

## Brief Report

## MALE HYPOGONADISM DUE TO A MUTATION IN THE GENE FOR THE $\beta$ -SUBUNIT OF FOLLICLE-STIMULATING HORMONE

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**N**ORMAL pubertal development and fertility depend on the intricate interplay of hypothalamic, pituitary, and gonadal factors. Crucial in this respect are normal secretory patterns of follicle-stimulating hormone and luteinizing hormone. These hormones stimulate the production of estrogen and ovulation in women and the production of testosterone and spermatogenesis in men. Secreted from common gonadotroph cells, the hormones are heterodimers composed of a common  $\alpha$ -subunit and a specific  $\beta$ -subunit, each encoded by a separate gene. Specificity of action depends on the recognition of these hormones by specific receptors on the surface of gonadal cells.

Various genetic defects of the hypothalamic-pituitary-gonadal axis that cause hypogonadism have been identified.<sup>1</sup> At the level of the hypothalamus, secretion of gonadotropin-releasing hormone is disturbed by mutations in the *KAL* gene,<sup>2</sup> leading to Kallmann's syndrome, and in the *DAX-1* gene,<sup>3</sup> causing X-linked adrenal hypoplasia and hypogonadotropic hypogonadism. At the pituitary level, mutations in the gene for the  $\beta$ -subunit of luteinizing hormone<sup>4</sup> cause hypogonadotropic hypogonadism, and at the gonadal level, loss-of-function mutations in the genes that encode the receptors for follicle-stimulating hormone and luteinizing hormone cause hypergonadotropic hypogonadism.<sup>5,6</sup> Specifically, mutations in the gene for luteinizing hormone receptors result in Leydig-cell hypoplasia and undermasculinization in genetic males,<sup>5,7,8</sup> whereas mutations in the gene for follicle-stimulating hormone receptors cause primary gonadal failure and hypergonadotropic hypogonadism in genetic females.<sup>6</sup>

Two female patients with follicle-stimulating hor-

mone deficiency caused by mutations in the gene for the  $\beta$ -subunit of follicle-stimulating hormone have been described. One presented with primary amenorrhea and infertility,<sup>9</sup> and the other with delayed puberty.<sup>10</sup> In this report, we describe a man with impaired secretion of follicle-stimulating hormone caused by a homozygous mutation in the gene for the  $\beta$ -subunit of follicle-stimulating hormone, as well as two asymptomatic heterozygous male members of his family.

### METHODS

#### Subjects

The proband was referred to our center at the age of 18 years for evaluation of delayed puberty. He reported normal erections and ejaculatory orgasms. He had a prepubertal physique and underdeveloped muscles. He was 178 cm tall (69th percentile) and weighed 59 kg. He had pubic hair (Tanner stage 4), scant axillary hair, and no facial hair. No breast tissue was palpated. The scrotum was thin, and two small, soft testicles (testicular volume, 1 to 2 ml) were palpated. There was no family history of consanguinity, infertility, or delayed puberty.

Laboratory studies revealed low serum testosterone and follicle-stimulating hormone concentrations and high serum luteinizing hormone concentrations (Table 1). Serum thyrotropin, prolactin, and cortisol concentrations were normal. Chromosomal analysis revealed a 46,XY karyotype. After intravenous administration of 100  $\mu$ g of gonadotropin-releasing hormone, the patient's serum luteinizing hormone concentration increased from 24.5 mIU per milliliter to 66.6, 73.3, 74.5, and 70.2 mIU per milliliter at 15, 30, 45, and 60 minutes, respectively. Serum follicle-stimulating hormone concentrations were less than 0.5 mIU per milliliter before and after the administration of gonadotropin-releasing hormone. Semen analysis on two occasions showed white ejaculates (2.5 and 2.9 ml) with no sperm. Bone age was 16 years, and the findings on magnetic resonance imaging of the brain and pituitary were normal.

The proband's 17-year-old brother was 179 cm tall with Tanner stage 5 pubic hair. His testicular volume was 25 ml bilaterally. He had normal libido, with normal erections and ejaculations. His serum follicle-stimulating hormone, luteinizing hormone, and testosterone concentrations were 3.3 mIU per milliliter, 5.7 mIU per milliliter, and 1020 ng per deciliter (35.4 nmol per liter), respectively.

The father, who was 41 years old, had normal libido and sexual function. His pubic hair was Tanner stage 5, and his testicular volume was 25 ml bilaterally. His serum follicle-stimulating hormone, luteinizing hormone, and testosterone concentrations were 3.9 mIU per milliliter, 4.7 mIU per milliliter, and 990 ng per deciliter (34.3 nmol per liter), respectively. The mother's age at menarche was 12.5 years. She had given birth to three children: the brothers described above and a three-year-old girl from a second marriage. She had chronic autoimmune thyroiditis, which was treated with thyroxine.

