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BRIEF REPORT

A Mutation in the Fibroblast Growth Factor Receptor 1 Gene Causes Fully Penetrant Normosmic Isolated Hypogonadotropic Hypogonadism

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Context: Kallmann syndrome (KS) consists of idiopathic hypogonadotropic hypogonadism (IHH) and anosmia/hyposmia. Currently, the fibroblast growth factor receptor 1 (FGFR1) gene is the only known autosomal dominant cause of KS, which is also associated with synkinesia, midfacial defects, and dental agenesis.

Objective: Mutations in FGFR1 typically demonstrate reduced penetrance, variable expressivity, and until recently have been exclusively identified in families with anosmia. The purpose of this study was to determine whether FGFR1 mutations were present in a unique family with autosomal dominant, fully penetrant, normosmic IHH.

Design: The study is a review of detailed clinical findings, dynamic endocrine studies, and performance of a molecular analysis of the FGFR1 gene.

Setting: The study was carried out in an academic medical center.

Patients: All four affected individuals have complete IHH with full penetrance but no anosmia/hyposmia, and they have none of the FGFR1-associated anomalies. In addition, no other family member has anosmia.

Inverventions: Interventions included detailed phenotype characterization including history, physical exam, smell testing, dynamic pituitary testing, brain imaging, and molecular analysis.

Main Outcome Measures: Outcome was measured by the determination of the severity of IHH, olfactory function, and sequence of the FGFR1 gene.

Results: The same heterozygous nonsense mutation, Arg622X, was present in all four affected members, but not in three unaffected members or 100 controls. The mutation is predicted to encode a truncated protein or result in nonsense-mediated decay.

Conclusions: Our findings indicate that mutations in the FGFR1 gene can cause normosmic, fully penetrant, complete IHH with little or no variable expressivity, and without the other FGFR1-associated anomalies typically found in KS. **(*J Clin Endocrinol Metab* 92: 1155–1158, 2007)**

KALLMANN SYNDROME (KS) is a developmental disorder characterized by idiopathic hypogonadotropic hypogonadism (IHH) and anosmia/hyposmia (1). IHH patients usually have irreversible delayed puberty and low serum gonadotropins without a hypothalamic/pituitary lesion. Because GnRH neurons migrate with olfactory neurons from the olfactory placode region to the hypothalamus, disruption of this pathway results in KS (2–5).

IHH is genetically heterogeneous with X-linked recessive (KAL1) (2, 3), autosomal dominant [fibroblast growth factor receptor 1 (FGFR1)] (6), and autosomal recessive (GNRHR most commonly) modes. Presently, FGFR1 is the only gene known to cause autosomal dominant KS. However, characteristics of individuals with FGFR1 mutations include reduced penetrance, extreme variable expressivity, anosmia, synkinesia, cleft palate, and dental agenesis (6–12). Recently, an FGFR1 mutation was reported in a family with both normosmia and anosmic IHH (9). No FGFR1 mutations have been reported in families with pure normosmic, fully penetrant IHH. We present the findings and molecular analysis of a family with autosomal dominant, normosmic IHH.

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Abbreviations: BMI, Body mass index; FGFR1, fibroblast growth factor receptor 1; HMG, human menopausal gonadotropin; IHH, idiopathic hypogonadotropic hypogonadism; KS, Kallmann syndrome; MRI, magnetic resonance imaging; UP-SIT, University of Pennsylvania Smell Identification Test.

Patients and Methods

Proband II-2

The proband II-2 (Fig. 1), now a 44-yr-old normosmic, African-American female G2P3003, was first seen at age 18 for primary amenorrhea at the Reproductive Endocrine and Genetics Section at The Medical College of Georgia (MCG). She denied a family history of pubertal delay, cleft palate, dental agenesis, renal agenesis, or anosmia. Her height was

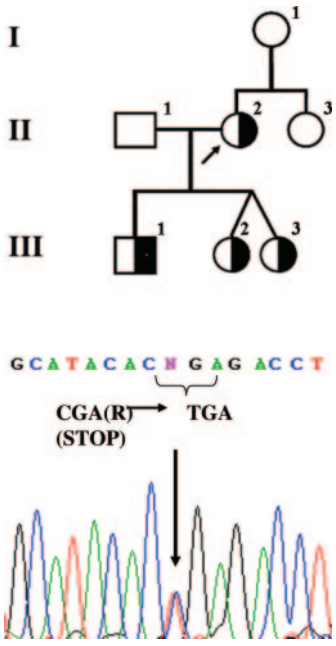

FIG. 1. A, All available family members are shown. Normosmic IHH is shown as *half-shaded* and unaffected members are *unshaded*. It should be noted that no history of anosmia or anomalies was present in an extended family history (not shown here). The proband is indicated with an *arrow*. B, The heterozygous FGFR1 nonsense mutation is shown in which a CGA (Arg = R) is converted to a TGA (STOP = X) codon.

