

ORIGINAL ARTICLE

A Genetic Basis for Functional Hypothalamic Amenorrhea

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ABSTRACT

BACKGROUND

Functional hypothalamic amenorrhea is a reversible form of gonadotropin-releasing hormone (GnRH) deficiency commonly triggered by stressors such as excessive exercise, nutritional deficits, or psychological distress. Women vary in their susceptibility to inhibition of the reproductive axis by such stressors, but it is unknown whether this variability reflects a genetic predisposition to hypothalamic amenorrhea. We hypothesized that mutations in genes involved in idiopathic hypogonadotropic hypogonadism, a congenital form of GnRH deficiency, are associated with hypothalamic amenorrhea.

METHODS

We analyzed the coding sequence of genes associated with idiopathic hypogonadotropic hypogonadism in 55 women with hypothalamic amenorrhea and performed in vitro studies of the identified mutations.

RESULTS

Six heterozygous mutations were identified in 7 of the 55 patients with hypothalamic amenorrhea: two variants in the fibroblast growth factor receptor 1 gene *FGFR1* (G260E and R756H), two in the prokineticin receptor 2 gene *PROKR2* (R85H and L173R), one in the GnRH receptor gene *GNRHR* (R262Q), and one in the Kallmann syndrome 1 sequence gene *KAL1* (V371I). No mutations were found in a cohort of 422 controls with normal menstrual cycles. In vitro studies showed that *FGFR1* G260E, *FGFR1* R756H, and *PROKR2* R85H are loss-of-function mutations, as has been previously shown for *PROKR2* L173R and *GNRHR* R262Q.

CONCLUSIONS

Rare variants in genes associated with idiopathic hypogonadotropic hypogonadism are found in women with hypothalamic amenorrhea, suggesting that these mutations may contribute to the variable susceptibility of women to the functional changes in GnRH secretion that characterize hypothalamic amenorrhea. Our observations provide evidence for the role of rare variants in common multifactorial disease. (Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development and others; ClinicalTrials.gov number, NCT00494169.)

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REPRODUCTION IS AN ENERGETICALLY costly process for women, and defense mechanisms have evolved that temporarily inhibit reproduction under adverse conditions. Stressors such as weight loss,¹ excessive exercise,² eating disorders,³ and psychological distress⁴ suppress the hypothalamic–pituitary–gonadal axis by inhibiting hypothalamic pulsatile secretion of gonadotropin-releasing hormone (GnRH).⁵ This frequent cause of female infertility is diagnosed as functional hypothalamic amenorrhea, defined as the absence of menses, low or normal gonadotropin levels, and hypoestrogenemia without organic abnormality.⁶ Hypothalamic amenorrhea is associated with a spectrum of abnormal GnRH-secretion patterns, and administration of exogenous pulsatile GnRH can restore functionality of the hypothalamic–pituitary–gonadal axis.⁷ The exquisite sensitivity of the GnRH pulse generator to energy deficits is evidenced by the fact that serum levels of leptin, a signal of fat reserves, are often low in patients with hypothalamic amenorrhea and that leptin replacement can restore GnRH pulsatility.^{8–10} After the underlying stressors have been eliminated, normal reproductive function resumes in most cases.¹¹ Among both women and female nonhuman primates, sensitivity to the inhibition of the hypothalamic–pituitary–gonadal axis by such stressors varies substantially.^{12,13} However, it is unknown whether this susceptibility reflects a genetic predisposition to hypothalamic amenorrhea.

Much is known about the genetics of congenital GnRH deficiency (idiopathic hypogonadotropic hypogonadism), in contrast to hypothalamic amenorrhea. Idiopathic hypogonadotropic hypogonadism is characterized by an absence of puberty and by infertility, caused by defects in the secretion of GnRH from the hypothalamus or defects in the action of GnRH on the pituitary.^{14,15} The disease is genetically heterogeneous, with several associated loci that account for approximately 40% of cases.¹⁵ The involved genes encode proteins essential for GnRH neuron development and GnRH secretion and action.^{16–18} The variable expressivity of the clinical features of GnRH deficiency most likely reflects the contributions of multiple genetic defects or epigenetic perturbations. We hypothesized that mutations in genes involved in idiopathic hypogonadotropic hypogonadism confer susceptibility to the functional deficiency in GnRH secretion that characterizes hypothalamic amenorrhea.

METHODS

STUDY PARTICIPANTS

All participants provided written informed consent.