The study protocol was reviewed and approved by the hospital review committee, and informed consent was obtained from all the subjects.

#### DNA Analysis

DNA was extracted from peripheral-blood leukocytes by standard methods. All three exons of the gene for the  $\beta$ -subunit of follicle-stimulating hormone were amplified by a polymerase-chain-reaction (PCR) assay with the use of primer pairs designed to amplify the exons and the exon-intron junctions on the basis of the gene sequence.<sup>13</sup> The primers used were FSH-1-F 5'AATTTGAGAAGGTAAAGGAG3' and FSH-1-R 5'GCATAAATTTCC-TACACAAC3' for exon 1, FSH-2-F 5'GGCTTCATTGTTTG-

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**TABLE 1. SERUM HORMONE VALUES IN A MAN WITH FOLLICLE-STIMULATING HORMONE DEFICIENCY CAUSED BY A MUTATION IN THE GENE FOR THE  $\beta$ -SUBUNIT OF FOLLICLE-STIMULATING HORMONE.\***

HORMONE	VALUE IN PATIENT	REFERENCE RANGE
Testosterone (ng/dl)		
Total	130	270–1070
Free	25	120–240
Sex hormone-binding globulin (nmol/liter)	79	13–71
Follicle-stimulating hormone (mIU/ml)	<0.5	1.6–11.0
$\beta$ -Subunit of follicle-stimulating hormone ( $\mu$ g/liter)	1.1	3.1–5.5
Luteinizing hormone (mIU/ml)	24.5	0.8–6.1
Estradiol-17 $\beta$ (pg/ml)	<10	10–45
Free $\alpha$ -subunit (ng/ml)	0.43	<1.8
Inhibin B (pg/ml)	15.6	100–140

\*Radioimmunoassays were used to measure total testosterone (Diagnostic Products, Los Angeles), follicle-stimulating hormone, luteinizing hormone, estradiol-17 $\beta$  (Boehringer Mannheim, Mannheim, Germany), and free  $\alpha$ -subunit (Biomerica, Newport Beach, Calif.). Free testosterone was measured by radioimmunoassay after precipitation with saturated ammonium sulfate.<sup>11</sup> Sex hormone-binding globulin was measured by a chemiluminescence immunometric assay (Diagnostic Products). The  $\beta$ -subunit of follicle-stimulating hormone was measured by a fluoroimmunoassay.<sup>12</sup> Inhibin B was measured by a solid-phase sandwich enzyme-linked immunosorbent assay (Serotec, Oxford, United Kingdom). To convert the values for testosterone to nanomoles per liter, multiply by 0.03467. To convert the values for estradiol-17 $\beta$  to nanomoles per liter, multiply by 0.03671.

CTTCC3' and FSH-2-R 5'AAACCCCGGTAATACAGAC3' for exon 2, and FSH-3-F 5'AACTTCCACAATACCATAACC3' and FSH-3-R 5'CAGACTTTTGAATATCTTGG3' for exon 3. FSH-3-R2 5'ACAGTACAATCAGTGCTGTCG3' was used instead of FSH-3-R for analyses of single-strand conformation polymorphisms and restriction analyses.

The PCR assay was performed with 2.5 mM magnesium chloride, 0.2 mM deoxynucleoside triphosphate, 0.5  $\mu$ M of each primer, and 1 unit of Taq polymerase (MBI Fermentas, Vilnius, Lithuania) with the manufacturer's buffer. Cycling conditions were as follows: one minute at 94°C, one minute at the annealing temperature, and one minute at 72°C for 30 cycles, followed by five minutes at 72°C. Annealing temperatures were 45°C for the first exon and 55°C or 52°C for the second and third exons. PCR products were purified with the Quiaquick gel-extraction kit (Quiagen, Hilden, Germany), and 3  $\mu$ g of DNA from three separate PCR reactions (1  $\mu$ g of DNA from each) were combined and subjected to sequencing with the use of DNA sequencer ABI 310 (Perkin Elmer, Foster City, Calif.) on both strands. Analysis of single-strand conformation polymorphisms was performed as previously described.<sup>14</sup>

For restriction analysis, *Tsp*RI (New England Biolabs, Beverly, Mass.) was used as recommended by the manufacturer. Products were separated on 4 percent agarose gels (3 percent NuSieve GTG and 1 percent SeaKem LE [FMC Bioproducts, Rockland, Me.]) in parallel with a 1-kb-ladder marker (Boehringer Mannheim, Mannheim, Germany) and visualized by staining with ethidium bromide (Sigma, St. Louis).

