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RAPID COMMUNICATION

Novel Fibroblast Growth Factor Receptor 1 Mutations in Patients with Congenital Hypogonadotropic Hypogonadism with and without Anosmia

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Context: Kallmann syndrome is a clinically and genetically heterogeneous disorder. To date, loss-of-function mutations in the genes encoding anosmin-1 (*KAL1*) and fibroblast growth factor receptor 1 (*FGFR1*) have been described in the X-linked and autosomal dominant forms of this syndrome, respectively.

Objective: The objective was to investigate genetic defects in the *KAL1* and *FGFR1* genes in patients with congenital isolated hypogonadotropic hypogonadism (IHH).

Patients: Eighty patients (71 males and nine females) with IHH were studied, of which 30 were familial. Forty-six of them had olfactory abnormalities.

Methods: The coding regions of both *KAL1* and *FGFR1* genes were amplified and automatically sequenced. The *KAL1* mutations were investigated only in patients with olfactory abnormalities, whereas *FGFR1* was studied in the entire group.

Results: Two novel *KAL1* mutations, an intragenic deletion of exons 3–6 and a splicing mutation IVS7 + 1G>A, were identified in two of 46 patients with Kallmann syndrome. Eight novel heterozygous *FGFR1* mutations (G48S, L245P, R250W, A343V, P366L, K618fsX654, P722S, and V795I) were identified in nine of 80 patients with IHH. Eight of them had olfactory abnormalities. Interestingly, the G48S mutation was identified in a normosmic IHH patient. Two unrelated females, who carried *FGFR1* mutations, had anosmia and normal reproductive function.

Conclusion: We identified novel mutations in *KAL1* and *FGFR1* genes in IHH patients. *FGFR1* mutations were identified in 17% of the patients with olfactory abnormalities and in one of 34 normosmic IHH patients. In addition, isolated anosmia was identified in two unrelated females as a partial phenotypic manifestation of *FGFR1* defects. **(*J Clin Endocrinol Metab* 91: 4006–4012, 2006)**

CONGENITAL ISOLATED hypogonadotropic hypogonadism (IHH) is characterized by complete or partial failure of pubertal development due to the impaired secretion of LH and FSH, in the absence of any hypothalamic-pituitary organic cause (1). When IHH is associated with impaired olfactory function (anosmia/hyposmia), it is defined as Kallmann syndrome. This condition is genetically heterogeneous, with reports indicating autosomal dominant, recessive, and X-linked transmission (1). To date, two distinct genes have been implicated in the molecular basis of Kallmann syndrome, the genes encoding anosmin-1 (*KAL1*) and the fibroblast growth factor receptor 1 (*FGFR1*) (2).

The *KAL1* gene (ENSG0011201) is located at Xp22.3 and comprises 14 exons (3, 4). Anosmin-1 is an extracellular matrix glycoprotein that shows significant homologies with molecules known to play specific roles in neuronal development (5, 6). Experiments *in vitro* revealed a role for anosmin-1 in the control of different cell function, including cell adhesion and neurite/axonal elongation and fasciculation, and in the migratory activity of GnRH-producing neurons (5–7). Several *KAL1* gene abnormalities (missense, nonsense and splice site mutations, intragenic deletion, and complete gene deletion) have been identified in approximately 8–11% of the sporadic form and in 14–50% of the familial form of Kallmann syndrome (8, 9). Other nonreproductive features are associated with *KAL1* gene abnormalities, such as unilateral renal aplasia, mirror movements, sensorineural deafness, high-arched palate, and eye-movement abnormalities (9–12).

The *FGFR1* gene (ENSG0077782), also called *KAL2,* is located at chromosome 8p12 and comprises 18 exons (13). Dode *et al.* (14), studying two patients with contiguous gene syndrome due to interstitial deletions at chromosome

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Abbreviations: FGF, Fibroblast growth factor; FGFR1, FGF receptor 1; IHH, isolated hypogonadotropic hypogonadism; MRI, magnetic resonance imaging; TK, tyrosine kinase.