54.5 cm and her weight was 58.4 kg [body mass index (BMI) = 25.1]. She had Tanner I breasts, Tanner II pubic hair, and a normal patent vagina. Serum FSH was 2.2 mIU/ml (5–20 mIU/ml) and LH was 2.5 mIU/ml (2–30 mIU/ml), T_4 was 11.3 μg/dl (4.5–12.5), prolactin was 11.3 ng/ml (2–20 ng/ml), and tomography of the sella was normal. A bone age was 14.7 yr. She underwent simultaneous iv infusions of 100 μg GnRH, 250 μg TRH, and 0.1 U/kg of insulin (13), and had normal TSH, prolactin, GH, and plasma corticoid responses (Table 1). Her FSH did not change, and LH peaked at 10 mIU/ml. She was diagnosed with IHH and was placed on estrogen to induce breast development, and later was placed on oral contraceptives.

At 20 yr of age, she underwent ovulation induction using human menopausal gonadotropins (HMG). She took 20 ampoules of 75 IU HMG im over 10 d and received hCG with peak total urinary estrogens of 201 μg, but did not conceive. One year later, she took HMG (150 IU increased to 375 IU, 43 ampoules/13 d), with a peak serum estradiol of 250 pg/ml and two preovulatory follicles on the day of hCG. She conceived and had a vaginal delivery of a son at 34 wk (III-1).

A year later, she underwent ovulation induction with pulsatile GnRH by pump sc up to a dose of 8.4 μg/pulse (140 ng/kg/pulse), but it was discontinued because of a poor response after 8 d of treatment (estradiol < 10 pg/ml and no follicles). HMG was instituted at a does of 300 IU/d and increased to 450 IU/d (62 total amps); hCG was given when her estradiol was 1034 pg/ml and two preovulatory follicles were present. She conceived, and an ultrasound demonstrated a twin pregnancy with separate sacs.

Subject III-1

Her son, now a 22-yr-old African-American male, was born at 34 wk weighing 1920 g (third percentile) and a 46, XY karyotype. At 6 months of age, he was referred to the Reproductive Endocrine and Genetics Section at MCG for micropenis. Growth parameters included a height of 63.5 cm, weight of 7.5 kg, and head circumference of 41.5 cm, all less than 5%. His penis was 1.5 × 0.5 cm (length × width) with a penile urethra, a descended, retractable right testis, and a left inguinal testis. He was suspected to have IHH, and underwent dynamic testing (Table 2) before and after iv GnRH (40 μg), TRH (100 μg), and arginine (4 g). GH, TSH, and prolactin responses were normal, whereas gonadotropin responses were blunted (maximal FSH = 4 mIU/ml; maximal LH = 6 mIU/ml). Basal testosterone was less than 1.2 ng/dl; dehydroepiandrosterone sulfate was 58.6 μg/dl, and androstenedione was 32 ng/dl. Basal cortisol of 13 μg/dl rose to 20 μg/dl 1 h after ACTH.

At 17 months, he underwent a left orchiopexy, but he was subsequently lost to follow-up until 20 yr of age when he reported no voice change, facial hair, or anosmia. On physical exam, his height was 167 cm and weight was 60 kg (BMI = 21.5). He had no cleft palate, dental agenesis, gynecomastia, synkinesia, or axillary or facial hair. His optic discs were sharp and his neurological exam was normal. He had Tanner V pubic hair, right testis of 1–2 cc, left testis of 3–4 cc, and a small penis. His serum testosterone was 51 ng/dl, LH was 0.2 mIU/ml, and FSH was less than 0.7 mIU/ml. His serum cortisol was 12.3 μg/dl (8.7–22.4 μg/dl), prolactin was 3 ng/ml, TSH was 0.4 μU/ml, and thyroxine was 8.1 μg/dl (4.6–12.4 μg/dl). Pituitary magnetic resonance imaging (MRI) was negative for tumor, with normal olfactory bulbs. He was normosmic by the University of Pennsylvania Smell Identification Test (UP-SIT), with a score of 33 of 40 (>30 questions indicates normosmia) (14). He was diagnosed with IHH and begun on testosterone injections.

