Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism


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Mutations in KAL1 and FGFR1 cause Kallmann syndrome (KS), whereas mutations in the GNRHR and GPR54 genes cause idiopathic hypogonadotropic hypogonadism with normal olfaction (nIHH). Mixed pedigrees containing both KS and nIHH have also been described; however, the genetic cause of these rare cases is unknown. We examined the FGFR1 gene in seven nIHH subjects who either belonged to a mixed pedigree (n = 5) or who had associated midline defects (n = 2). Heterozygous FGFR1 mutations were found in three of seven unrelated nIHH probands with normal MRI of the olfactory system: (i) G237S in an nIHH female and a KS brother; (ii) P722H and N724K in an nIHH male missing two teeth and his mother with isolated hyposmia; and (iii) Q680X in a nIHH male with cleft lip/palate and missing teeth, his brother with nIHH, and his father with delayed puberty. We show that these mutations lead to receptor loss-of-function. The Q680X leads to an inactive FGFR1, which lacks a major portion of the tyrosine kinase domain (TKD). The G237S mutation inhibits proper folding of D2 of the FGFR1 and likely leads to the loss of cell-surface expression of FGFR1. In contrast, the (P722H and N724K) double mutation causes structural perturbations in TKD, reducing the catalytic activity of TKD. We conclude that loss-of-function mutations in FGFR1 cause nIHH with normal MRI of the olfactory system. These mutations also account for some of the mixed pedigrees, thus challenging the current idea that KS and nIHH are distinct entities.

normosmia | FGFR1

Diopathic hypogonadotropic hypogonadism (IHH) occurs either with anosmia [Kallmann syndrome (KS)] (1) or with a normal sense of smell [normosmic IHH (nIHH)] (2). Although KS and nIHH are thought to represent two distinct entities, there are documented pedigrees containing both KS and nIHH (3, 4). Varying degrees of pubertal development exist in association with IHH, although the majority of patients lack evidence of puberty (5). In addition, nonreproductive phenotypes, such as synkinesia, hearing loss, labial or palatine cleft, and dental anomalies, are commonly reported in KS (6, 7) but can also occur in nIHH (8–10). In our experience, 11% of nIHH probands have associated phenotypes.

Genetic heterogeneity also exists in IHH. Approximately one-third of cases are familial, whereas the remainder are sporadic (11). Genetic defects have been mapped in ~30% of the IHH population: (i) GNRHR and GPR54 mutations underlie nIHH (12–16), and (ii) KAL1 and FGFR1 mutations cause KS. Mutations in KAL1 cause the X-linked form of KS (17, 18) associated with a high frequency of renal anomalies and synkinesia (6), whereas mutations in FGFR1 are one cause of the autosomal dominant form of KS associated with cleft palate and dental agenesis (19–22).

Human FGFR1 is a member of the receptor tyrosine kinase superfamily. FGFR1 signaling regulates cell proliferation, migration, differentiation, and survival and thus is essential for various stages of human development (23). The prototypical FGFR comprises an extracellular region consisting of three Ig-like domains (D1, D2, and D3), a single transmembrane helix, and an intracellular region containing the tyrosine kinase domain (TKD). D2, D3, and the short D2–D3 linker of FGFR house all of the determinants of ligand (FGF) binding and specificity. In the presence of heparan sulfate (HS), FGF binds with high affinity to FGFR1 and induces receptor dimerization, which allows for the subsequent transautophosphorylation of tyrosine residues in the intracellular domain and activates downstream signaling pathways (24). Heterozygous mutations in FGFR1 have previously been identified within D1, D2, D3, and the TKD of the protein in subjects with KS (19–22). In addition, one KS patient with severe associated phenotypes (cleft palate, hearing loss, and agenesis of the corpus callosum) harbored a homozygous mutation in D2 (19).

To date, no genetic cause has been identified for the mixed pedigrees in which KS and nIHH subjects coexist. Variable expressivity (19, 22) within KS pedigrees harboring the same FGFR1 mutation led us to hypothesize that FGFR1 mutations might also account for both mixed pedigrees and nIHH with concurrent midline defects. Therefore, we expanded our genetic screening for FGFR1 to this cohort and performed genotype–phenotype correlations. Importantly, through structural and biochemical studies using recombinant FGFR1 proteins, we provide evidence that these FGFR1 mutations impair receptor activity.