8p11.2–12, first reported the association of loss-of-function mutations in *FGFR1* with the dominant form of Kallmann syndrome. Since then, several *FGFR1* heterozygous mutations were identified in approximately 10% of individuals with Kallmann syndrome (11, 14, 15). In addition to hypogonadotropic hypogonadism, different nonreproductive features including cleft palate, mirror movements, and dental agenesis have also been identified in these patients, with variable phenotypic expression (14, 15). Moreover, a rare case of Kallmann syndrome with reversible hypogonadism was recently associated with a *FGFR1* mutation (16). It is noteworthy that most patients with *FGFR1* defects described so far were anosmic or hyposmic (11, 14–16). It has been suggested that anosmin-1 is involved in fibroblast growth factor signaling through FGFR1 (14, 17–19).

In the present study, we investigated *KAL1* and *FGFR1* gene mutations in a cohort of Brazilian patients with Kallmann syndrome. Molecular analysis of *FGFR1* was also carried out in patients with IHH and normal olfaction to determine the frequency of *FGFR1* defects in these patients.

Patients and Methods

Eighty unrelated Brazilian patients (71 males and nine females, aged 17–50 yr) with IHH were selected from three university institutions of Sao Paulo, Brazil. Permanent IHH was documented based on the following criteria: age older than 17 yr, clinical signs and symptoms of hypogonadism, prepubertal testosterone or estradiol levels, low or inappropriately normal gonadotropin levels, normal baseline and stimulated levels of the other anterior pituitary hormones, and normal hypothalamic-pituitary imaging. All patients were questioned regarding their sense of smell. In addition, two objective olfactory tests, Smell Identification Test or Alcohol Sniff Test, were performed in 55 patients with IHH (20). Based on the olfactory questionnaire and objective olfactory test results, 46 individuals were found to have olfactory abnormalities, whereas 34 had a normal sense of smell. GnRH receptor mutations were previously excluded in all normosmic IHH patients (21, 22).

Thirty patients (21 with Kallmann and nine normosmic IHH) had a positive familial history of IHH. Among the 21 patients with familial Kallmann syndrome, autosomal dominant inheritance was predicted in six of them, based on the presence of affected male and female relatives, as well as the direct transmission of the phenotype across generations. Four families had a pattern of transmission suggestive of autosomal recessive inheritance, where both sexes were affected at the same generation. A clear recessive X-linked transmission, characterized by the presence of affected males only and maternal transmission, was observed in one family with Kallmann syndrome. Three of the nine families with normosmic IHH showed an autosomal recessive mode of inheritance. Given the limited sample size and/or only one generation being affected, it was not possible to determine the mode of inheritance in 16 familial IHH. The remaining 50 IHH patients were considered to be sporadic cases.

One hundred adult individuals of both sexes (50 males and 50 females) with normal sexual development at the appropriate chronological age and no history of abnormal sense of smell were used as the control group. The study was approved by the ethical committee of each institution. Informed and written consent was obtained from all patients.

DNA analysis of KAL1 and FGFR1 genes

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. The *KAL1* gene was studied in all Kallmann syndrome patients. DNA was amplified by PCR using previously described primers (12). The PCR amplifications were performed in 20 μl reaction mixes containing 200–500 ng of genomic DNA, 0.2 mM deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, 0.6 pmol of each of the primers, 1 × PCR buffer, and 1 U *Taq* polymerase (Amersham Biosciences, Piscataway, NJ). After a first denaturation step (10 min, 95 C), 30 PCR amplification cycles of 30 sec at 95 C, 30 sec at 57 C (except for exon 1, 62 C), and 1 min at 72 C were carried out, followed by a final extension of 10 min at 72 C. The PCR products were electrophoresed on 1.0% agarose gel, stained with ethidium bromide, and photographed. If no amplification product of *KAL1* exons was detected, PCR was repeated with the inclusion of primers for the *SRY* gene, as internal positive control (21).

Exons 2–18 of the *FGFR1* gene were amplified in *KAL1* mutation-negative cases and normosmic IHH patients. *FGFR1* oligonucleotides are shown in Table 1. The *FGFR1* amplification conditions were similar to those used in *KAL1* gene amplification.