Controls

Controls were 422 women from the greater Boston area, recruited by means of advertising, who had undergone normal puberty (menarche at ≥ 10 but < 15 years), had had a normal menstrual cycle (27 to 32 days' duration) for the previous 2 years, and had a body-mass index (BMI, the weight in kilograms divided by the square of the height in meters) between 18 and 35. No predisposing factors for hypothalamic amenorrhea were reported for 375 of the 422 women; the remaining 47 exercised for more than 5 hours per week, which is a predisposing factor.

Patients with Hypothalamic Amenorrhea

Hypothalamic amenorrhea was diagnosed in 55 women presenting to the Massachusetts General Hospital or Newcastle upon Tyne Hospital with a history of secondary amenorrhea for 6 months or more, low or normal gonadotropin levels, low serum estradiol levels, and one or more predisposing factors. These factors included excessive exercise (> 5 hours per week),¹⁹ loss of more than 15% of body weight, and a subclinical eating disorder as ascertained with the use of the Eating Attitudes Test.²⁰ None of the patients met the diagnostic criteria for anorexia nervosa at presentation.²¹

All 55 patients with hypothalamic amenorrhea had completed puberty spontaneously. The mean (\pm SD) age at diagnosis was 22.4 ± 6.1 years, and the mean BMI was 19.4 ± 2.2 . The mean age at menarche was 13.5 ± 1.8 years, with 13 patients reporting delayed menarche (age at onset, ≥ 15 years) at a time when no factors predisposing them to hypothalamic amenorrhea were present. Twenty-five patients reported exercising excessively, 20 had weight loss, and 28 had a subclinical eating disorder characterized by dietary restriction and preoccupation with weight.^{20,21} Six patients had a family history of delayed puberty, and 9 a family history of hypothalamic amenorrhea. The mean serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol in the group with hypothalamic amenorrhea were 4.1 ± 3.0 IU per liter, 6.7 ± 3.3 IU per liter, and 39 ± 25 pg per milliliter (143 ± 92 pmol per liter), respectively. All patients had normal results on neuroimaging, and none had symptoms or

biochemical signs of the polycystic ovary syndrome (hirsutism, acne, hyperandrogenemia, or an LH-to-FSH ratio >1). A subgroup of the patients underwent LH-secretion studies involving blood sampling every 10 minutes over a 24-hour period.^{11,22}

Patients with Idiopathic Hypogonadotropic Hypogonadism

We evaluated 160 women with idiopathic hypogonadotropic hypogonadism. All had absent or incomplete puberty as of 18 years of age, low or normal serum gonadotropin levels, low serum estradiol levels, otherwise normal anterior pituitary function, and normal results on neuroimaging.

GENETIC STUDIES

Genomic DNA was extracted from peripheral-blood samples obtained from all participants. Exonic and proximal intronic sequences (located at least 15 bp from the splice sites) of seven genes implicated in the cause of idiopathic hypogonadotropic hypogonadism were determined in all samples from patients with hypothalamic amenorrhea: the Kallmann syndrome 1 sequence gene *KAL1*,²³ the GnRH receptor gene *GNRHR*,²⁴ the G protein-coupled receptor 54 gene *GPR54*,²⁵ the fibroblast growth factor receptor 1 gene *FGFR1*,²⁶ the fibroblast growth factor 8 gene *FGF8*,²⁷ the prokineticin 2 gene *PROK2*,²⁸ and the prokineticin receptor 2 gene *PROKR2*.²⁹ Sequence variations were found on both strands and were confirmed in separate polymerase-chain-reaction assays. No rare sequence variants were found in a cohort of 422 controls with normal menstrual cycles.

FUNCTIONAL CHARACTERIZATION OF NEW *FGFR1* AND *PROKR2* MUTATIONS

Gene-Reporter Assays

The *FGFR1* G260E and R756H mutations and the *PROKR2* R85H mutation were introduced into previously described expression vectors by means of a QuickChange XLII Kit (Stratagene)^{28,29} and were studied in L6 myoblasts and human embryonic kidney (HEK) 293 cells, respectively. The ability to activate downstream signaling was compared between the mutated receptor and its wild-type counterpart in transient transfection assays, as previously described.^{29,30} The osteocalcin fibroblast growth factor (FGF) response element (OCFRE) reporter and the murine early growth response 1 (Egr-1) reporter served as indicators of *FGFR1*-induced and *PROKR2*-induced

mitogen-activated protein kinase (MAPK) signaling, respectively. Each assay was performed twice in triplicate. Four-parameter sigmoidal dose-response curves were generated and analyzed with the use of Prism 4 statistical software (GraphPad).