Results

Molecular Analysis of FGFR1 Gene. Four rare sequence variants of the FGFR1 gene were found in three unrelated probands (cases 2-03, 4-03, and 7-03) (Table 1 and Fig. 1). A transversion c.709 G → A in exon 6 is predicted to substitute a glycine for serine at position 237 (G237S) in D2 within the extracellular region of FGFR1 (case 2-03; see Figs. 1 and 2). Two rare sequence variants on the same allele, a transversion c.2165 C → A and a transition c.2172 C → G in exon 16, lead to a substitution of proline for histidine at position 722 (P722H) and asparagine for lysine at position 724 (N724K) in the TKD, respectively (case 4-03; see Figs. 1 and 3). Finally, a transversion c.2038 C → T in exon 15 leads to a stop codon (Q680X) in the TKD (case 7-03, Figs. 1 and 3). These changes, segregated

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Abbreviations: IHH, idiopathic hypogonadotropic hypogonadism; GnRH, gonadotropin-releasing hormone; KS, Kallmann syndrome; LH, luteinizing hormone; nIHH, normosmic hypogonadotropic hypogonadism; FGF, fibroblast growth factor; T, testosterone; TKD, tyrosine kinase domain.

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appropriately within each pedigree (Fig. 1), were not detected in ethnically matched controls (340 Caucasian chromosomes and 130 Hispanic chromosomes), and were highly conserved both across species and within the family of FGFRs (Figs. 2B and 3B).

**Table 1. Clinical characteristics of the affected family members with an FGFR1 mutation**

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>G237S pedigree no. 2</th>
<th>P722H and N724K pedigree no. 4</th>
<th>Q680X pedigree no. 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Father</td>
<td>Brother</td>
</tr>
<tr>
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<td>F</td>
<td>M</td>
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<tr>
<td>Diagnosis</td>
<td>nlHH</td>
<td>Anosmia</td>
<td>Anosmia</td>
</tr>
<tr>
<td>Sense of smell</td>
<td>Normal</td>
<td>Anosmia</td>
<td>Anosmia</td>
</tr>
<tr>
<td>Formal smell test</td>
<td>39/40</td>
<td>16/40</td>
<td>12/40</td>
</tr>
<tr>
<td>History of puberty</td>
<td>Absent</td>
<td>Normal</td>
<td>Absent</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>None</td>
<td>Bilateral</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Dental agenesis</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>MRI of the OB</td>
<td>Normal</td>
<td>Absent</td>
<td>Normal</td>
</tr>
</tbody>
</table>

|                | Proband               | Mother                          | Brother             |
| Gender         | M                     | M                               | M                   |
| Diagnosis      | nlHH                  | Hyposmia                        | Hyposmia            |
| Sense of smell | Normal                | Normal                          | Normal              |
| Formal smell test | 36/40              | 36/40                           | 31/35               |
| History of puberty | Partial            | Normal                          | Absent              |
| Cryptorchidism | None                  | None                            | None                |
| Dental agenesis | No                   | Yes                             | No                  |
| MRI of the OB  | Normal                | Yes                             | Normal              |

DP, delayed puberty; F, female; M, male; OB, olfactory bulbs.

**Pedigree no. 4 (P722H and N724K).** The proband (case 4-05; see Table 1 and Fig. 1) is a 25-yr-old Hispanic male with unilateral cryptorchidism and two congenital missing teeth. By age 14, he reported occasional morning erections, an increased libido, and ejaculations but no virilization. He grew steadily until age 18. At that time, he presented with knee pain. He was unvirilized and eunuchoidal (height of 190 cm; arm span of 201 cm). His testicular volume was 6 ml. He had short fourth metacarpal bones bilaterally and no synkinesia. He had severe bone loss (T score of −3.7 SD at the spine; T score of −3 SD at the hip) in the setting of a low serum testosterone (T) (50 ng/dl) and inappropriately normal gonadotropin levels. We diagnosed him with partial nlHH. The 12-h frequent sampling study revealed an eneefied pattern of pulsatile LH secretion with mean LH of 4.7 units/liter (Fig. 4A). Additional exams included a formal smell test (36/40), normal MRI of the olfactory system, and renal ultrasound. His mother, who has congenital hyposmia (case 4-02; see Fig. 1 and Table 1), had normal puberty. His father (case 4-01), sister (case 4-04), and brother (case 4-05) had a normal puberty.

