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Novel *FSHβ* mutation in a male patient with isolated FSH deficiency and infertility

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ABSTRACT

Isolated follicle stimulating hormone (FSH) deficiency due to mutations in *FSHβ* is an extremely rare autosomal recessive disease that has only been reported in ten patients to date. Symptoms of the disease include amenorrhoea and hypogonadism in women and azoospermia and normal testosterone levels in men. This study describes a Chinese male patient who presented with cryptorchidism and infertility. His serum hormonal profile revealed low FSH, elevated LH and normal testosterone levels. Sequence analysis identified a novel homozygous mutation in the *FSHβ* gene (c.343C > T) predicted to result in a premature termination codon and a truncated FSH protein (p.R115X). Both parents were heterozygous carriers of the mutation with normal pubertal development and fertility. The patient's testicular volume increased after one year of exogenous FSH replacement therapy at which point spermatocytes were detected in seminal samples, indicating potential future spermatogenesis. The expanded spectrum of *FSHβ* mutations and associated clinical manifestations described in this study may improve the diagnosis and treatment of this disease.

1. Introduction

The hypothalamic-pituitary-gonadal (HPG) axis plays important roles in pubertal development and reproductive functions. The coordination of the HPG axis is regulated by the pulsatile release of gonadotropin-releasing hormone (GnRH) in the hypothalamus. In response to GnRH stimulation, the pituitary releases the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), into the bloodstream (Krsmanovic et al., 2009). FSH is required for Sertoli cell proliferation and sperm quality in men and for follicular development and sex steroid production in women and thus essential for fertility (Simoni et al., 1997).

FSH is a dimeric glycoprotein hormone composed of a common α-subunit, which is identical to that of LH, human chorionic gonadotropin and thyroid-stimulating hormone, and a unique β-subunit, which drives hormone specificity (Fox et al., 2001; Fiddes and Goodman, 1981). The α-subunit is noncovalently joined to the β-subunit to form the biologically active hormone. Internal disulfide bonds between a "backbone" of conserved cysteine residues present in both subunits are critical for the correct tertiary structure and function of FSH. In addition, glycosylation at specific asparagine residues can modulate hormone action by drastically changing its stability and bioactivity (Fox et al., 2001).

The *FSHβ* gene (OMIM 136530) is located on chromosome 11p14.1 and has 3 exons which encode the 129-amino acid (aa) FSH β-subunit, consisting of an 18-aa signal peptide and the 111-aa mature protein (Jameson et al., 1988). Isolated FSH deficiency linked to homozygous or compound heterozygous *FSHβ* gene mutations (OMIM 229070) is an extremely rare autosomal recessive disease. To date, only 4 male and 6 female patients have been reported in the literature. The disease manifestations include amenorrhoea and hypogonadism in women and azoospermia and normal testosterone levels in men.

Here, we describe a man with isolated FSH deficiency who mainly presented with cryptorchidism and infertility. Sequence analysis of *FSHβ* identified a novel homozygous nonsense mutation predicted to encode a truncated FSH protein.

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1.1. Patient report

This study was approved by the ethics board of the Peking Union Medical College Hospital. Informed consent was obtained from both the patient and his parents.

The patient was a 32-year-old male referred to our clinic for the diagnosis of infertility despite having had regular unprotected intercourse for at least one year. The patient was delivered full term at the gestational age of 36 weeks. He appeared anatomically male at birth with no noted abnormalities except that his right testis was not palpable in the scrotum which was not attended to further. At the age of 12, he experienced normal pubertal development followed by regular erections and ejaculations. His sense of smell was normal. His parents were first cousins with normal pubertal development and fertility. The patient had no siblings. Delayed puberty or infertility had not been reported in any other family members.

Upon physical examination, the patient's height and weight were 174 cm and 72 kg, respectively. His appearance was fully virilized with normal penis size and pubic hair development (Tanner stage V). The right testis was not palpable in the scrotum, and subsequent ultrasound revealed a possible testis located in the inguinal region. The left testicular volume measured by a Prader orchidometer was 2 ml. No gynecomastia was observed.