Subjects III-2 and -3

III-2. These fraternal twins were seen at 16 months of age because of the potential autosomal dominant inheritance pattern of IHH. Patient III-2 appeared normal and had a serum FSH of 0.7 mIU/ml and LH less than 0.5 mIU/ml. IHH was suspected, but further supportive evidence was obtained when she presented with primary amenorrhea at age 14 yr. She had no breast development and denied anosmia. On physical exam, her height was 156.2 cm, weight was 61.1 kg, and BMI was 25.4, with Tanner I breasts and Tanner III pubic hair. Her serum estradiol was less than 20 pg/ml, FSH was 0.7 mIU/ml, LH was less than 0.2 mIU/ml, prolactin was 3 ng/ml, and TSH was 1.28 μU/ml. Her MRI was unremarkable with normal olfactory bulbs/tracts. She was normosmic (10 of 12 by Brief UP-SIT; >9 normosmic). She was diagnosed as IHH and treated with estrogen. At 15 yr of age, she had Tanner IV breasts with one episode of vaginal bleeding, so she was switched to oral contraceptives.

III-3. She was seen at 16 months with a normal exam. Her serum FSH was 0.6 mIU/ml and LH was less than 0.5 mIU/ml. She was not seen again until age 14, when she presented with primary amenorrhea. On physical exam, her height was 149.2 cm, weight was 43.6 kg, BMI was 19.6, and she had Tanner I breasts and Tanner I pubic hair. Her serum

TABLE 1. Subject II-2 underwent dynamic pituitary testing to exclude multiple pituitary deficiencies (insulin tolerance test, TRH, and GnRH stimulation)

Time (min)	TSH (μU/ml)	Prolactin (ng/ml)	Corticoids (μg/%)	GH (μg/ml)	FSH (mIU/ml)	LH (mIU/ml)	Glucose (mg/dl)
0	0	3.3	11	<0.5	<2	<2	87
15	2.9	21	15.5	<0.5	2	8	31
30	0.8	17	20.5	3	2	10	42
45	0.78	14.5	24.5	14.5	2	7	50
60	0.5	15	25	20	2	9	54
90	0	7.5	23	16			66
120	0	5.5	16.5	5.4	2	4	71
150	0	6	11.5	6	<2	4	79
180	0	6	10	4.6	<2	4	81

TABLE 2. Subject III-1 underwent GnRH and TRH stimulation and arginine infusion at age 7 months, 13 d

Time (min)	FSH (mIU/ml)	LH (mIU/ml)	Prolactin (ng/ml)	TSH (μIU/ml)	GH (mg/dl)
0	<1.0	1	16.5	1.6	9.4
15	2.4	5.5		6.2	
30	3.4	6			
60	3.2	4	18.5	8.8	5.7
75	4	3.5			
90	3	3	16.5	5.7	5
120	2.5	2.3	12.3		
150	2.4	1.5			

estradiol was less than 20 pg/ml, FSH was 0.7 mIU/ml, LH was less than 0.2 mIU/ml, prolactin was 3 ng/ml, and TSH was 1.48 μU/ml. Her MRI was unremarkable. By UP-SIT, she was normosmic (36 of 40). She was diagnosed as presumptive IHH and treated with estrogen. At 15 yr of age, she still had only Tanner III breasts, so the dose of conjugated estrogen was increased. Both twins had sharp optic discs and no other findings such as synkinesia, dental agenesis, or clefting.

Other family members

The husband (II-1) of patient II-2 is normosmic and had normal puberty. Family members I-1 and II-3 are also normosmic with normal puberty. There are no family members with delayed puberty, anosmia, cleft palate, neurological disorders, or dental agenesis.

