Fertile Eunuch Syndrome with the Mutations (Trp8Arg and Ile15Thr) in the β Subunit of Luteinizing Hormone

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Abstract. Fertile eunuch syndrome is caused by isolated LH deficiency, but its pathophysiology still remains controversial. We report a case of fertile eunuch syndrome with homozygous Trp8Arg and Ile15Thr mutations in the LH β subunit gene. An 18-year-old man was admitted to our hospital for hypogonadism. Examination of genitalia revealed Tanner G1PH1, whereas both testes were elastically palpated and developed up to 18 ml. Endocrinological evaluations revealed normogonadotropic hypogonadism and there were normal responses after GnRH and hCG stimulation. Intratesticular testosterone concentration was almost normal (1.34×10^3 ng/g). By PCR direct sequencing, homozygous Trp (8) Arg and Ile (15) Thr mutations in exon 2 of LH β were detected. Normal virilization and improved semen parameters were achieved after hCG supplementation. To our knowledge, this is the first case of fertile eunuch syndrome with homozygous Trp (8) Arg and Ile (15) Thr mutations in β subunit of LH gene.

Key words: fertile eunuch syndrome, LH β , mutation

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MEN with fertile eunuch syndrome have eunuchoid proportions, but characteristically have developed testes with sperm, often oligoasthenozoospermia, in their ejaculate. Although it is considered to be an isolated luteinizing hormone (LH) deficiency caused by disturbed gonadotropin-releasing hormone (GnRH) secretion in hypothalamus [1], the pathophysiology of fertile eunuch syndrome still remains controversial, and altered function of Leydig cells [2] and mutation in GnRH receptor in gonadotrophs [3, 4] have also been reported.

LH is a heterodimer composed of a common α -subunit and a specific β subunit that confers biological specificity for the receptor in Leydig cells in men [5]. The most common LH β allele variant is the Trp8Arg and Ile15Thr mutation in exon 2, which has a carrier frequency ranging from 0 to 53% in various populations [6], including 4.7% in Japanese men [7].

Homozygous mutation was observed 2% of azoospermic individuals [7]. There has been a report of a homozygous mutation of LH β gene, which is an amino acid substitution Gln54Arg in exon 3, in an adolescent with delayed puberty, increased serum concentration of LH, normal FSH and low testosterone [8], whereas the associations of variant LH (Trp8Arg and Ile15Thr in exon 2) and male infertility or hypogonadism are unclear [9, 10].

To our knowledge, there is no information on the association of fertile eunuch syndrome and variant LH. In this report, we describe a case of fertile eunuch syndrome with homozygous mutation of LH β gene.

Case Report

An 18-year-old man consulted our clinic on March 9, 2001 for delayed puberty. Physical examination revealed a thin young male with weight of 60.5 kg, height was 166 cm and arm span was 170 cm. There were no abnormalities in breast, olfactory function and mental development. Examination of genitalia revealed Tanner G1PH1, whereas both testes were

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Fig. 1. Photograph of external genitalia showing prepubertal stage, while testes are comparatively well developed.

elastically palpated and developed up to 18 ml as evaluated by punched-out orchidometer (Fig. 1). By interviewing the family members of the patient, no phenotypic abnormalities were detected. Semen analysis was oligoasthenozoospermia, with a sperm count of $4.5 \times 10^6/\text{ml}$, 30% of motility, and 60% of deformity in 1.5 ml of ejaculate. Endocrinological examination revealed normogonadotropic hypogonadism: total testosterone was 9.8 ng/dl (250–1100 ng/dl), LH was 2.57 mIU/l (1.7–8.6 mIU/l), and FSH was 3.76 mIU/l (1.6–11.0 mIU/l). Serum prolactin was 178.8 ng/ml (57–357 ng/ml). LH increased up to 52.4 mIU/l and FSH up to 11.9 mIU/l 30 and 60 minutes after gonadotropin-releasing hormone (GnRH) stimulation (100 μg of LH-RH intravenously), respectively. Serum testosterone increased up to 453.0 ng/dl after daily 5000 IU human chorionic gonadotropin (hCG) stimulation for 3 days. Episodic secretion of LH was recognized up to 5.72 mIU/l (4.5 ± 0.9 mIU/l; mean $\pm$ SD) during the night. The blood karyotype was 46, XY. Testicular biopsy demonstrated that more than half of the tubules showed normal degrees of spermatogenesis. There were a few small tubules with marked thickening of the basement membrane and only a few cells in the lumen. The interstitial tissue was of moderately increased density with relative rarity of mature Leydig cells (Fig. 2).

Intramuscular injection of hCG (5000 IU) twice a week (Tuesday and Friday) was started at March 23, 2001. Six months after the treatment, he achieved good virilization up to Tanner G3PH4, and increased testicular volume (both 22 ml). Serum testosterone concentration was elevated up to 385 ng/dl. Ejaculate

Fig. 2. Photomicrograph of testicular biopsy specimen ($\times 400$). Many tubules showed normal degrees of spermatogenesis. The interstitial tissue was moderately increased with relative rarity of mature Leydig cells.

volume increased up to 2.8 ml and semen parameters became normal, with sperm concentration of $25.5 \times 10^6/\text{ml}$, 60% of motility, and deformity was 30%. In September 2002, six months after discontinuation of hCG supplementation, serum testosterone fell to 38.2 ng/dl. He was followed up without any treatment because virilization was achieved and maintained.