TABLE 1. Oligonucleotides used for amplification of the *FGFR1* coding exons and splice site junctions, annealing temperatures, and product sizes

Primers	Sequence (5′→3′)	Annealing temperature (C)	Amplified product size (bp)
FGFR1_2F	CTT TAA GCA GCC ACC ACA TGG	60	399
FGFR1_2R	GCT CCA CTT GGG AAG GAG CC		
FGFR1_3F	GCT CAG TAG CCT CCA GTA AGT G	59	493
FGFR1_3R	GGT TCA CCT TCC TCT GAA ACT G		
FGFR1_4–5 F	CGT GTT CAT CTG GAA CTG CAC	59	672
FGFR1_4–5R	GCA TGT AAT CAG GAC TTC C		
FGFR1_6F	CCA CCA GGC TCT GAT ATG GAG	59	346
FGFR1_6R	GAA GTG CCA ATC GCT ATC CTG		
FGFR1_7F	CAT GAG CGA CTT ACT GTG ACT G	59	549
FGFR1_7R	CGT GAG GAA TGA TCC CAT TCG		
FGFR1_8F	CAG CAT TTC TTC CTC TAG TC	58	328
FGFR1_8R	AGC CTG GAA ATG CAT GCT CC		
FGFR1_9F	AGT CCT AGC TAG AAC TTG CC	59	502
FGFR1_9R	AGA CTC TAG AGC ACT TAG TTC		
FGFR1_10–11F	TAC ACA GAC ATG TGC CTC TG	59	698
FGFR1_10–11R	CAG AGA AGC TGT TCT GCT GG		
FGFR1_12F	TAT TGC AAC GGC TCC C	59	352
FGFR1_12R	TTG GGA CTG ATA CCC CAG C		
FGFR1_13F	GGG TTT CTT TGA GGT GAA GCC	58	374
FGFR1_13R	GTG CTC AGT GCA TCC ACA ACG		
FGFR1_14–15F	AAG TCG GCT AGT TGC ATG GG	59	491
FGFR1_14–15R	CTA CAG TGC TAG AAG CTC TC		
FGFR1_16–17-18F	AGA TGT TGA AAG GCT GAT CT	59	938
FGFR1_16–17-18R	GGT GAA GGC AGG CCA CAC A		

The PCR products of *KAL1* and *FGFR1* genes were pretreated with an enzymatic combination of exonuclease I and shrimp alkaline phosphatase (United States Biochemical Corp., Cleveland, OH) and directly sequenced using the BigDye terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Foster City, CA) in an ABI PRISM 310 automatic sequencer (PerkinElmer Cetus, Shelton, CT). *KAL1* and *FGFR1* mutations identified were confirmed in three independent PCR products and sequencing reactions of both strands.

In silico analysis by RepeatMasker software (http://www.repeatmasker.org, accessed in February, 2006) was performed for the identification of repetitive sequences in the *KAL1* gene.

Results

Standard and multiplex PCR amplifications revealed an intragenic deletion involving exons 3–6 of the *KAL1* gene in a sporadic case of Kallmann syndrome (Fig. 1). *In silico* analysis of introns 2 and 6 of the *KAL1* gene, adjacent regions to this deletion, disclosed several elements of SINE and LINE family repeats (such as Alu, L1, and MIR) and the simple repeat CAAATT in both introns.

Automatic sequencing revealed a G to T transversion in the splice donor site of intron 7 of the *KAL1* gene, expected to result in a splicing aberration in a patient who had four maternal uncles with Kallmann syndrome (Fig. 2).

Eight novel heterozygous mutations of the FGFR1 (G48S, L245P, R250W, A343V, P366L, K618fsX654, P722S, and V795I) were identified in nine male patients with IHH, including one with normal olfactory status (Table 2, Fig. 3). The G48S mutation was found in patient 1 with sporadic IHH and normal olfactory sense established by objective olfactory test (score, 38/40). He also had a normal magnetic resonance imaging (MRI) of olfactory sulci and bulbs. The R250W mutation was identified in two apparently unrelated patients, cases 3 and 4, with familial and sporadic Kallmann syndrome, respectively (Table 2).

Among the nine patients with FGFR1 mutations, six had other first- and second-degree affected family members (Fig. 4). We were unable to perform additional DNA analysis in the affected members of families 1 and 2. The P366L mutation was identified in patient 6, two of his paternal aunts with Kallmann syndrome, and his normosmic father (family 3). The frameshift mutation at position 618 within the tyrosine kinase (TK) domain of the FGFR1 was identified in patient 7 and his sister, both with Kallmann syndrome. This defect was also identified in their anosmic mother (family 4). The P722S mutation was identified in patient 8 and his maternal first-degree male cousin with Kallmann syndrome (family 5). The V795I mutation was identified in patient 9 with Kallmann syndrome as well as in his sister, who had anosmia and normal reproductive function demonstrated by normal basal and stimulated gonadotropin levels (family 6).

