FSHβ gene mutation in a female with delayed puberty and hypogonadism: response to recombinant human FSH

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Abstract: We report a woman with primary amenorrhoea and infertility associated with an isolated deficiency of pituitary FSH that does not respond to GnRH administration. Serum inhibin B was undetectable and antimullerian hormone (AMH) was within the normal range. Ultra sound examination revealed a small uterus and small ovaries with few small follicles. We identified an homozygous 1-bp (G) deletion at codon 79 in FSHβ gene suggesting a complete loss of function. The patient underwent studies of ovarian responsiveness to recombinant human FSH according to the following protocol: 150UI/d for five days following by 75 UI/d for 10 days. Estradiol plasma level started to increase from day 5 associated to a sharp increase of inhibine B and a decrease of LH. During the same time, we observed an excessive development of multiple follicles resulting in an arrest of the treatment to avoid hyperstimulation. The present study confirm that follicles up to 5 mm in diameter had developed in the absence of FSH and that FSH is required for the growth of follicles beyond the two-layer granulose stage.

Key words: FSHβ mutation, hypogonadism, recombinant hFSH

Introduction

Human FSH is a heterodimeric pituitary glycoprotein that regulates gonadal function in both sexes. In females, FSH regulates the follicular development and sex-steroid production, whereas in males, FSH stimulates Sertoli cell proliferation and maintenance of sperm quality. These functions are necessary for fertility in both sexes and are supported by studies involving knockout of the FSHβ gene. Several mutations in FSHβ gene have been described in humans, and all of those studied by functional analysis demonstrated complete FSH deficiency with absent puberty and infertility.

We report a woman with primary amenorrhoea and infertility associated with an isolated deficiency of pituitary FSH. We identified a new mutation at the homozygous state. Recombinant human FSH was administered in order to study the effect of FSH on follicle development and hormonal production.

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Patient and Methods

Patient. The proband presented at 29 years with primary amenorrhoea. She is 1.62 m tall and weighed 62 kg. At clinical examination, she is Tanner stage mammary gland 2 and pubic hair 4. There was no family history of delayed puberty and infertility but she was born from consanguineous parents.

The following laboratory test results were obtained: serum FSH was undetectable: <0.5mIU/ml (normal, 2.0 to 17.2 mIU/ml) using three different IRMA or EIA assays with monoclonal antibodies and did not response to GnRH administration (100μg); LH was elevated: 50 mIU/ml (normal, 1.6 to 9.3 mIU/ml) with an explosive response to GnRH. Estradiol was low, between 5 to 15 pg/ml (normal range in follicular phase 30-90 pg/ml). Prolactin as well as androgens levels were within the normal range. Serum inhibine B was undetectable and antimullerian hormone (AMH) was within the normal range (26 pmol/ml).

Ultra sound examination revealed a small uterus and small ovaries with few small follicles. The patient and her parents gave their consent for genetic screening.

DNA sequencing and analysis. Genomic DNA was extracted from peripheral blood leukocytes using Amersham guidelines. The regions spanning exon 2 and exon 3 which encodes for the FSHβ-subunit protein were amplified using PCR assay, with the following primers: exon 2 forward (AGT TTC TAG TGG GCT TCA TTG TTT G); exon 2 reverse (TGG CTA AAG GAC TCA TGG CTG)
as previously described [1]; exon 3 forward (AGG AAC TTC CAC
AAT ACC ATA ACC TAA C) and exon 3 reverse (CTG CCA GTA
GAC CAG GGA TCA GTA GAG).

Briefly, 30 cycles of PCR were performed at 95°C for 30 sec,
60°C for 30 sec, and 72°C for 40 sec. The MgCl₂ concentration
was 1.5 mM. A negative control, containing all reagents except
DNA, was included in each PCR. The PCR products were then
electrophoresed on agarose gel, purified with Exo-SAP-IT (usb,
Ohio, USA) according to manufacture's guidelines, and subjected
to dideoxy DNA sequencing using GenomeLab™ Dye Terminator
Cycle Sequencing Kit (DTCS, Beckman Coulter).

For restriction analysis, MscI (New England
BioLabs, Ozyme, france) was used as recommended by the manu-
facturer and the products were then analyzed on 1.5% agarose gel
electrophoresis with ethidium bromide.

**Results**

**DNA analysis**

Automated DNA sequencing of exon 2 and exon 3 of
FSHβ gene in the proband, revealed a homozygous
1-bp (G) deletion at codon 79 (Fig. 1A). This dele-
tion results in a frame shift that completely alters the
amino acid sequence of FSHβ subunit from 80 to
108 before leading to a premature stop codon which
means that the predicted protein is shorter (Fig. 1B).

The deletion of one base pair in codon 79 eliminates
a MscI restriction site in exon 3. Intra familial
screening for the mutation was performed using
restriction analysis (Fig 2). As expected, the ampli-
fied PCR fragment of exon 3 from the proband was
not digested by MscI (Fig. 2B, lane III.1-D), where-
as wild type fragment of exon 3 was digested into
two fragments (Fig. 2B, lane WT-D). Both parents
were heterozygous for this variation (Fig. 2B, lanes
II.1-D and II.2-D).

**Stimulation by r-hFSH**

Estradiol plasma level started to increase from day
5 associated to a sharp increase of inhibin B and a
decrease of LH. During the same time, we observed an
excessive development of multiple follicles resulting
in an arrest of the treatment to avoid hyperstimulation.

