GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism

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Abstract

Idiopathic hypogonadotropic hypogonadism (IHH) is a condition characterized by failure to undergo puberty in the setting of low sex steroids and low gonadotropins. IHH is due to abnormal secretion or action of the master reproductive hormone gonadotropin-releasing hormone (GnRH). Several genes have been found to be mutated in patients with IHH, yet to date no mutations have been identified in the most obvious candidate gene, GNRH1 itself, which encodes the preprohormone that is ultimately processed to produce GnRH. We screened DNA from 310 patients with normosmic IHH (nIHH) and 192 healthy control subjects for sequence changes in GNRH1. In 1 patient with severe congenital nIHH (with micropenis, bilateral cryptorchidism, and absent puberty), a homozygous frameshift mutation that is predicted to disrupt the 3 C-terminal amino acids of the GnRH decapeptide and to produce a premature stop codon was identified. Heterozygous variants not seen in controls were identified in 4 patients with nIHH: 1 nonsynonymous missense mutation in the eighth amino acid of the GnRH decapeptide, 1 nonsense mutation that causes premature termination within the GnRH-associated peptide (GAP), which lies C-terminal to the GnRH decapeptide within the GnRH precursor, and 2 sequence variants that cause nonsynonymous amino-acid substitutions in the signal peptide and in GnRH-associated peptide. Our results establish mutations in GNRH1 as a genetic cause of nIHH.
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Idiopathic hypogonadotropic hypogonadism (IHH) is a condition characterized by failure to undergo puberty in the setting of low sex steroids and low gonadotropins. IHH is due to abnormal secretion or action of the master reproductive hormone gonadotropin-releasing hormone (GnRH). Several genes have been found to be mutated in patients with IHH, yet to date no mutations have been identified in the most obvious candidate gene, GNRH1 itself, which encodes the preprohormone that is ultimately processed to produce GnRH. We screened DNA from 310 patients with normosmic IHH (nIHH) and 192 healthy control subjects for sequence changes in GNRH1. In 1 patient with severe congenital nIHH (with microgenital, bilateral cryptorchidism, and absent puberty), a homozygous frameshift mutation that is predicted to disrupt the C-terminal amino acids of the GnRH decapetide and to produce a premature stop codon was identified. Heterozygous variants not seen in controls were identified in 4 patients with nIHH: 1 nonsynonymous missense mutation in the eighth amino acid of the GnRH decapetide, 1 nonsense mutation that causes premature termination within the GnRH-associated peptide (GAP), which lies C-terminal to the GnRH decapetide within the GnRH precursor, and 2 sequence variants that cause nonsynonymous amino-acid substitutions in the signal peptide and in GnRH-associated peptide. Our results establish mutations in GNRH1 as a genetic cause of nIHH.

Gonadotropin-releasing hormone (GnRH) is the master hormone of the reproductive endocrine system. The existence of central hormones that regulate reproduction was postulated a century ago (reviewed in ref. 1). In 1910, Crowe et al. (2) demonstrated that disruption of the hypothalamic-pituitary connection in dogs prevented the onset of puberty. Subsequent studies led to the hypothesis that the pituitary is controlled by a hypothalamic factor (3–6). It was not until 1971, however, that a hypothalamic factor (3–6). It was not until 1971, however, that connection in dogs prevented the onset of puberty. Subsequent studies led to the hypothesis that the pituitary is controlled by a hypothalamic factor (3–6). It was not until 1971, however, that the hypothalamic factor (H2O841) was identified, which encodes the Gnrh1 (17) that encodes the preprohormone that is ultimately processed to produce GnRH. We screened DNA from 310 patients with normosmic IHH (nIHH) and 192 healthy control subjects for sequence changes in GNRH1. In 1 patient with severe congenital nIHH (with microgenital, bilateral cryptorchidism, and absent puberty), a homozygous frameshift mutation that is predicted to disrupt the C-terminal amino acids of the GnRH decapetide and to produce a premature stop codon was identified. Heterozygous variants not seen in controls were identified in 4 patients with nIHH: 1 nonsynonymous missense mutation in the eighth amino acid of the GnRH decapetide, 1 nonsense mutation that causes premature termination within the GnRH-associated peptide (GAP), which lies C-terminal to the GnRH decapetide within the GnRH precursor, and 2 sequence variants that cause nonsynonymous amino-acid substitutions in the signal peptide and in GnRH-associated peptide. Our results establish mutations in GNRH1 as a genetic cause of nIHH.