Automatic sequencing of 200 alleles from the 100 normal Brazilian males and females did not reveal any of the *FGFR1* mutations described in this study.

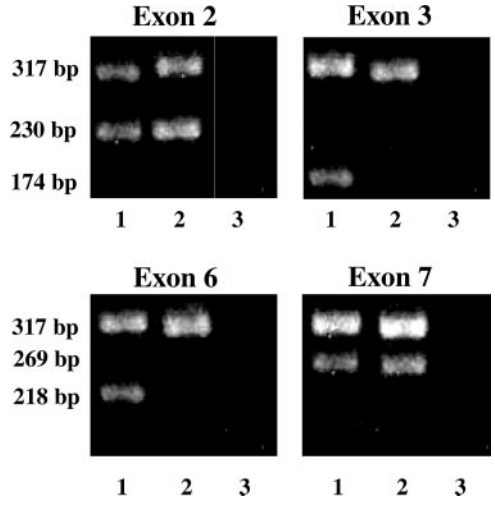

FIG. 1. PCR amplification of exons 2 (230 bp), 3 (174 bp), 6 (218 bp), and 7 (269 bp) of *KAL1* gene in a male patient with Kallmann syndrome. Lanes 1–3 correspond to a normal 46,XY male, affected patient, and negative control, respectively. Exons 2 and 7 were amplified in the patient (lane 2) and control male (lane 1), whereas exons 3 and 6 were amplified only in the male control (lane 1). A product of the *SRY* gene (317bp) was used as control of amplification.

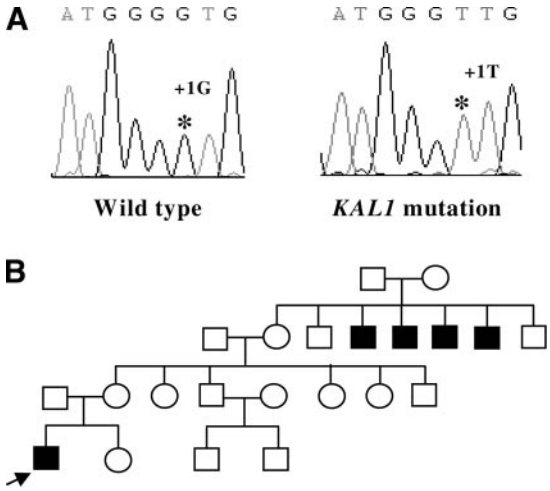

FIG. 2. A, Automatic sequencing showing a *KAL1* mutation IVS7 + 1G>T in a familial case of Kallmann syndrome (*left*) and a normal sequencing (*right*). *Asterisks* indicate the mutated base. B, The pedigree is consistent with X-linked transmission. *Arrow* indicates the proband. *Solid symbols* indicate IHH and olfactory abnormalities.

Discussion

Hypothalamic GnRH plays a key role in gonadotropin secretion induction from the anterior pituitary. The altered GnRH function may be caused by failure in the embryonic migration of GnRH neurons, defective synthesis, secretion, or action of GnRH. The identification of *KAL1*, *FGFR1*, GnRH receptor, and *GPR54* mutations in patients with IHH provided new insights in the developmental organization and regulation of hypothalamic GnRH neurons (2).

Here, we describe novel *KAL1* and *FGFR1* mutations in 11 of 80 IHH patients studied. An intragenic deletion encompassing exons 3–6 and one splicing mutation +1G>T in intron 7 were identified in *KAL1* in two patients with sporadic and familial Kallmann syndrome, respectively. We have previously studied *KAL1* mutations in Brazilian patients with IHH and olfactory abnormalities (22–24). Considering the data from previous and current studies, the prevalence of *KAL1* mutations in Brazilian male patients with Kallmann syndrome was approximately 22% (27% familial and 16% sporadic cases).