## RESULTS

No changes in sequence were found in exons 1 and 2 of the patient's gene for the  $\beta$ -subunit of follicle-stimulating hormone. Sequencing of exon 3 re-

vealed that the patient was homozygous for a deletion of the second and third nucleotides (thymidine and guanine) in codon 61 (Fig. 1A). This mutation would be expected to lead to a frame shift in transcription so that the  $\beta$ -subunit of follicle-stimulating hormone would contain the first 60 amino acids of the third exon and 26 amino acids in a frame shift until the stop codon (TGA) was reached; the last 51 amino acids of the  $\beta$ -subunit would be missing.

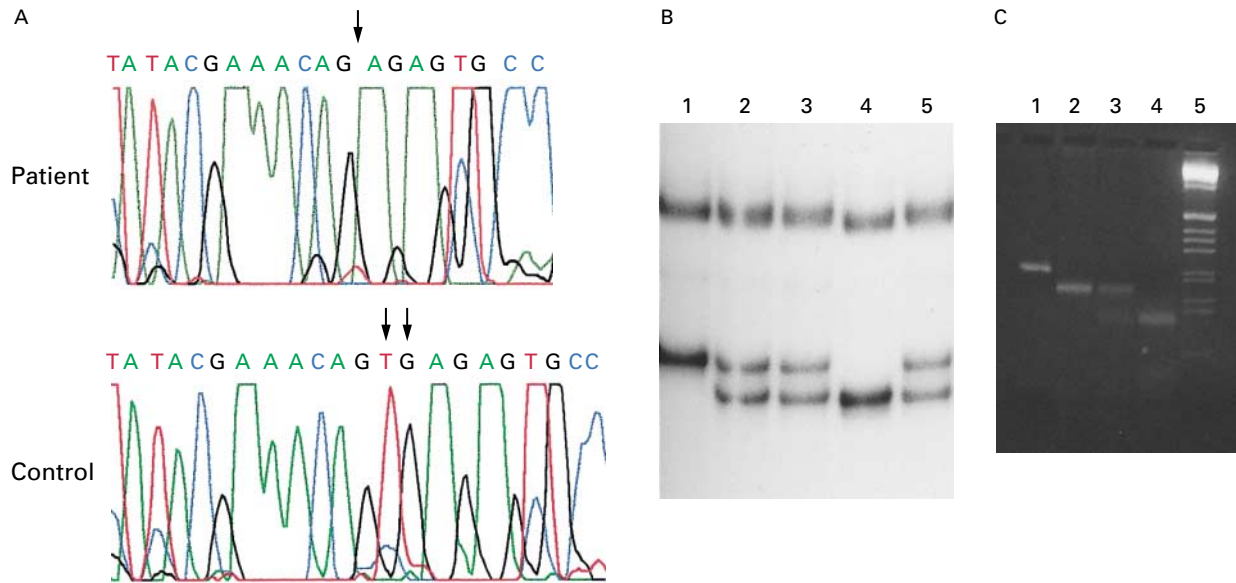
To verify the source of the mutation, PCR products of exon 3 from the patient, his brother, and his parents were analyzed for single-strand conformation polymorphism. As expected from the sequencing results, the patient's DNA migrated as a single band, indicating homozygosity, whereas DNA from his parents and brother migrated as two bands, indicating heterozygosity (Fig. 1B). The deletion of two base pairs in codon 61 was predicted to eliminate one of the two *Tsp*RI restriction sites in exon 3. As expected, the amplified PCR fragment of exon 3 from the patient was digested by *Tsp*RI into two fragments (Fig. 1C).

## DISCUSSION

Normal adolescent development begins with an increased amplitude of pulsatile gonadotropin-releasing hormone leading to increased secretion of luteinizing hormone and follicle-stimulating hormone. In men, follicle-stimulating hormone supports the growth and proliferation of seminiferous tubules and spermatogenesis, whereas luteinizing hormone mainly affects the production of testosterone by testicular Leydig cells.