Total Expression and Cell-Surface–Receptor Expression

Total expression of wild-type and mutant *FGFR1* and *PROKR2* transfected into COS-7 cells was determined by means of Western blotting involving whole-cell extracts, as previously described.^{29,30} The cell-surface expression of *FGFR1* was quantified as previously described,³⁰ and the cell-surface expression of *PROKR2* was also quantified, with the use of a 3xHA tag sequence fused with the N-terminal end of *PROKR2* to facilitate recognition by the antibody (Clone HA-7, Sigma). Antibody-binding assays were performed three times in quadruplicate. Expression levels of mutant and wild-type receptors were compared by means of Student's two-tailed t-test.

RESULTS

Heterozygous mutations in genes associated with idiopathic hypogonadotropic hypogonadism (*FGFR1*, *PROKR2*, *GNRHR*, and *KAL1*) were identified in 7 of the 55 patients with hypothalamic amenorrhea (13%; 95% confidence interval, 5 to 24) (Fig. 1 and Table 1). All 7 patients were white, as reported by study physicians. These genetic variants were absent among the controls (the 375 women without risk factors for hypothalamic amenorrhea and the 47 women who exercised >5 hours per week). The variants alter amino acids that are highly conserved across species (Fig. 2C, 2D, and 2E) and cause considerable loss of function,^{29,31,32} findings that are consistent with disease-associated mutations.

FGFR1 mutants (G260E and R756H) in the ligand domain and tyrosine kinase domain of the receptor, respectively, were found in Patients 1 and 2. The *FGFR1* G260E and R756H mutants showed expression levels similar to those of wild-type *FGFR1*, both overall (Fig. 2F and 2G) and on the cell surface (Fig. 2I and 2J). However, results of a transcriptional assay show that the G260E and R756H mutants result in loss of function, as demonstrated by a decrease in FGF-induced MAPK reporter activity (reflected by OCFRE activity) ($P < 0.001$) (Fig. 2L and 2M).