TABLE 2. Clinical features, familial history, and *FGFR1* mutations of male patients with hypogonadotropic hypogonadism with and without olfactory abnormalities

Patient	Olfactory status	Age (yr)	Exon	Nucleotide change	Amino acid change	Protein (domain)	MRI of bulbs and sulci	Other clinical features	Familial history
1	Normosmic	17	3	142G>A	G48S	IgI	Normal		Sporadic
2	Hyposmic	23	6	734T>C	L245P	IgII-IgIII linker	Normal	Cleft lip and palate	Sporadic
3	Anosmic	19	7	748C>T	R250W	IgII-IgIII linker	Agenesis of the olfactory bulbs and sulci	Mental deficiency, epilepsy	Maternal cousin with hypogonadism
4	Hyposmic	23	7	748C>T	R250W	IgII-IgIII linker			Sporadic
5	Anosmic	41	8	1028C>T	A343V	IgIII	Hypoplasic bulbs		Sister with hypogonadism and anosmia and anosmic brother
6	Anosmic	17	9	1097C>T	P366 liter	IgIII-TM linker		Obesity, sleep disorder	Two paternal aunts[a] with hypogonadism and anosmia
7	Anosmic	27	13	1852–1853delAA	K618fsX654	TK		Cubitus valgus	Sister[a] with hypogonadism and anosmia and anosmic mother[a]
8	Hyposmic	22	16	2164C>T	P722S	TK		Cleft lip, bimanual synkinesis	Maternal cousin[a] with hypogonadism and hyposmia and affected brother
9	Anosmic	19	18	2383G>A	V795I	C-terminal tail	Normal		Sister[a] with anosmia and normal reproductive axis

C, Carboxyl; TM, transmembrane domain.
[a] *FGFR1* mutations were also found in these affected relatives.

To date, several point mutations, small deletions, and a few single-exon deletions have been identified in Kallmann syndrome patients (9, 12). Large deletions involving more than one exon of *KAL1* have been reported for exons 13–14 (25), 3–5 (26), 5–10 (24, 27), and 3–13 (28). Although the breakpoints were not determined in most cases, the flanking intronic regions of these deletions containing repeated elements might promote nonallelic recombination (24). We were able to identify several elements of SINE and LINE family repeats (such as Alu, L1 and MIR) within intronic sequences that are adjacent to the *KAL1* exons 3–6 deletion using *in silico* analysis. Direct copies of the simple repeat CAAATT, previously identified in the deletion breakpoints involving exons 13–14 of *KAL1*, were also identified (25). These elements have been suggested as putative recombination-promoting factors throughout the genome, which have been associated with several exon and/or gene deletions in humans (29, 30).

Eight novel *FGFR1* heterozygous defects (one frameshift and seven missense mutations) were identified in nine of 80 patients (11.2%) with sporadic or familial IHH. Among the Kallmann syndrome patients, the overall incidence of *FGFR1* mutations was 17% (28% familial and 8% sporadic). In the familial cases, mutations were identified in two autosomal dominant pedigrees and in four families with undetermined inheritance form. The frequency of *FGFR1* mutations observed in this Brazilian series with Kallmann syndrome was significantly higher in the familial than in sporadic cases and slightly higher than the ones found in other populations (11, 14, 15).

The mature FGFR1 protein, one of the four transmembrane receptors for fibroblast growth factor (FGF) ligands, consists of three extracellular Ig-like loops (IgI, IgII, and IgIII), an acid box between the first two Ig loops, a transmembrane domain, and an intracellular split TK domain (31). The FGFR1 mutations described here were widely distributed along the distinct domains of the receptor (Fig. 5). They involved amino acid sequences that are highly conserved across species as well as within the FGFR family, suggesting a critical role of these residues in the receptor signaling pathway. None of these *FGFR1* mutations were found in 200 alleles from the control individuals, supporting the functional significance of these mutations.