We report a case of secondary hypogonadism associated with an isolated deficiency of follicle-stimulating hormone in a young man. The hormonal deficiency was due to a two-nucleotide deletion in the coding sequence for the  $\beta$ -subunit of follicle-stimulating hormone, resulting in a truncated polypeptide lacking the last 51 amino acids at the C-terminal end of the subunit. Layman et al. recently described a teenage girl with delayed puberty, hypogonadism, and isolated follicle-stimulating hormone deficiency due to compound heterozygous mutations in the gene for the  $\beta$ -subunit, including the deletion of thymidine and guanine in codon 61, as noted in our patient.<sup>10</sup> In the study by Layman et al., transfection of the patient's  $\beta$ -subunit DNA and  $\alpha$ -subunit DNA resulted in the production of follicle-stimulating hormone with no immunoreactive or biologic activity.

The severe deficiency of follicle-stimulating hormone in our patient provided an opportunity to evaluate this hormone's action on male sexual maturation and fertility. The patient had bilaterally descended small, soft testes; clinical evidence of androgen deficiency; high serum luteinizing hormone concentrations and low serum total and free testosterone concentrations; high-normal serum sex hor-



**Figure 1.** Characterization of the Mutation in the Gene for the  $\beta$ -Subunit of Follicle-Stimulating Hormone in a Man with Hypogonadism. Sequence analysis of exon 3 of the gene for the  $\beta$ -subunit, amplified from the patient's DNA and compared with control DNA (Panel A), revealed a deletion of two base pairs (TG) in codon 61 (arrows). An autoradiograph of single-strand conformation polymorphism (Panel B) shows that the patient's DNA (lane 4) migrated both faster than control DNA (lane 1) and as a single band, indicating homozygosity. DNA from his mother (lane 2), father (lane 3), and brother (lane 5) migrated as two bands, indicating heterozygosity. Panel C shows the results of restriction analysis of exon 3 with *TspRI*. Uncut DNA (lane 1) migrates as a single band in an ethidium bromide-stained agarose gel. Control DNA (lane 4) is cut into three fragments of 123, 52, and 43 bp because there are two *TspRI* sites within the gene. The codon 61 TG deletion in the patient's DNA (lane 2) eliminates the *TspRI* site located 123 bp from the 5' end of the gene, so that the DNA is cut into only two fragments of 175 and 43 bp. Note the heterozygous pattern of the father's DNA (lane 3). Lane 5 contains a 1-kb ladder. The lower five bands are 220, 201, 154, 134, and 75 bp.

mone-binding globulin concentrations; low serum inhibin B concentrations; and azoospermia on two occasions.

There have been several reports of males with isolated follicle-stimulating hormone deficiency diagnosed by biochemical methods. Some of the patients had associated disorders, such as cryptorchidism, hypospadias, omphalocele, deafness, the olfactory-genital dysplasia syndrome, chromosomal alterations, or short stature.<sup>15-17</sup> Others had a normal habitus without any malformations or chromosomal alterations.<sup>18</sup> In all male patients previously described, basal serum luteinizing hormone and testosterone concentrations were normal. The variable phenotypes and other disorders may represent additional disorders or a partial rather than total deficiency of follicle-stimulating hormone. It is also conceivable that another mutation in the coding or regulatory sequences of the gene for the  $\beta$ -subunit of follicle-stimulating hormone leads to low serum follicle-stimulating hormone concentrations or to undetectable yet partially bioactive hormone, resulting in a different phenotype. Mutations in the gene for the follicle-stimulating hormone receptor also lead to various degrees of oligospermia and normal-

to-elevated serum luteinizing hormone concentrations, representing different phenotypes with the same genotype.<sup>19</sup>

The low serum total and free testosterone concentrations and high serum luteinizing hormone concentrations in our patient are curious findings. Leydig cells do not have follicle-stimulating hormone receptors, and the low serum testosterone concentrations are therefore not readily explained. Supernatants of Sertoli cells incubated with follicle-stimulating hormone stimulate testosterone secretion by Leydig cells and testicular explants from rats,<sup>20,21</sup> hamsters,<sup>22</sup> and humans.<sup>23,24</sup> These findings suggest that our patient's low serum testosterone concentrations may have been due to the absence of a Leydig-cell-stimulating substance that is normally produced by Sertoli cells when they are stimulated by follicle-stimulating hormone. The patient's serum luteinizing hormone concentration was high because of the impaired testosterone secretion. His low serum inhibin B concentrations were probably due to Sertoli-cell hypofunction.<sup>25</sup>

The prevalence of mutations in the gene for the  $\beta$ -subunit of follicle-stimulating hormone remains to be determined. Ours is the third report of the

same mutation in the  $\beta$ -subunit gene; the other two reports involved women, one from the United Kingdom<sup>9</sup> and the other from the United States.<sup>10</sup> Our finding of the same mutation in two additional nonconsanguineous subjects, our patient's parents, suggests that this mutation may be more prevalent than previously suspected.

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