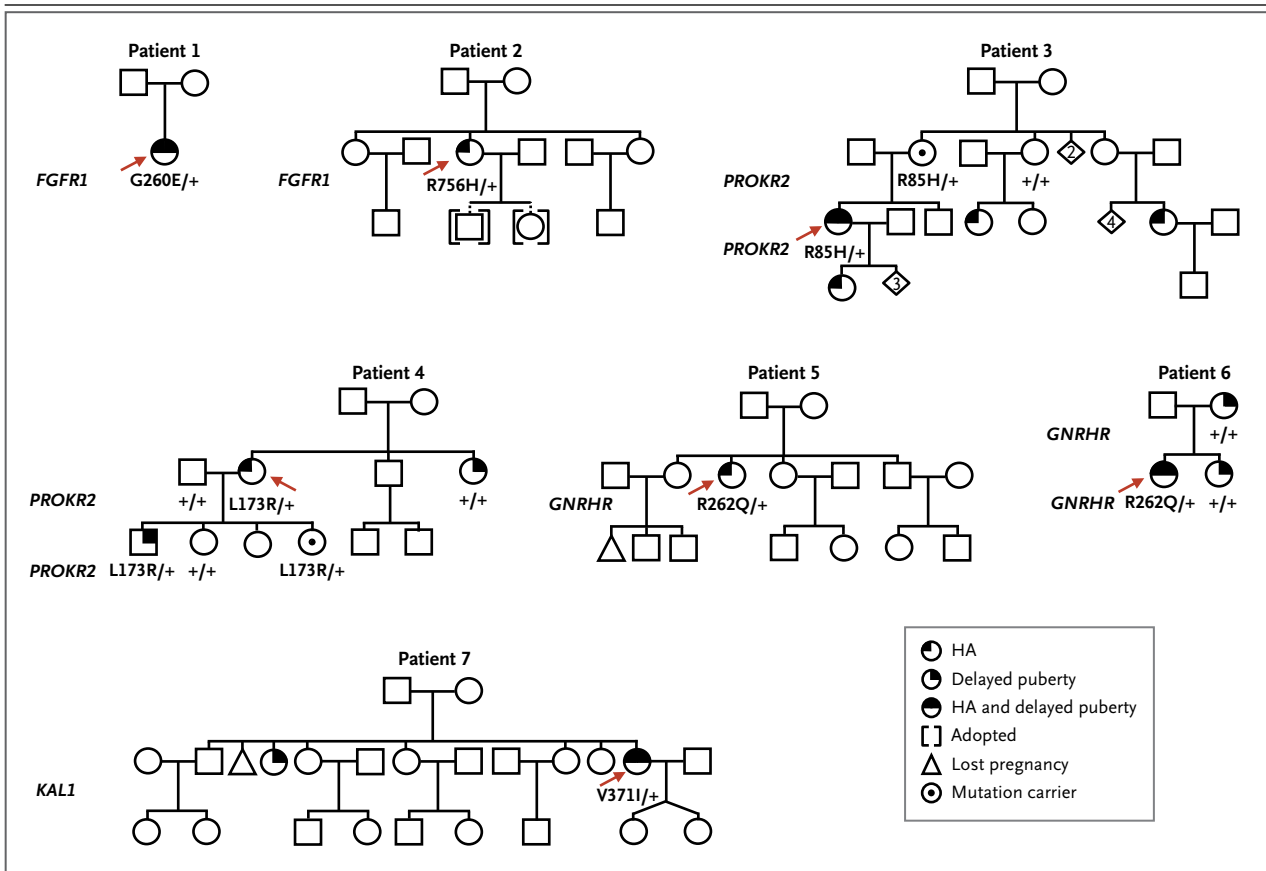


Figure 1. Pedigrees of the Seven Patients with Hypothalamic Amenorrhea (HA) Found to Have Mutations.

For each pedigree, the patient with the mutation is indicated by a red arrow. The mutated gene is indicated in bold to the left of the pedigree, and the allele status is given below the proband or affected family members, with plus signs indicating wild-type and G260E, R756H, R85H, L173R, R262Q, and V371I indicating the amino acid mutations. Squares indicate male family members, circles female family members, and diamonds offspring whose sex is not shown (with the numbers of persons given within the diamond).

The *PROKR2* mutant R85H identified in Patient 3 is also a loss-of-function mutant, as evidenced by decreased overall and cell-surface expression and decreased signaling activity as compared with wild-type *PROKR2* ($P < 0.001$ for cell-surface expression and signaling activity) (Fig. 2H, 2K, and 2N).³¹ The *PROKR2* L173R mutant seen in Patient 4 and the *GNRHR* R262Q mutant in Patients 5 and 6 have previously been reported as loss-of-function mutants (Table 2).^{29,31,32} Finally, characteristics of the *KAL1* V371I mutant in Patient 7 could not be assessed (Table 2) owing to the scarcity of *in vitro* functional assays available for *KAL1*.

All seven patients with hypothalamic amenorrhea who had mutations had secondary amenorrhea for at least 6 months and at least one risk factor for hypothalamic amenorrhea (Table 2).

Four of the seven reported a family history of hypothalamic amenorrhea or delayed puberty (Fig. 1 and Table 2). Among the seven patients, the age at diagnosis ranged from 18 to 34 years (mean, 24.4 ± 6.2), and the BMI at diagnosis ranged from 18 to 22 (mean, 19.4 ± 1.9). Four of the seven patients had attempted to conceive; three of the attempts were successful, with one patient conceiving without assisted reproductive treatment. Two of the seven patients continued to receive long-term hormone-replacement therapy. The other five discontinued hormonal therapy and had recovery of menses. A more detailed summary of the seven patients is given in the Supplementary Appendix (available with the full text of this article at NEJM.org).

Our patients with hypothalamic amenorrhea who had the *PROKR2* R85H or *FGFR1* R756H mu-

Table 1. Frequency of Loss-of-Function Mutations in the Study Participants, According to Group.

Mutation	In Vitro Functionality*	Patients with Hypothalamic Amenorrhea (N=55)	Patients with Idiopathic Hypogonadotropic Hypogonadism (N=160)	Controls Who Were Menstruating but Not Exercising (N=375)	Controls Who Were Menstruating and Exercising >5 Hr/ Wk (N=47)
FGFR1					
R756H	Decreased	1	0	0	0
G260E	Decreased	1	0	0	0
PROKR2					
R85H	Decreased	1	0	0	0
L173R	Decreased	1	5	0	0
GNRHR R262Q	Decreased	2	3	0	0
KAL1 V371I	Not assessed	1	0	0	0

* The functional activity of the FGFR1 R756H and G260E mutants and the PROKR2 R85H mutant was assessed in this study. The functional activity of PROKR2 L173R was evaluated by Cole and colleagues²⁹ and Monnier and colleagues.³¹ The GNRHR R262Q mutant was studied by de Roux and colleagues.³²

tation also had abnormal patterns of endogenous GnRH-induced LH secretion (Fig. 3).