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GnRH | luteinizing hormone-releasing hormone | LHRH


The authors declare no conflict of interest.

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hypogonadism makes the absence of human GNRH1 mutations as a cause of nIHH all the more puzzling.

We herein report a homozygous mutation in a male patient with severe congenital nIHH. This single base-pair deletion produces a frameshift that is predicted to disrupt the GnRH decapeptide. We also identified rare heterozygous GNRH1 sequence variants in 4 patients with nIHH.

Results

Patient Phenotypes. Patient 1. Patient 1 was evaluated at 8 years, 8 months, for cryptorchidism and microphallus. His parents had normal pubertal timing, come from the same village in Armenia, and deny consanguinity (Fig. 1A). The patient’s examination was notable for height between the 10th and 25th percentiles, weight between the 75th and 90th percentiles, nonpalpable testes, microphallus (<3 cm), and absence of midline or skeletal defects. FSH and LH were both <0.5 international units (IU)/L, and anti-Müllerian hormone was 99 pmol/L (reference range for prepubertal boys, 100–300 pmol/L), indicating the presence of testicular Sertoli cells. A human chorionic gonadotropin (hCG) stimulation test at 9 years produced no change in serum testosterone (from 0.38 to 0.4 nmol/L). Ultrasound identified inguinal testes with calculated volumes of 0.13 and 0.11 mL. Shortly thereafter, he underwent bilateral orchiopexy. Intraoperative bilateral testicular biopsies showed immature seminiferous tubules with no lumen, gonocyte-like cells, immature Sertoli cells, and interstitial fibrosis with spindle-shaped myofibroblasts (Fig. 1B).

At age 13 years, 6 months, a GnRH stimulation test was performed because of lack of pubertal development. Baseline FSH and LH were both <0.5 IU/L and rose minimally to 1.3 and 0.8 IU/L, respectively. Formal smell testing with a set of odorants revealed a normal sense of smell. He began treatment with testosterone enanthate 100 mg every 4 weeks, which resulted in linear growth and development of secondary sexual characteristics. Currently, at age 15 years, 6 months, his height is 168 cm (50th percentile), his weight is 81 kg, and he has developed facial hair and Tanner V pubic hair.

Patient 2. Patient 2 presented at 19 years with absence of breast development and was diagnosed with nIHH. She conceived 1 singleton and 1 twin pregnancy after gonadotropin treatment.

Fig. 1. Features of patient 1, who has a homozygous frameshift mutation in GNRH1. (A) Pedigree. Arrow, proband. (B) Testicular biopsy of patient 1, stained with hematoxylin and eosin. [Magnification: 1,000×.] Arrows, gonocyte-like cells; asterisks, interstitial cells.

Fig. 2. Pedigrees of patients with nIHH found to have heterozygous sequence variants in GNRH1. Arrows, probands; +, wild type.
LH, but his testosterone level remained low (Fig. S1). Pulsatile GnRH resulted in progressive increases in FSH and showed absence of LH pulses (Fig. S1).

Patient 2 was evaluated at 17 years, 6 months, for absent puberty. His physical examination was notable for a high-pitched voice, mild acne on the chin, absence of pubic hair, sparse axillary hair, a few strands of pubic hair, microphallus (length 2.5 cm), right testicle with length of 2 cm and soft consistency, left testicle with length of 1 cm, and intact olfaction as tested by serial dilutions of pyridine solutions. Laboratory evaluation was normal with positive human chorionic gonadotropin (hCG) stimulation test (5,000 IU daily for 5 days), testosterone rose from 1.9 to 3.0 nmol/L. After a clomiphene stimulation test, FSH, LH, and testosterone were essentially unchanged. Bone age was 15 years. The patient was treated with escalating doses of intramuscular testosterone.

Patient 3 was a product of a normal delivery at term (Fig. 2). At age 42 years, she exhibited reversal of IHH, with normal menstrual cycles after stopping hormonal therapy. The patient’s eldest daughter presented at 14 years with primary amenorrhea and Tanner III breast development, whereas her younger twin daughters were started on estrogen therapy at 13 years for absent puberty (Fig. 2). The diagnosis of nIHH was confirmed in her daughters when they failed to resume menses after discontinuation of hormonal therapy at age 18. Patient 2’s two paternal aunts also have nIHH, and her niece has hypotalamic amenorrhea (Fig. 2).