**Discussion**

The FSHβ gene (MIM 136530) that codes for the FSH
β-subunit is located on 11p13 and consists of one non-
coding exon plus two translated exons that encode the
129-aminoacid preprotein. The mature protein consists
in 111 amino-acids. To day only 8 subjects (both men
and women) with inactivating FSHβ mutations have
been described [1,3-7].

We reported a novel mutation in the β-subunit FSH
gene. If translated, this mutation is predicted to pro-
duce a frameshift, altering amino acids 79-108, and
then to introduce a stop codon at position 108. Accord-
ing to the crystal structure of hFSH, this variant should
interfere with the cystine knot of FSHβ, important for
the dimer formation and intracellular stability [2]. In
addition, the Ala79Profs108X mutant would be lack-
ning the noose (determinant loop)-amino acids 87-94-
involved in FSH receptor specificity and the seat belt
region contained in amino acids 84-104 that enables
the β-subunit to wrap around the α-subunit [2]. Taken
together these data suggest a complete loss of function.
These finding are similar to that observed for Val61X
mutant, excepted for amino acids 61-76, which are
conserved with Ala79Profs108X mutant.

Four inactivating mutations, either altering single
amino acids or deleting nucleotides leading to prema-
ture stop codons and truncated FSH β-subunit protein
(Table 1), have been reported in five female and three
male patients [1,3-7]. The phenotypic characteristics
caused by these mutations in both sexes are severe,
resulting in absent, or incomplete pubertal develop-
ment and infertility.

This patient was hypoestrogenic with secondary LH excess but not excessive androgen production.

Table 1. Inactivating mutations described in β-subunit FSH gene. Prot βFSH: 111AA (without SP); SP, signal peptide; PTC, premature termination codon

<table>
<thead>
<tr>
<th>Nucleotides</th>
<th>Exon</th>
<th>Amino acids change (without SP)</th>
<th>Amino acids change with SP (18 AA)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.236_237delTG</td>
<td>3</td>
<td>Val61X (frameshift, PTC at 86)</td>
<td>Val79X</td>
<td>[1,5,6,12]</td>
</tr>
<tr>
<td>c.205T&gt;G</td>
<td>3</td>
<td>Cys51Gly</td>
<td>Cys69Gly</td>
<td>[1]</td>
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<tr>
<td>c.298T&gt;C</td>
<td>3</td>
<td>Cys82Arg</td>
<td>Cys100Arg</td>
<td>[4]</td>
</tr>
<tr>
<td>c.282C&gt;A</td>
<td>3</td>
<td>Tyr76X (stop codon)</td>
<td>Tyr94X</td>
<td>[3,7]</td>
</tr>
<tr>
<td>c.289delG</td>
<td>3</td>
<td>Ala79X (frameshift, PTC at 108)</td>
<td>Ala97X</td>
<td>Present study</td>
</tr>
</tbody>
</table>

**In vivo FSH administration.** After giving informed consent and
discontinuing the oral contraceptive for one months, the patient
underwent studies of ovarian responsiveness to gonadotropins.

The patient was treated by recombinant human FSH (Gonal-F,
Serono) according to the following protocol: 150UI/d for five days
following by 75 UI/d for 10 days. Hormonal evaluation was made
every 5 days and the growth of follicles was monitored by ultra
sound examination.

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Fig. 1. (A) Characterization of the mutation in the gene for the β-subunit of Follicle-Stimulating Hormone in a woman with hypogonadism. Sequence analysis of exon 3 of the gene for the β-subunit, amplified from the patient's DNA III.1 and compared with wild type (WT) DNA, revealed a deletion of one base pair (G) in codon 79 (arrows, c.289delG mutation). (B) Protein alignments between wild type and predicted β-subunit peptide of patient III.1 prot; protein.; WT; wild type

Fig. 2. (A) Pedigrees of the family with FSH deficiency All individuals studied are designated as homozygous (completely black), heterozygous (half-black), or not studied (indicated by question mark). The proband (Subject III.1) is denoted by an arrow. (B) Electrophoresis of a restriction digestion by MscI of exon 3 PCR product of the FSHβ gene on 1.5% agarose gel (with ethidium bromide staining). A restriction site for MscI exists in exon 3 of intact fragment resulting to 241- and 179-bp short fragment. The variation c.289delG eliminates this restriction site resulting to a resistance of mutated fragment to this enzyme. Note the heterozygous pattern of the parental's DNAs (II.1 and II.2). M, mass ladder; WT, wild type; U, undigested; D, MscI digested.
This finding argues against strict compartmentalization of the action of FSH and LH and suggests the role of paracrine factors (inhibin B being one possibility) stimulated by FSH which in turn stimulate the production of androgen by theca cells in response to LH [8]. Similar positive regulatory effect of FSH on healthy LH-stimulated Leydig cells was reported in a man with isolated FSH deficiency due to an FSHβ mutation [9], probably mediated by its primary action on Sertoli cells, in a paracrine mechanism.

We also document the presence of antral follicular development in spite of lack of FSH.

The point at which FSH is necessary for follicular development in the human ovary has been debated for a long time. The present study confirm that follicles up to 5 mm in diameter had developed in the absence of FSH and that FSH is required for the growth of follicles beyond the two-layer granulose stage [10]. Last, follicles had apparently developed normally since estradiol and inhibin B increased promptly after FSH administration. Our finding is similar to those in other patients with isolated FSH deficiency who ovulated and had successful pregnancy [5,8,11,12].

References