DISCUSSION

We found genetic defects in several patients with hypothalamic amenorrhea. The affected genes play fundamental roles in GnRH ontogeny and function: *GNRHR* encodes the unique receptor that is activated by gonadotropin-releasing hormone 1 (GnRH1) in the pituitary³³; *KAL1* and *PROKR2* are critical for the migration of GnRH-secreting neurons^{34,35}; and *FGFR1* controls the fate specification, migration, and survival of GnRH-secreting neurons.¹⁷ In humans, mutations in these genes underlie severe congenital GnRH deficiency (idiopathic hypogonadotropic hypogonadism).¹⁵ In fact, the *GNRHR* R262Q mutation and the *PROKR2* R85H and L173R mutations described here have previously been associated with idiopathic hypogonadotropic hypogonadism.^{29,31,32,36,37}

Patients who had hypothalamic amenorrhea as well as the *PROKR2* R85H or *FGFR1* R756H mutation in our study were also shown to have abnormal patterns of endogenous GnRH-induced LH secretion, as previously described in women with hypothalamic amenorrhea.⁷ We speculate that decreased *PROKR2* or *FGFR1* signaling leads to a partially compromised GnRH neuronal network owing to a smaller-than-normal number of GnRH-producing cells that have successfully

completed embryonic migration to the hypothalamus, a suboptimal maturation of the GnRH network during puberty, or a defective regulation of GnRH secretion — since both proteins are expressed not only during development but also in the adult hypothalamus.^{16,28} This would, in turn, predispose persons to abnormal GnRH secretion under the influence of factors that stress the reproductive system. *FGFR1* and *PROKR2* signaling also modifies eating behavior in mice.^{38,39} Thus, we speculate that genetic defects in these pathways may also contribute to the abnormal eating patterns seen in many patients with hypothalamic amenorrhea.

We found that genes mutated in patients with idiopathic hypogonadotropic hypogonadism also are mutated in those with hypothalamic amenorrhea. This finding expands our understanding of the genetics of GnRH-deficiency disorders. Idiopathic hypogonadotropic hypogonadism was traditionally considered a genetically determined, congenital, and lifelong form of GnRH deficiency. However, as many as 10% of patients with idiopathic hypogonadotropic hypogonadism resume normal reproductive function after treatment is discontinued, even if they have genetic defects.⁴⁰ This reversal of idiopathic hypogonadotropic hypogonadism indicates the plasticity of the GnRH network and its sensitivity to nongenetic factors. Conversely, idiopathic hypogonadotropic hypogonadism occasionally is present in