**Pedigree no. 7 (Q680X).** The Caucasian male proband (case 7-03; see Table 1 and Fig. 1) is a 25-yr-old Hispanic male with unilateral cryptorchidism and two congenital missing teeth. By age 14, he reported occasional morning erections, an increased libido, and ejaculations but no virilization. He grew steadily until age 18. At that time, he presented with knee pain. He was unvirilized and eunuchoidal (height of 190 cm; arm span of 201 cm). His testicular volume was 6 ml. He had short fourth metacarpal bones bilaterally and no synkinesia. He had severe bone loss (T score of −3.7 SD at the spine; T score of −3 SD at the hip) in the setting of a low serum testosterone (T) (50 ng/dl) and inappropriately normal gonadotropin levels. We diagnosed him with partial nlHH. The 12-h frequent sampling study revealed an eneefied pattern of pulsatile LH secretion with mean LH of 4.7 units/liter (Fig. 4A). Additional exams included a formal smell test (36/40), normal MRI of the olfactory system, and renal ultrasound. His mother, who has congenital hyposmia (case 4-02; see Fig. 1 and Table 1), had normal puberty. His father (case 4-01), sister (case 4-04), and brother (case 4-05) had a normal puberty.

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**Detection of FGFR1 mutations in pedigrees no. 2, 4, and 7.** Pedigree no. 2 reveals a mixed pedigree, with both the proband and her brother carrying a heterozygous missense mutation in FGFR1 (G237S). Pedigree no. 4 reveals a mixed pedigree, with the proband and his mother carrying a heterozygous double mutation in FGFR1 (P722H and N724K). Pedigree no. 7 is consistent with familial nlHH and an autosomal dominant mode of inheritance. The proband, his brother, and his father carry a heterozygous mutation in FGFR1 (Q680X). The proband is identified by the arrow. Circles denote females; squares denote males. Phenotypes are as described in the text.
stage II–III axillary and pubic hair, and a testicular volume of 2 ml bilaterally (normal >12 ml). He had bilateral nystagmus on lateral gaze, and there was no evidence of synkinesia. His serum T was 16 ng/dl with undetectable gonadotropins and otherwise normal pituitary function. He had normal olfaction (36/40) and was diagnosed with a severe form of nIHH. His neuroendocrine evaluation revealed undetectable serum LH and follicle-stimulating hormone (Fig. 4). A renal ultrasound and MRI of the olfactory system were normal, and a dual energy x-ray absorbometry scan revealed osteopenia of the spine (T score of −1.5) with normal bone density at the femoral neck (T score of −0.6).

The proband’s 19-yr-old brother (case 7-05; see Fig. 1 and Table 1) was diagnosed with absent puberty (prepubertal testes, hypogonadal serum T, and undetectable serum gonadotropins) but no associated phenotypes. He had a normal smell (score of 31), consistent with nIHH. The proband’s father (case 7-01; see Fig. 1 and Table 1) reported having delayed puberty, with initiation of shaving at age 18, and no other associated phenotypes. The proband’s mother (case 7-02; see Fig. 1) and sister (case 7-04; see Fig. 1 and Table 1) reported having delayed puberty, with initiation of shaving at age 18, and no other associated phenotypes. The proband’s mother (case 7-02; see Fig. 1) and sister (case 7-04; see Fig. 1) experienced menarche at age 12 and both have regular menstrual cycles.

**Structural and Biochemical Analysis of the FGFR1 Mutations Responsible for nIHH Show That These Mutations Result in Receptor Loss-of-Function.** The G680X nonsense mutation maps to the beginning of the α-helix (αF) in the larger, mostly helical C-terminal lobe of the FGFR1 TKD (Fig. 3A). Hence, this mutation will result in the deletion of a catalytically essential portion of the TKD and lead to a “kinase dead” receptor.