2. Material and methods

2.1. Hormonal test

FSH, LH, and testosterone levels were measured using chemiluminescent immunoassays (Bayer Diagnostic Corporation, USA) according to the manufacturer's instructions. A GnRH analogue (GnRHa, triptorelin) stimulation test was conducted to evaluate the potential reservoir of HPG axis function. After 100 μg triptorelin was intramuscularly injected, LH and FSH were measured prior to and 60 min after triptorelin administration.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes collected from the patient and his parents using a QIAGEN DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Primers for the polymerase chain reaction (PCR) amplification of the *FSHβ* coding region (exons 2 and 3) were designed using Primer 3.0 (http://primer3.ut.ee/) and were as follows: Exon 2 sense: 5′-TATATTCCCACCCTGACCCA-3′; Exon 2 anti-sense: 5′-GATATCGTGGTCATTAACCC-3′; Exon 3 sense: 5′-ATGGGTAAATGTTAGAGCAA-3′; Exon 3 anti-sense: 5′-TGAATCCCCTGATCCAGT-3′. The *FSHβ* coding region was amplified using the TaqMix DNA polymerase (Biomed, China). Direct sequencing of PCR products was performed using a Taq Big Dye Terminator Sequencing Kit and an ABI3730 automated sequencer (Applied Biosystems, Forster City, CA, USA). DNA sequences were compared to NCBI reference sequences (gDNA, NG_008144.1; cDNA, NM_000510.2; protein, NP_000501). The A of the ATG start codon was designated as +1. The presence of a SNP was confirmed by searching the NCBI dbSNP database. Novel mutations were confirmed by comparison with the database of human gene mutation data (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php).

3. Results

3.1. Laboratory test

The patient's serum hormone levels prior to GnRHa administration were: LH, 32.79 IU/L (normal range: 1.27–19.26 IU/L); FSH, 0.05 IU/L (normal range: 1.24–8.62 IU/L); total testosterone, 9.4 nmol/L (normal range: 6.0–27.1 nmol/L); estradiol, 28 pg/mL (normal: <47 pg/mL); and inhibin B, <10 pg/mL (normal range: 47–308 pg/mL). After GnRHa administration, his LH level increased from 32.79 IU/L to 41.22 IU/L and his FSH level from 0.05 IU/L to 0.06 IU/L (Table 1).

The patient's thyroid hormone, growth hormone, insulin-like growth factor 1 (IGF-1), prolactin, adrenocorticotrophin and cortisol levels were within the normal range. No sperm were observed in semen analysis on two separate occasions. His chromosomal karyotype was 46, XY. No deletions in the AZF a, b or c loci of the Y chromosome were revealed by fluorescent *in situ* hybridization (Fish). Magnetic resonance imaging of the brain and pituitary were normal.

3.2. Mutational analysis

Based on the clinical manifestations and sex hormone levels, the patient's condition was suspected to be an isolated FSH deficiency. Further analysis of the underlying genetic cause revealed a novel homozygous substitution of thymine for cytosine at the 343rd nucleotide of the *FSHβ* cDNA resulting in a premature termination codon at the 115th amino acid of FSH-β subunit (p.R115X). Both parents of the patient harbored a single copy of the same mutation (Fig. 1). The novel mutation in the *FSHB* gene has been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/; accession number SCV000494015).

3.3. Treatment and follow-up

The ideal treatment to restore fertility in this patient should be purified or recombinant FSH, but he declined it due to unaffordable high cost. Thus, considering his desire for fertility, 75 IU of Human Menopausal Gonadotrophin (HMG, Livzon Pharmaceutical Co, Guangdong, China) was administered intramuscularly twice a week for 12 months. One year later, the patient's serum FSH was 6.64 IU/L, LH 36.64 IU/L and total testosterone 12.3 nmol/L. His left testicular volume increased from 2 to 5 ml. After four days of sexual abstinence, semen was obtained by masturbation and semen analysis was performed according to WHO guidelines (World, 2010). The semen volume was 4.8 mL, PH was 7.2, and liquefaction was one hour, but no sperm were found. Then semen analysis by Diff-Quik stain was performed, and spermatocytes, mainly primary spermatocytes, presented in the semen, while spermatids were not observed. The patient refused testicular sperm extraction by biopsy due to worrisome for potential surgery related complication. During follow-up, he underwent right orchidopexy.

4. Discussion

This study presents the fifth male patient with isolated FSH deficiency due to *FSHβ* gene mutation. In contrast to previous reports, the patient presented with cryptorchidism. Genetic analysis of the *FSHβ* gene revealed a novel homozygous mutation (p.R115X) predicted to result in a truncated FSHβ protein, and this was considered to be the underlying cause of the patient's condition.

To date, six other *FSHβ* mutations have been reported in four male and six female patients (Berger et al., 2005; Kottler et al., 2010; Layman et al., 1997, 2002; Lindstedt et al., 1998; Matthews et al., 1993; Matthews and Chatterjee, 1997; Phillip et al., 1998; Simsek et al., 2016). As shown in Table 2, two are frameshift mutations (p.Val79fsX105 and p.Ala97fsX127), three are missense mutations occurring at the highly conserved cysteine residues (p.Cys69Gly, p.Cys100Arg and p.Cys122Arg), and one is a nonsense