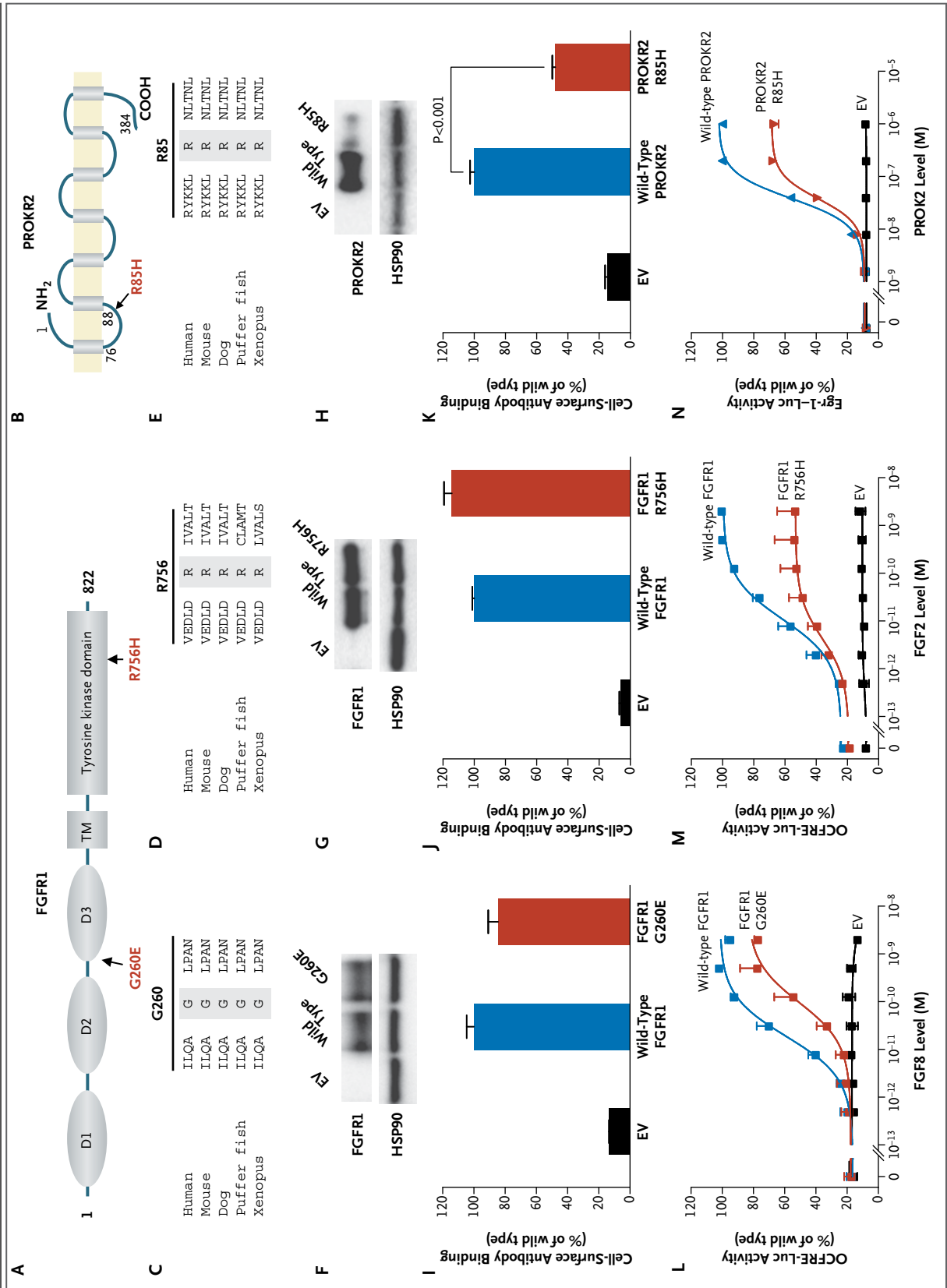


Figure 2 (facing page). Loss-of-Function Mutations in Patients with Hypothalamic Amenorrhea.

Panels A and B show the structures of FGFR1 and PROKR2, respectively, as well as the mutations of interest. Panels C, D, and E show that the FGFR1 G260 and R756 amino acids and the PROKR2 R85 amino acid are highly conserved across vertebrate species. Panels F, G, and H show that the overall expression levels of FGFR1 G260E and R756H were normal, whereas PROKR2 R85H expression levels were decreased ($P < 0.01$), as compared with wild-type levels. Heat-shock protein 90 (HSP90) was a positive control for gel loading. An empty vector (EV) was used as a negative control for protein expression. Panels I, J, and K show that the receptor cell-surface expression levels in COS-7 cells were similar to the wild-type levels for both FGFR1 mutants but were significantly decreased for PROKR2 R85H ($P < 0.001$). Panels L, M, and N show that the FGFR1 G260E mutant has decreased fibroblast growth factor 8 (FGF8)-induced osteocalcin FGF response element (OCFRE) activity as compared with the wild type ($P < 0.001$), that the FGFR1 R756H mutant has decreased FGF2-induced OCFRE activity as compared with the wild type ($P < 0.001$), and that the PROKR2 R85H mutant has decreased PROKR2-induced early growth response 1 (Egr-1) activity as compared with the wild type ($P < 0.001$). D1, D2, and D3 denote the ligand-binding domains of FGFR1; Luc the luciferase reporter vector; and TM the transmembrane domain of FGFR1. T and I bars indicate standard errors of the means of two experiments performed in triplicate for the gene-reporter assays or the means of three experiments performed in quadruplicate for the antibody-binding assays.

adult men (in which case it is called adult-onset idiopathic hypogonadotropic hypogonadism).⁴¹ Some men with the disease who carry mutations in genes underlying idiopathic hypogonadotropic hypogonadism²⁷ have normal reproductive function before onset, suggesting that their GnRH deficiency results from a combination of genetic and environmental influences.

In contrast to idiopathic hypogonadotropic hypogonadism, hypothalamic amenorrhea has been traditionally viewed as a functional form of GnRH deficiency resulting from insufficient energy availability, psychological stress, or both. Studies of humans and nonhuman primates have shown that stress can induce reproductive dysfunction, including amenorrhea, in many, although not all, females with normal menstrual cycles.^{12,13,19} Our findings may help to explain the variable susceptibility of women to inhibition of the hypothalamic-pituitary-gonadal axis. Since patients with mutations resumed regular menses after discontinuing hormone-replace-

ment therapy, the genetic component of hypothalamic amenorrhea predisposes one to, but is not sufficient to cause, GnRH deficiency.