The G237S mutation maps to the βF–βG turn in D2 of FGFR1 and therefore is distant from either the ligand or heparin binding sites (Fig. 2A). However, Gly-237 residue plays an important role in determining the conformation of the βF–βG turn and thus contributes to the overall structural integrity of D2. Specifically, both oxygen and nitrogen atoms of Gly-237 make hydrogen bonds that define the end and the beginning of β-strands F and G, respectively. Importantly, because of spatial constraints, only glycine (no side chain) is allowed at this location. Substitution to serine would cause steric conflicts at this region, resulting in the instability of βF–βG turn and ultimately destabilizing the entire D2 fold. The importance of the Gly-237 for D2 fold is underscored by the fact that this residue is invariant among all FGFRs (Fig. 2B). Like the wild-type protein, the G237S mutant extracellular domain was also expressed in large quantities in bacterial inclusion bodies; however, we were unable to refold in vitro the G237S mutant extracellular domain. Importantly, our inability to refold this mutant protein is consistent with our structural analysis, suggesting that the G237S mutation is harmful to D2 folding.

The (P722H and N724K) double mutation maps to the loop connecting the α-helices αG and αH in the FGFR1 TKD (Fig. 3A). Pro-722 is in a cis conformation, and its side chain is engaged in numerous hydrophobic contacts with residues from neighboring αF and αH helices at the bottom corner of the kinase domain. The P722H substitution should weaken these hydrophobic contacts and induce structural perturbations, which at the active site of kinase domain lead to a reduction in tyrosine kinase activity of FGFR1. In contrast, Asn-724 is solvent-exposed and appears to play a subtle role in preserving the loop conformation. Consistent with these structural observations, Pro-722 is invariably conserved among all FGFRs and across species, whereas Asn-724 is highly variable (Fig. 3B). In addition, the double mutant FGFR1 (P722H and N724K) exhibited a marked decreased in the tyrosine kinase activity in vitro as compared with the wild type (Fig. 3C), demonstrating that these mutations result in FGFR1 loss-of-function. It is noteworthy that the expression level of the mutant kinase was only slightly lower as compared with the wild type (Fig. 3C).

**Discussion**

Herein, we report three FGFR1 mutations in subjects with nIHH either with midline defects or belonging to mixed pedigrees containing both KS and nIHH. This report thus expands the phenotype of FGFR1 mutations beyond anosmic cases of IHH. Importantly, we present in vitro biochemical evidence that these FGFR1 mutations represent loss-of-function mutations.
A nonsense mutation (Q680X) in the catalytic TKD of FGFR1 was identified in pedigree no. 7. This mutation should result in the synthesis of a truncated inactive receptor lacking an essential portion of the catalytic TKD (Fig. 3A), which could act as a dominant negative mutant by forming heterodimers with the normal FGFR1 allele. Alternatively, the FGFR1 mRNA containing this termination codon could be degraded by way of nonsense-mediated decay, leading to haploinsufficiency (25). Pedigree no. 4 harbored the heterozygous double missense mutation (P722H and N724K) in the TKD of FGFR1. Structural and biochemical studies showed that this double mutation leads to loss-of-function of FGFR1 by reducing the tyrosine kinase activity of FGFR1 (Fig. 3A and C). Finally, a missense mutation (G237S) in D2 of FGFR1 was identified in pedigree no. 2. Structural and in vitro biochemical studies revealed that this mutation leads to receptor loss-of-function by inhibiting the proper folding of D2 (Fig. 2A). Overall, our data, which show that these FGFR1 mutations in nIHH are loss-of-function mutations, agree with a report showing a deletion of one allele of the FGFR1 gene in two KS patients (19).

Deletions and nonsense and missense mutations in FGFR1 cause familial or sporadic KS (19–22). It is proposed that a reduction in FGF signaling abolishes olfactory bulb development, resulting in abnormal GnRH neuronal migration. Consistent with this hypothesis, targeted disruption of FGFR1 expression in mice telecephalon results in aplasia of the olfactory bulbs (26). Furthermore, MRI studies have previously identified aplasia or hypoplasia of the olfactory bulb in KS patients carrying an FGFR1 mutation (20, 22).