Genetic analysis of this patient identified a heterozygous mutation in FGFR1 (p.L299T) and a heterozygous variant in PROKR2 (p.S202G) (Table 1 and Fig. 2). The patient's twin daughters and niece do not carry the PROKR2 variant, and one of the patient's daughters carries the FGFR1 mutation (Fig. 2). Patient 3. Patient 3 was a product of a normal delivery at term after a benign pregnancy. He had descended testes at birth, but testicular biopsy performed at age 22 years, 11 months, showed small seminiferous tubules and complete absence of Leydig cells, consistent with gonadotropin deficiency. Bone age was 17 years. Subsequent treatment with pulsatile GnRH therapy for >1 year resulted in normalization of serum testosterone levels, elevated gonadotropin levels, and increase in testicular size to 5–6 mL bilaterally.

Patient 4. Patient 4 was born to Chinese/Cambodian parents who were first cousins (Fig. 2), and he had a small penis at birth. He was evaluated at 16 years for absence of secondary sexual characteristics. Treatment with escalating doses of intramuscular testosterone enanthate induced development of secondary sexual characteristics. At 26 years, testes were 3 mL bilaterally. Smell testing with the University of Pennsylvania Smell Identification Test revealed a normal sense of smell. The patient also has progressive visual loss that started at 9 years because of retinitis pigmentosa.

Patient 5. Patient 5’s detailed medical and family history has been reported in ref. 26. The patient and his nephew are hemizygous for a frameshift mutation in NR0B1 (also called DAX1) (Table 1 and Fig. 2) and consequently have X-linked adrenal hypoplasia congenita, with primary adrenal insufficiency and nIHH. The patient’s mother, sister, and niece had delayed puberty and are heterozygous for the NR0B1/DAX1 mutation (Fig. 2 and Table 1).

**Sequence Changes in GNRH1.** GNRH1 consists of 3 coding exons (Fig. 3), which encode a 92-aa preprohormone that is processed to produce the GnRH decapeptide and the GnRH-associated peptide (GAP) (1). DNA from 310 patients with nIHH and 192 control subjects underwent sequencing of the GNRH1 exons and exon–intron boundaries. A homozygous frameshift mutation ([c.87delA] + [c.87delA], [p.G29GfsX12] + [p.G29GfsX12]) was identified in patient 1 (Table 1 and Fig. 3). This change lies in the codon encoding the sixth amino acid of the GnRH decapeptide and is predicted to alter all amino acids C-terminal to this residue, with premature termination after 11 aa (Fig. 3).

Four heterozygous sequence variants not seen in control subjects were found in nIHH patients (Table 1 and Fig. 3). Patient 2 has a rare variant resulting in an amino-acid substitution in the eighth residue of the GnRH decapeptide ([c.91C>T] + [=], [p.R31C] + [=]) (Table 1 and Fig. 3). The same heterozygous change in GNRH1 was found in the patient’s twin daughters and niece (Fig. 2). Patient 3 carries a heterozygous rare variant ([c.217C>T] + [=], [p.R73X] + [=]) that causes premature termination in the GAP region of the GnRH precursor (Table 1 and Fig. 3). This change was also found in his sister (Fig. 2). Patient 4 is heterozygous for the variant ([c.172A>T] + [=], [p.T58S] + [=]), which causes an amino acid substitution in a nonconserved residue in the GAP region.

Patient 5 carries a heterozygous rare variant ([c.52G>A] + [=], [p.V18M] + [=]) that alters an amino acid in the signal peptide. This amino acid change is not predicted to alter signal peptide recognition or cleavage. The patient’s mother, sister, and niece also carry this variant (Fig. 2).

SNP rs6185 was seen in 135 (22%) of the 620 patient alleles and 133 (35%) of the 384 control alleles; the reported allele frequency of this SNP is 18–30% in Caucasians and 52–61% in Asians.
Asians. One patient and one control subject were found to be heterozygous for SNP rs6186, which has an allele frequency of 1–4%.

Discussion

We have identified a homozygous frameshift mutation in GNRH1 in a patient with severe nIHH. This establishes GNRH1 as a cause of nIHH. We also identified rare heterozygous sequence variants in GNRH1 in 4 nIHH patients of 310 patients screened.