If hypothalamic amenorrhea has a genetic basis partially in common with idiopathic hypogonadotropic hypogonadism, what are the factors that ultimately generate these divergent clinical phenotypes? The total load of mutations in genes related to GnRH ontogeny and action might be less in hypothalamic amenorrhea than in idiopathic hypogonadotropic hypogonadism. Patients with idiopathic hypogonadotropic hypogonadism frequently show homozygosity and compound heterozygosity for mutations at the disease-causing loci.^{25,32,36,42} Furthermore, digenic inheritance of mutations associated with idiopathic hypogonadotropic hypogonadism (FGFR1 in combination with GNRHR, NELF [the gene encoding the nasal embryonic LH-releasing hormone factor], or PROKR2, or other digenic pairs) has been reported.^{30,36,37,43}

All six mutations associated with hypothalamic amenorrhea in our study were heterozygous. We speculate that such heterozygous mutations, while not sufficient to cause idiopathic hypogonadotropic hypogonadism, could set a lower threshold for functional inhibition of the hypothalamic-pituitary-gonadal axis under adverse hormonal, nutritional, or psychological conditions and thereby lead to hypothalamic amenorrhea. Such a lower threshold for inhibition might also confer a selective advantage to female carriers during famine, helping to balance survival against the metabolic needs of pregnancy. This explanation would be consistent with the presence of mutations associated with idiopathic hypogonadotropic hypogonadism and hypothalamic amenorrhea in persons who do not have symptoms. The PROKR2 L173R mutation appears to be an example: it has been reported in more than a dozen patients with idiopathic hypogonadotropic hypogonadism, mostly in the heterozygous state, and in many of these cases was inherited from an asymptomatic parent.^{9,31,36,37}

To elucidate the genetics of hypothalamic amenorrhea more completely, it will be important to undertake comprehensive sequencing of the genes associated with idiopathic hypogonadotropic hypogonadism in larger cohorts of patients with hypothalamic amenorrhea. Twenty-five percent of women with hypothalamic amenorrhea in our study had a history of delayed

Table 2. Results of Clinical and Genetic Studies in the Seven Patients with Hypothalamic Amenorrhea with Rare Variants in Genes Associated with Idiopathic Hypogonadotropic Hypogonadism.*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Clinical characteristics							
Age (yr)							
At menarche	16	13.5	15	12	14	16.5	15
At diagnosis of hypothalamic amenorrhea	19	28	26	18	34	18	28
BMI at diagnosis†	18.5	17	19	18	22	22	19
Predisposing factors							
Weight loss	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Subclinical eating disorder	No	Yes	Yes	Yes	No	No	Yes
Excessive exercise	No	Yes	No	No	No	No	Yes
Fertility status	No attempt at conception	Failed to conceive while receiving GnRH therapy	Conceived while receiving pulsatile GnRH therapy	Conceived without therapy	No attempt at conception	No attempt at conception	Conceived while receiving gonadotropin therapy
Recovery of menses	NA	Yes	Yes	Yes	Yes	NA	Yes
Family history of hypothalamic amenorrhea	No	No	Yes	No	No	Yes	Yes
Genetic and functional characteristics							
Gene and variant identified	FGFR1 G260E	FGFR1 R756H	PROKR2 R85H	PROKR2 L173R	GNRHR R262Q	GNRHR R262Q	KALL1 V371I
Overall protein expression‡	Similar to wild type	Similar to wild type	Decreased	Decreased§	NA	NA	NA
Cell-surface expression‡	Similar to wild type	Similar to wild type	Decreased	Decreased§	NA	NA	NA
Signaling activity‡	Decreased	Decreased	Decreased	Decreased§	Decreased¶	Decreased¶	NA