Herein, we demonstrate that FGFR1 mutations can cause nIHH with apparent normal olfactory bulbs and normal quantitative smell tests. Interestingly, a male IHH patient with cleft lip and palate and without a history of frank anosmia was recently reported to harbor a reciprocal translocation t(7,8) (p12.3, p11.2), disrupting the FGFR1 gene (27), although no formal smell test or MRI of the olfactory bulbs were performed in this patient. In subjects with nIHH, FGFR1 mutations may lead to an isolated defect in GnRH neuron migration. Indeed, disruptive GnRH neuronal migration in the presence of normal olfactory bulbs and sulci has been demonstrated in the knockout mouse model for another gene, Ebf2 (28). Alternatively, FGFR1 mutations could cause a defect in differentiation or survival of GnRH neurons within the hypothalamus. Notably, FGFR1 expression in the mice can be detected in the nasal placode, in developing olfactory bulbs, and along the GnRH migratory pathways, as well as in mature hypothalamic GnRH neurons and their projection (29). Furthermore, mice expressing dominant negative FGFR1 mutants targeted to GnRH neurons demonstrated a 30% reduction in the GnRH neuronal population of the hypothalamus (30). These mice, although fertile, display subtler reproductive phenotypes, such as delayed puberty and premature ovarian senescence. Thus, it is clear that more IHH subjects with normal olfaction should be screened for mutations in FGFR1.
anomalies and cleft lip. Hypothesize that defects in endogenous GnRH secretion. It is thus tempting to of reproductive phenotypes suggests an equally wide range of ranging from absent puberty to partial puberty. This wide spectrum humans (33) and mouse models (34). Mice homozygous for a function mutations in FGFR2 cause cleft palate associated with craniosynostosis in both and palate development.

FGFR1 mutations in mixed pedigrees with both KS and nIHH. This finding is informative because it is thought that KS and nIHH are two distinct entities. KS is thought to result from embryonic defects in GnRH neuron migration associated with a disruption in olfactory bulb formation, whereas nIHH is thought to arise from defects in GnRH synthesis, processing, or action. Additionally, previous molecular genetic studies have identified different loci for KS (KAL1 and FGFR1) (17, 19, 31, 32) and nIHH (GNRHR and GPR54) (12–14). Therefore, our finding challenges the absolute nature of this clinical distinction and provides a genetic explanation for some of these mixed pedigrees.

Our data also reveals variable degrees of pubertal development within and across families carrying identical FGFR1 mutations ranging from absent puberty to partial puberty. This wide spectrum of reproductive phenotypes suggests an equally wide range of defects in endogenous GnRH secretion. It is thus tempting to hypothesize that FGFR1 mutations might therefore underlie other milder reproductive phenotypes, such as delayed puberty or perhaps hypothalamic amenorrhea.

Finally, this report confirms the association of IHH with dental anomalies and cleft lip/palate in IHH subjects carrying an FGFR1 mutation (19). FGFRs have been shown to play a key role in dental and palate development. FGFR1 and FGFR2 are differentially expressed in the mesenchyme and epithelium of fusing palatal shelves and in dental follicle development (33). Whereas loss-of-function mutations in FGFR1 cause IHH with a high frequency of cleft palate, somewhat paradoxically, gain-of-function mutations in FGFR2 cause cleft palate associated with craniostenosis in both humans (33) and mouse models (34). Mice homozygous for a hypomorphic allele for FGFR1 display open palatine shelves in 80% of cases (35).

In summary, we have identified three heterozygous mutations in the FGFR1 gene in nIHH subjects with associated midline defects or belonging to mixed pedigrees. These results expand the phenotypic spectrum of FGFR1 mutations beyond KS and suggest a broader biologic role of FGFR1 in reproduction. Mutations in FGFR1 causing mixed pedigrees suggest that KS and nIHH are part of the same spectrum of disease. Furthermore, we present structural and in vitro biochemical evidences that these FGFR1 missense mutations causing IHH are loss-of-function mutations. Finally, the variable expressivity of the disease within pedigree harboring the same FGFR1 mutation suggests an interaction of FGFR1 with other gene products versus environmental influences on FGFR1 expression. More patients with nIHH should be screened to assess the frequency of heterozygous FGFR1 mutations in nIHH and to further define the role of FGFR1 in human reproduction.