The frameshift mutation in patient 1 is unquestionably null because the C terminus of GnRH is essential for its function (reviewed in ref. 27). Patient 1’s severe phenotype is consistent with complete loss of GnRH activity. His minimal rise in gonadotropin or pulsatile GnRH therapy to achieve Leydig cell maturation and normalization of serum testosterone (33). Thus, his Leydig cell dysfunction could be a result of his cryptorchidism or his hypogonadotropism during development, because patients with severe nIHH require months of gonadotropin or pulsatile GnRH therapy to achieve Leydig cell maturation and normalization of serum testosterone (33). Thus, his poor response to hCG does not necessarily indicate a direct function of GnRH in the testes. To the contrary, his testicular biopsy did not reveal any obvious abnormalities.

The R31C change in patient 2 alters the eighth amino acid of the GnRH decapeptide. Substitutions of histidine, glutamine, leucine, serine, tyrosine, or tryptophan at this position markedly reduce the ability of GnRH to bind and activate mammalian GnRH receptors (34, 35). Lu et al. (35) propose that the human GnRH receptor has evolved to specifically recognize this arginine residue, and they have identified a residue in the human GnRH receptor (Asn7.45) that confers this specificity. Thus, the R31C change is likely to cause significant loss of GnRH function. The patient’s pedigree suggests an autosomal dominant mode of inheritance, with individuals from 3 generations with nIHH. Two of the patient’s daughters (DNA was not available for her eldest daughter) and her niece are all heterozygous for the R31C mutation, consistent with the possibility that the mutation is responsible for their reproductive phenotypes. However, the reproductive phenotypes in this family appear somewhat less severe, because patient 2 exhibited reversal of IHH, and the patient’s niece had hypothalamic amenorrhea, with her reproductive phenotype becoming apparent only in the presence of an external stressor.

The R73X variant in patient 3 causes truncation of the GAP but is predicted to leave the GnRH decapeptide intact. It is possible that the resulting transcript is rapidly degraded, although the proximity of the premature stop codon to a splice junction may allow for escape from nonsense-mediated decay (36). Thus, the effect of this variant on GnRH synthesis is unclear. Nevertheless, this patient’s pedigree suggests an autosomal dominant inheritance pattern with variable expressivity, because his father had the milder reproductive phenotype of delayed puberty. The patient’s father is inferred to have carried the R73X mutation; the fact that the patient’s sister carries the mutation, consistent with the possibility that the mutation is responsible for their reproductive phenotypes in this family appear somewhat less severe, because patient 2 exhibited reversal of IHH, and the patient’s niece had hypothalamic amenorrhea, with her reproductive phenotype becoming apparent only in the presence of an external stressor.

The T58S change in patient 4 lies in a residue in the GAP region of the prohormone that is not conserved across species, and it is unclear whether this conservative amino acid substitution disrupts GnRH synthesis or function. Also, the consanguinity of his parents implies a recessive mode of inheritance of his
disease. Furthermore, it is unclear how a mutation in GNRH1 could contribute to the patient’s retinitis pigmentosa, although it is intriguing that GNRH-immunoreactive fibers have been observed in the mammalian retina (37).

The V18M variant in patient 5, which lies in the signal peptide, is not predicted to alter signal peptide function. Furthermore, his nIHH and adrenal insufficiency are readily attributable to his frameshift mutation in NR0B1/DAX1 (26). Nevertheless, it is notable that 3 female carriers of the NR0B1/DAX1 mutation in patient 5’s family had delayed puberty (26) and are also heterozygous for the GNRH1 variant. This raises the possibility that the GNRH1 variant contributed to their reproductive phenotypes.

How might heterozygous variants in GNRH1 cause or contribute to nIHH? It is possible that our screening strategy failed to identify mutations in other regions, such as transcriptional regulatory elements, that disrupt the function of the seemingly unaffected GNRH1 allele. It is also possible that the heterozygous mutations in GNRH1 do not contribute to the pathogenesis of nIHH and that only the homozygous frameshift mutation is causal. However, there is clear precedent for association of nIHH/KS with heterozygous mutations in FGF8 and PROK2, which also encode secreted ligands (9, 10, 14, 15). Another possibility is that heterozygous GNRH1 mutations act in conjunction with mutations in other genes to cause nIHH, a mechanism that has been observed for several other nIHH/KS genes (15, 38–40). It is also possible that some mutations in GNRH1 have dominant effects. One potential mechanism is exemplified by mutations in AVP that cause autosomal dominant neurohypophyseal diabetes insipidus because of neurotoxicity of the mutant gene products (reviewed in ref. 41); mutations in GNRH1 may have similar effects. In particular, the R31C mutation in patient 2 could potentially form inappropriate disulfide bonds, resulting in misfolded peptides or aggregates that interfere with the function of the endoplasmic reticulum and cause GnRH neuronal dysfunction.