DNA amplification and sequencing analysis of LH β gene

Informed consent was obtained from the patient and his family and all examinations followed a protocol approved by the Ethics Committee in Yamaguchi University. Blood samples were collected from the patient. Genomic DNA was isolated from peripheral blood lymphocytes by isolation reagents (DNA extraction kit, Takara Bio. Inc.) and was used as a polymerase chain reaction (PCR) template. The primers used in this study spanned exon 2, intron 2 and exon 3 of the LH β gene. The 5' primer was 5'-GGGAATTC TCTTTGTGGGTGGTGTACCACGC-3' and 3' primer was 5'-GGAGGATCCGGGTGTCAGGGCTCCA-3'. The PCR amplification was performed in a total volume of 25 μL reaction mixture containing 1.5 mM of MgCl_2 , 0.2 ml of each dNTP, 10% dimethylsulfoxide, 100 ng of genomic DNA, 10 pmol of each primer, and 1 U of *Taq* DNA polymerase. The cycling profile consisted of denaturation at 94°C for 1 minute, annealing at 65°C for 40 seconds, and extension at 72°C for 1 minute. The amplified DNA was sequenced with a fluorescence-based automated se-

Fig. 3. DNA sequencing showing the patient's nucleotide mutations and substitutions of amino acid.

quencer (ABI PRISM version 3.2 Genetic analyzer, Perkin Elmer, Foster City, CA).

DNA sequence of β subunit of LH gene revealed two heterozygous point mutations: a T to C transition at Trp⁸ (TGG) resulting in an amino acid substitution to Arg⁸ (CGG) and a T to C at Ile¹⁵ (ATC) resulting in an amino acid substitution to Thr¹⁵ (ACC) (Fig. 3).

Measurement of intratesticular testosterone concentration

A bit of testicular biopsy specimen was homogenized in 0.1 M phosphate buffer and centrifuged at 2000 rpm for 10 min. Supernatant was transferred to tubes and stored at -80°C until the assay. Testosterone concentration of the supernatant was measured by RIA (Total testosterone kit, Nippon DPC Corp.) [11]. Intratesticular testosterone concentration was 1.34×10^3 ng/g.

Discussion

This is the first report of homozygous mutation of LH β (Trp8Arg and Ile15Thr) in a patient presenting fertile eunuch syndrome. Conventional endocrinological evaluations, including episodic secretion of LH during the night time and responses for Gn-RH and hCG, revealed that there were no disorders in the hypothalamus-pituitary-Leydig cell axis. Achievement of favorable virilization after hCG supplementation suggests normal response in androgen-dependent organs. In men, variant LH has been reported to affect the course of puberty [9]; on the other hand, it was not associated with male infertility [10]. These observations suggest that homozygous mutation of LH β (Trp8Arg and Ile15Thr) may be a possible cause of fertile eunuch syndrome.

Many patients of this disease show normal or low

normal LH secretion, but not isolated LH deficiency [12–14], therefore another cause has to be considered. Variant LH β is undetectable in a radioreceptor assay compared with the wild-type hormone [8] and it has lower receptor-binding activity [15]. Inactivating human LH β mutation results in absence or decreased number of Leydig cells [6] because LH is necessary for normal development of Leydig cells. Additionally, there are several differences in intracellular trafficking by variant LH stimulation in Leydig cells [16]. Testicular biopsy finding (decreased number of mature Leydig cells) suggests the deterioration of ligand stimulation, following insufficient testosterone production. However, the prevalence of these LH β polymorphisms was reported to be up to 53% [6], since not all men with variant LH β present hypogonadism. Contrary to our case, a boy with homozygous mutation detected by immunological assays had normal pubertal onset [7]. It is unclear whether these genetic alterations represent causative mutations or simply polymorphisms [17, 18]. In different from Gln54Arg mutation in exon 3 [8], hypogonadism cannot be explained clearly only by Trp8Arg and Ile15Thr mutations in exon 2. Possible alterations in LH action (e.g., binding affinity to LH receptor, alterations in intracellular signal transduction) should also be clarified.

The initiation of spermatogenesis normally occurs under the influence of LH and FSH. Even a little testosterone can initiate spermatogenesis under sufficient FSH concentration, because spermatogenesis can be induced hypogonadotropic hypogonadism patients with human menopausal gonadotropins (hMG) [19]. One study showed that intratesticular testosterone concentration was $1.54 \pm 1.18 \times 10^3$ ng/g (mean $\pm$ SD) in adult and infertile men without any pubertal delay [11]. In our case, there was adequate LH to stimulate intratesticular testosterone (1.34×10^3 ng/g) with resultant spermatogenesis but insufficient testosterone to promote virilization [1].

Usually, low concentrations of serum testosterone provoke hypergonadotropic hypogonadism through negative feedback. Why did our case not represent hypergonadotropic hypogonadism? The variant LH was found to have a shorter half-life than that of the wild-type LH [20]. If production of the variant LH in gonadotroph is accelerated, the serum LH may appear normal or low. Additionally, Gn-RH secretion is also regulated by inhibin, which is produced from Sertoli cells under the regulation of FSH [21]. Raivio *et al.*

suggested that the variant LH was involved in the regulation of the GH-insulin-like growth factor I axis [9].

However, the spermiogram of fertile eunuch patients is oligoasthenozoospermia in many cases, we must be aware that it can be improved by proper hormonal supplementation. Hormonal therapy may provide a simpler and more inexpensive means of achieving sexual maturation and fertility. In an era of artificial reproductive technology (ART), men with sperm in their testes become "fertile", even if their spermiograms are azoospermia. The term "fertile

eunuch syndrome" remains somewhat of a misnomer that continues to invite misunderstanding. Evaluation of variant LH may help our understanding of unexplained hypogonadism and infertility.

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