Materials and Methods

Subjects. IHH population. Seven probands from unrelated pedigrees (five males and two females) with IHH and normal olfaction were included in this study. All subjects either had associated midline defects (n = 2) or belonged to a mixed pedigree (n = 5). IHH was characterized by (i) absence/incomplete puberty by age 18 yr in otherwise healthy patients; (ii) serum T ≤100 ng/dL in men or estradiol ≤20 pg/ml in women in association with low or normal levels of serum gonadotropins; (iii) otherwise normal pituitary function; (iv) normal serum ferritin concentrations; and (v) normal MRI of the hypothalamic-pituitary region. In addition, six of seven probands were tested for GNRHR mutations and were negative. If an FGFR1 mutation was found, affected and unaffected parents and siblings of the proband were studied.

Controls. The Caucasian control population consisted of 170 healthy Caucasian normal controls from Massachusetts General Hospital as assessed by history and clinical examination (n = 340 chromosomes). In addition, 115 healthy Mexican control subjects were selected either from an independently assembled cohort (200 chromosomes) (Coriell Laboratory, Camden, NJ) or from healthy Mexican controls from Massachusetts General Hospital (n = 30 chromosomes).

The study was approved by the Human Research Committee of Massachusetts General Hospital, and all subjects provided written informed consent before participation.

Mutation Analysis of the FGFR1 Gene. Sequencing of the coding regions of the FGFR1 gene (GenBank accession no. BC018128) was performed as described in ref. 20. Amplified products were sequenced in both directions by using the AmpliTaq Dye Terminator Cycle Sequencing kit and an ABI Prism 377 DNA sequencer (PerkinElmer). Subsequent sequence analysis was performed by using Mutation Surveyor (Version 2.2; SoftGenetics, State College, PA) and visual inspection. All sequence variations were found on both strands and confirmed in a separate PCR. Nonsense changes resulting in a truncated protein, frameshift, insertion, or deletion were categorized as definite mutations. Nucleotide changes which were (i) absent from the dbSNP and ESTs; (ii) absent in 200 ethnically matched control chromosomes; (iii) evolutionarily conserved across species; and (iv) segregated appropriately in the family were also identified as disease-causing mutations. All genes and proteins are described by using standard nomenclature (36).

Clinical and Biochemical Studies. A detailed personal and family history of pubertal development (5) and associated nonreproductive phenotypes (i.e., dental agenesis, cleft lip/palate, hearing loss, synkinesia, etc.) was performed. Families including KS or congenital anosmia and nIHH subjects were termed mixed pedigrees. A detailed physical examination, including Tanner staging for pubic and axillary hair and breast development, testicular volume by using
a Prader Orichimeter, and mirror movement (37) were obtained. Olfactory acuity was assessed by history and confirmed with formal, quantitative smell testing [the University of Pennsylvania Smell Identification Test (UPSIT); Sensonics, Haddon Heights, NJ]. Thirty or more correct answers indicated a normal sense of smell (>5th percentile of 1,819 men and women between 15 and 50 yrs), 19–29 indicated hyposmia, and ≤18 indicated anosmia (38). In addition, a renal ultrasound and a dual energy x-ray absorptiometry scan were performed. Cranial 3D MRI was performed to assess the olfactory system. The examination included routine axial Tesla 2 fluid-attenuated inversion recovery images of the entire brain. This examination was followed by coronal 3D spoiled gradient recalled echo images with 1.5-mm-thick partitions that included the olfactory system and coronal 3-mm-thick Tesla 2 fast spin echo images focused on the frontal olfactory region from the posterior border of the frontal paranasal sinuses to the optic chiasma.

Five of the seven patients were admitted to the General Clinical Research Center of Massachusetts General Hospital for detailed neuroendocrine studies, including an overnight 12-h frequent blood-sampling study for LH (q10′′ × 12 h) after withdrawal of hormonal therapy for a suitable washout period (typically 8 weeks). Pulsatile hormone secretion was analyzed by using a validated modification of the method (39, 40). The pool of the 12-h sampling was also assayed for follicle-stimulating hormone, T, estradiol, and inhibin B. In men able to produce an ejaculate, semen fluid analysis was performed according to World Health Organization guidelines.

**Structural Analysis of the Effects of nHh Mutations on FGFR1 Function.** The crystal structures of the FGFR1 kinase domain (Protein Data Bank entry 1FGK) (41) and the extracellular ligand binding region of FGFR1 in complex with FGF ligand and heparin oligosaccharide (Protein Data Bank entry 1FQ9) (42) were used to explore the effects of the mutations. Crystal structures were visualized by using the program o (43).