GNRH1 is an obvious candidate gene for nIHH, so why have mutations in GNRH1 not been identified to date? One possibility is that functional mutations in genes encoding ligands arise less frequently than in genes encoding receptors because of differences in size between ligands and their cognate receptors. Encoding a peptide product of only 92 aa, GNRH1 represents a smaller “target” for mutation than the 328 aa encoded by GNRH. Indeed, for other ligand–receptor pairs implicated in nIHH/KS, fewer mutations have been reported in genes encoding ligands (FGF8 and PROK2) than in genes encoding receptors (FGFR1 and PROKR2; refs. 9, 10, and references therein). An alternative explanation for the rarity of GNRH1 mutations is that they are rapidly eliminated from the population. This could occur from inability to transmit mutations to future generations, as would be expected from mutations that cause a reduction in fertility. The expression of GNRH1 in the placenta (29) raises the possibility that mutations may cause lethality, which would also lead to elimination of mutations from the population, although

again patient 1’s normal gestation argues against an essential role for zygotic GNRH in the placenta.

Our study fills a long-standing gap in the genetics of nIHH. The finding of a homozygous mutation in a patient with nIHH firmly establishes mutations in GNRH1 as a rare cause of nIHH. Furthermore, the discovery of heterozygous GNRH1 changes in patients with familial nIHH suggests that mutations in GNRH1 may also act in a dominant fashion. These patients offer a rare opportunity to study the effects of human GnRH deficiency throughout the life cycle and may provide insight into functions of GnRH outside the hypothalamus.

Methods

Patients. All studies were approved by the Institutional Review Board (IRB) of MGH, and informed consent was obtained from all patients enrolled in the study. Participants in the study were either evaluated clinically by the Reproductive Endocrine Associates of MGH or were referred directly by their physicians to participate in our genetic studies. The diagnosis of nIHH was based on the absence of spontaneous puberty and low sex steroids (testosterone, ≤3.4 nmol/L; estradiol, ≤73 pmol/L) in the setting of normally normal or low gonadotropin levels and an intact sense of smell. Additional evidence for the diagnosis of nIHH was provided by the following: (i) absence of LH pulses during 12–24 h of blood sampling every 10 min (see SI Methods); (ii) normal basal and stimulated levels of thyroid-stimulating hormone, prolactin, growth hormone, and cortisol; and (iii) absence of abnormalities on imaging of the hypothalamic-pituitary region. Whenever possible, patients were interviewed by both a clinical investigator and a genetics counselor by using an IRB-approved questionnaire so that a full family pedigree could be obtained. Control subjects were also evaluated at MGH with detailed histories and physical examinations.

Of the 310 patients with nIHH screened, 212 were Caucasian, 12 African-American, 23 Asian, 1 Native American, 3 mixed race, and 59 not assessed for ethnicity. Of the 192 control subjects, 154 were Caucasian, 34 Asian, 2 Native Hawaiian/Pacific Islander, and 2 mixed race.

Sequence Analysis. Genomic DNA was extracted from peripheral blood leukocytes or cultured white blood cells. All exons of GNRH1 and 50 bp of intronic DNA flanking each exon were sequenced by Polymorphic DNA Technologies. Sequence variants found in patients but not controls were verified by amplification of the GNRH1 coding region by PCR by using Taq polymerase (Fisher Scientific) under standard conditions with the following primers: for exon 1, 5′-CCTGACTCTCAGTCCTG-3′ and 5′-GCTTCATCTACCATGGACG-3′ (annealing temperature, 60 °C); for exon 2, 5′-CTCGAATCTCGCAAGTCC-3′ and 5′-GAGGAGTCTAGGAAATGACG-3′ (annealing temperature, 55 °C); and for exon 3, 5′-CTATGACTCTGAGTACGG-3′ and 5′-GTGCAATACGGTAGTGAAGT-3′ (annealing temperature, 49 °C). PCR products were purified by using the QiAquick PCR Purification kit (Qiagen) and sequenced with the same primers by the MGH DNA Sequencing Core. FGFR1 and PROKR2 were sequenced as described in refs. 42 and 43, respectively. Signal peptide recognition and cleavage were predicted by using SignalP 3.0 (44) and Sig-Pred (45).

Note Added in Proof. Bougland et al. (46) recently reported a homozygous frameshift mutation in two siblings with IHH.

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