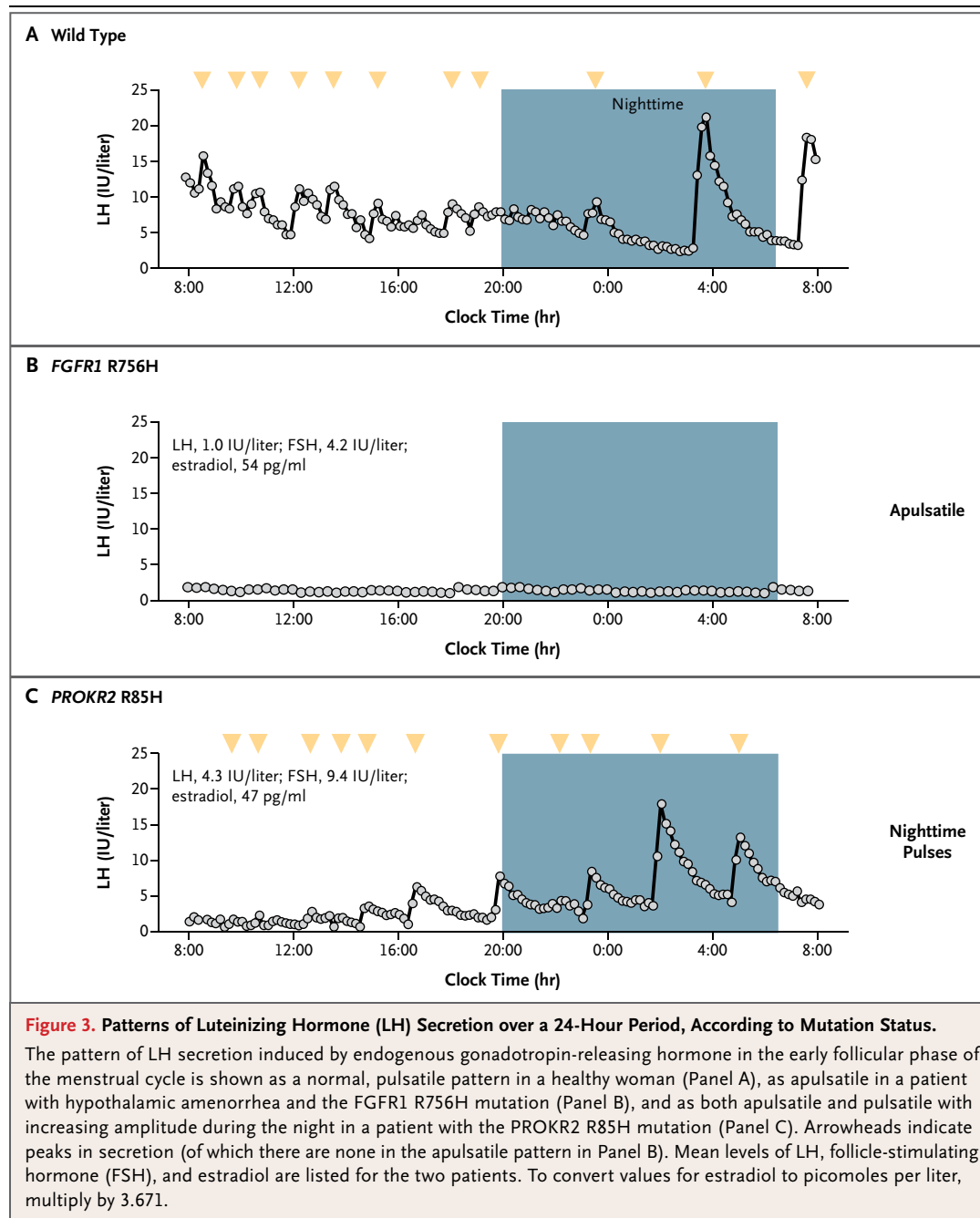
* GnRH denotes gonadotropin-releasing hormone, and NA not assessed.

† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

‡ Protein expression in Patients 5 and 6 was not assessed because of the loss-of-function nature of the mutations; and protein expression and signaling activity in Patient 7 were not assessed owing to the scarcity of in vitro functional assays for KALL1.

§ For Patient 4, overall protein expression, cell-surface-receptor expression, and transcriptional level are based on the studies by Cole and colleagues²⁹ and Monnier and colleagues.³¹

¶ For Patients 5 and 6, transcriptional level is based on the study by de Roux and colleagues.³²



puberty, which is frequently seen in family members of patients with idiopathic hypogonadotropic hypogonadism. It may be worthwhile to investigate whether rare variants in genes underlying idiopathic hypogonadotropic hypogonadism or hypothalamic amenorrhea also contribute to delayed puberty.

Genetic defects within pathways controlling appetite or stress-response systems might also

contribute to hypothalamic amenorrhea. The genetic susceptibility to anorexia or bulimia nervosa has been investigated in several association studies that examined candidate genes such as brain-derived neurotrophic factor, neurotrophic tyrosine kinase receptor types 2 and 3, serotonin, leptin, and hypocretin.^{44,45} Unlike our study, which sought rare genetic variants, the prior studies focused on common DNA polymor-

phisms. The results have been largely inconclusive, most likely owing to small sample sizes, heterogeneity in race and ethnic group, and variation in diagnostic criteria. It might prove more fruitful to investigate whether rare variants in genes associated with the response to stress and starvation also contribute to susceptibility to hypothalamic amenorrhea.

In conclusion, we demonstrated that patients with hypothalamic amenorrhea have mutations in genes regulating GnRH ontogeny and action. Given the limited size of the cohort with hypothalamic amenorrhea, we would not recommend that women with hypothalamic amenorrhea be routinely screened for mutations at loci known to underlie idiopathic hypogonadotropic hypogonadism, except in cases of clear familial in-

heritance of hypothalamic amenorrhea or idiopathic hypogonadotropic hypogonadism. Further elucidation of the genetic basis of hypothalamic amenorrhea and delineation of the relationship among genotype, environment, and phenotype are needed.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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