	c.1764dupT (hom)	p.A589Cfs17	TM6	Pseudohermaphroditism (46, XY)	[26]
32	c.1777G > C (hom)	p.A593P	TM6	Pseudohermaphroditism (46, XY)	[27]
34	c.1824_1829del (hom)	p.V609_L610del	TM7	Pseudohermaphroditism (46, XY)	[28]
35	c.1836 T > G (hom)	p.Y612Term	TM7	Pseudohermaphroditism (46, XY)	[29]
36	c.1847C > A (hom)	p.S616Y	TM7	Pseudohermaphroditism (46, XY)	[17]
37	c.1850delG (hom)	p.C617Lfs22	TM7	Pseudohermaphroditism (46, XY)	[4]
38	c.1874 T > A (hom)	p.I625K	TM7	Pseudohermaphroditism (46, XY)	[30]

EC Extracellular, *TM* transmembrane segment, *IL* intracellular loop, *fs* Frame shift, *Term* Terminal Codon, *hom* Homozygote, *het* Heterozygote

Most of the variants are in exon 11 of the *LHCGR* gene since this is its largest exon. Thus far, 21 inactivating mutations have been identified in exon 11 of this gene that can encode the receptor intracellular domains and the 7-transmembrane (TM). Various kinds of mutations can cause functional deactivation of the LH receptor gene product. A number of nonsense mutations have been identified in distinct regions of the transmembrane domain. Laue et al. have reported the first case of a nonsense mutation (A1635C) in exon 11 of the human luteinizing hormone receptor (hLHR) gene in two sisters with LCH [31]. They found that this mutation results in the receptor loss of function by introduction of a stop codon at residue 545 in transmembrane helix 5 of the hLHR. Richter-Unruh et al. have identified a LCH patient with premature termination codon at position 491 (TGG–TGA; W491*), truncated before the third intracellular loop that leads to deficiency in signaling pathways

[30]. Latronica et al. have reported two new homozygous missense and nonsense mutations in the LH-receptor gene ($Ser^{616} \rightarrow Tyr^{616}$ and $Arg^{554} \rightarrow$ stop $codon^{554}$ (TGA), respectively) in 3 46,XY siblings with Leydig-cell hypoplasia, a boy with micropenis and primary hypogonadism, and a 46,XX sister with amenorrhea [3]. They have shown that the found stop codon in the 3rd intracellular loop of the LH receptor interrupts the translation process of the LH-receptor mRNA and hence eliminates a large part of the receptor. It is further proposed that this truncated mutant receptor cannot transfer the hormonal signal even if expressed in the target cell membranes [3]. On the other hand, Newton et al. have reported on comparative and detailed functional analyses of deactivating disease-producing mutations of the LHR. They have performed a combined study by ligand-binding assays, in vitro signaling assays, and by determination of cell surface expression of mutant and wild-type receptors. They have shown that existence of proline residues within the TM helix regions (e.g., L502P and A593P) can produce helix kinks that disrupt the structure and the correct folding of the 7TM domain [32]. Consistent with these reports, it can be predicted that the frameshift mutations in c.1091dupT and L365P at the first TM produce truncated protein due to loss of its function and cannot transfer the hormonal signal.

Based on the modeling results in this study, the substitution of L365 with proline in the TM1 alters the structure and folding of the LHCGR receptor, results in a stop codon in codon 370, and produces a truncated protein without transmembrane domain. In our case, the performed analysis shows a novel duplication variant in exon 11, c.1091dupT, and pLeu365Profs*5. Two patients with 46, XY DSD and type 1 LCH are studied in this report and it is found that they have novel homozygous variants in their *LHCGR* gene. By introduction of a stop codon, this frameshift variant can result in premature termination and hence formation of a truncated protein with impaired protein function. We are of the opinion that the found frameshift variant in our case can lead to loss of function in the *LHCGR* protein that in turn influences the intracellular signaling cascades. Future studies must be directed towards the functional analysis investigations of this variant.

Conclusion

Leydig cell hypoplasia (LCH) is a rare autosomal recessive disorder presenting with abnormal development of male external genitalia in 46, XY individuals and one of the causes of disorder of sexual differentiation (DSD) in males. The present study discussed the phenotype and genotype of patients with LCH and demonstrated a novel homozygote variant among the Iranian population. By virtue of this case report, it can be said that Whole Exome Sequencing (WES) and genetic counseling should be considered in the diagnosis and management of patients with a disorder of sexual differentiation (DSD).

Abbreviations

LCH: Leydig cell hypoplasia; DSD: Disorder of sexual differentiation; LH: Luteinizing hormone; hCG: Human chorionic gonadotropin; LHCGR: Luteinizing hormone/chorionic gonadotropin receptor; SNVs: Single nucleotide variants.

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Author contributions

Conceptualization: SV, Methodology: MRA, Formal analysis and investigation: SS, Writing—original draft preparation: SV, SS and ML, Writing—review and editing: SS and SV, Supervision: MRA. All authors read and approved the final manuscript.

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Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

The manuscript had the approval of the Research Ethics Committee of the Mashhad University of Medical Sciences. Written informed consent was taken from the father of the patients as their official guardian for participation.

Consent for publication

Written informed consent was obtained from the father of the patients as their official guardian for publishing the results of the study.

Competing interests

The authors declare that they have no competing interests.

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