Molecular analysis of FGFR1 gene: PCR and DNA sequencing

This study was approved by the Human Assurance Committee at MCG. DNA was extracted from each of the members of the family and from 100 fertile controls. Previously, the proband was shown not to possess mutations in the GNRH1, GNRHR, or KAL1 genes. PCR was performed on exons 2–18, including the splicing junctions (primer sequence available upon request). All exons were amplified using one of two master mixes: the first with 1.5 mM $MgCl_2$ (exons 2, 3, upstream-8, downstream-8, 10/11, 13, 16/17, and 18) and the other with a 2.5 mM $MgCl_2$ (exons 4/5, 7, 9, 12, 14/15). After denaturation of 94 C for 2 min, 30 cycles of 94 C for 30 sec, 60 C for 30 sec, and 72 C for 30 sec were performed for each exon. A negative control containing all reagents except DNA was included in each PCR. PCR products were then electrophoresed on 1.2% agarose gels, ethanol was precipitated, sequenced forward and reverse using dideoxy methods, run on an automated DNA sequencer, and analyzed using CodonCode Aligner. Putative mutations were sequenced X6/affected patient and unaffected family members. Because Arg622X created a *Tsp*RI restriction enzyme-recognition site, additional controls were tested by overnight restriction digestion and gel electrophoresis.

Results

All affected IHH individuals had a heterozygous C1864T (exon 14) creating an Arg622X mutation. This nonsense mutation was absent in the proband's husband, two other unaffected relatives (Fig. 1), and 100 fertile controls.

Discussion

Heterozygous FGFR1 mutations were first reported in 9.3% of 129 unrelated KS patients (6). Reduced penetrance and variable expressivity were observed in this autosomal dominant transmission. Associated anomalies included cleft lip/palate, dental agenesis, corpus callosum agenesis, synkinesia, unilateral hearing loss, and fusion of the fourth/fifth metacarpals (6).

To date, all FGFR1 mutations were identified in families with anosmia (6–12). In this study, we report a normosmic, autosomal dominant family in which the proband clearly presented with complete IHH, but did not have anosmia, cleft palate, or dental or neurologic abnormalities. She conceived twice with gonadotropins, and delivered a son with unilateral cryptorchidism and micropenis. He was diagnosed with complete IHH based on testis size at age 20. In addition, II-2 had twin girls who presented at 14-yr with absent breast development (complete IHH). All three IHH children were normosmic by history and UP-SIT, and none had KS-associated anomalies (6–12). Although it is still possible the girls could initiate puberty spontaneously, breast development remains incomplete at age 15 despite estrogen administration.

All four affected individuals in this family were heterozygous for an FGFR1 nonsense mutation (Arg622X) not observed any unaffected members or 100 fertile controls. Unique features of this family include complete penetrance, minimal/no variable expressivity (all had complete IHH), normosmia, and no associated anomalies such as synkinesia, cleft palate or lip, or dental agenesis. This nonsense mutation in exon 14 may cause nonsense-mediated decay, and is predicted to truncate the FGFR1 protein, completely removing a tyrosine kinase domain (6, 9).

Interestingly, this same nonsense mutation has been reported twice previously in families with IHH, anosmia, and associated anomalies. Dode *et al.* (6) described a family with the Arg622X mutation in which there was reduced penetrance, anosmia, and cleft lip/palate. In this family, five individuals carried the heterozygous mutation: three had KS (IHH severity is not stated), one had only anosmia, and one was unaffected. Pitteloud *et al.* (9) described a male with incomplete KS who had 7 ml testes, his mother with delayed puberty only, and a maternal grandfather with only anosmia with the Arg622X mutation. Interestingly, this male had reversible KS, as evidenced by pulsatile LH secretion and normal testosterone levels after treatment discontinuation (9).

The impact of FGFR1 mutations may be more complicated than previously thought. In addition to inactivating mutations that cause KS, activating FGFR1 mutations produce a broad range of skeletal abnormalities including the craniosynostosis disorder Pfeiffer syndrome, trigonocephaly, osteoglophonic dysplasia, and Antley-Bixler syndrome (15), whereas inactivating mutations cause KS. FGF signaling is involved in both olfactory and GnRH neuron migration, but the pathogenesis of impaired FGF action with normal olfaction is unclear (16–19). It is interesting that Arg622X may cause either KS with/without associated anomalies or normosmic IHH (6, 9). These findings suggest that additional genetic, epigenetic, or environmental modifiers contribute to the phenotype with this mutation (5). In conclusion, we show that the Arg622X FGFR1 mutation causes fully penetrant, normosmic IHH without variable expressivity or associated anomalies.

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The authors have nothing to declare.

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