One of the major findings reported here is the presence of an *FGFR1* mutation (G48S) in one of 34 unrelated patients with IHH and normal olfaction. The conserved amino acid at position 48 is located in the IgI domain involved in the autoinhibitory function (32). This patient showed no midline defect and had normal sulci and olfactory bulbs at MRI. The apparent normal sense of smell in patients with *FGFR1* defects was previously reported in the description of *FGFR1* haploinsufficiency due to a balanced reciprocal translocation between chromosomes 7 and 8 in a male with IHH (33). However, the lack of olfactory abnormalities was not confirmed by objective test in this study. During the review course of this manuscript, Pitteloud *et al.* (34) reported the analysis of the *FGFR1* gene in seven selected normosmic IHH patients who either belonged to mixed pedigrees containing both Kallmann syndrome and IHH with normal olfaction or had associated midline defects. Heterozygous *FGFR1* point

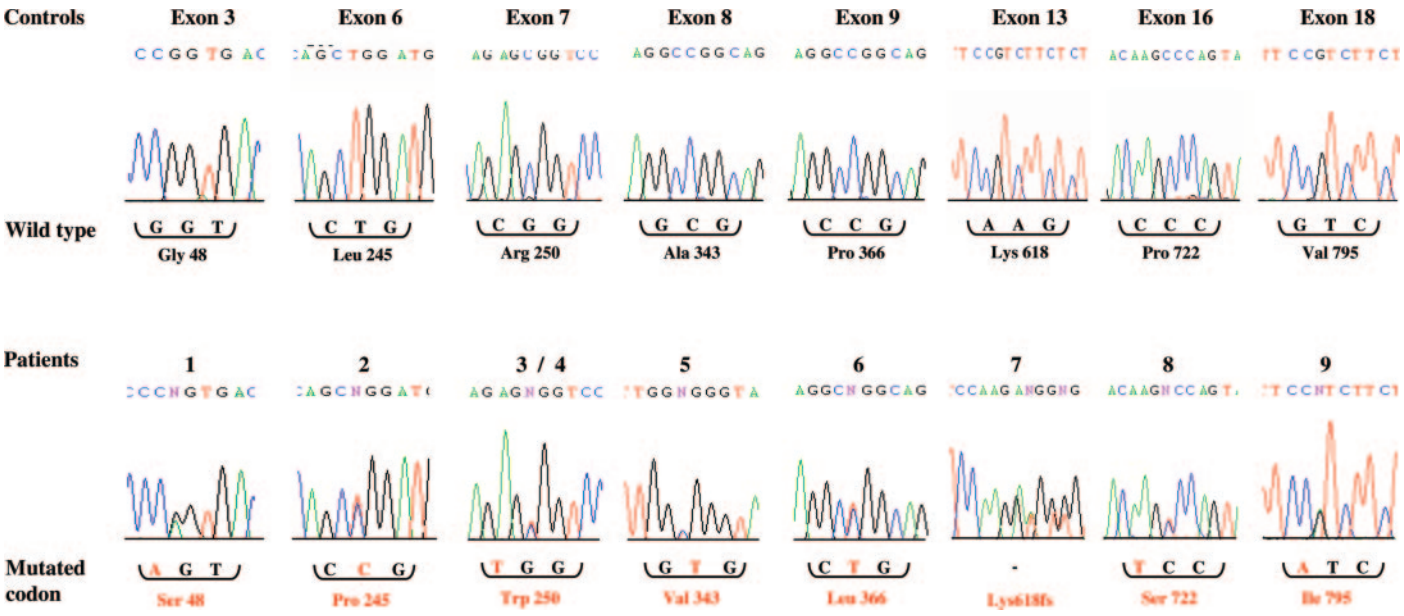

FIG. 3. Automatic sequencing showing heterozygous mutations in *FGFR1* of patients with Kallmann syndrome (cases 2, 3, 4, 5, 6, 7, 8, and 9) and normosmic hypogonadotropic hypogonadism (case 1). The corresponding amino acid is described *below* the codon sequence. Mutated codons and amino acids are indicated in *red*. Normal sequence of *FGFR1* is shown *above*.

mutations were identified in three familial cases of this study, indicating that *FGFR1* mutations also account for some of the mixed pedigrees.

Among the nine *FGFR1* mutations identified in Brazilian patients with IHH, a missense mutation at codon 366, characterized by the substitution of proline by leucine within the linker IgII-IgIII domain of the receptor, was identified in one familial patient (case 6) with Kallmann syndrome (Table 2). This mutation was also identified in his two paternal aunts with Kallmann syndrome as well as in his asymptomatic father. Incomplete penetrance of hypogonadism and/or anosmia and an inter- and intrafamilial variety of phenotypic

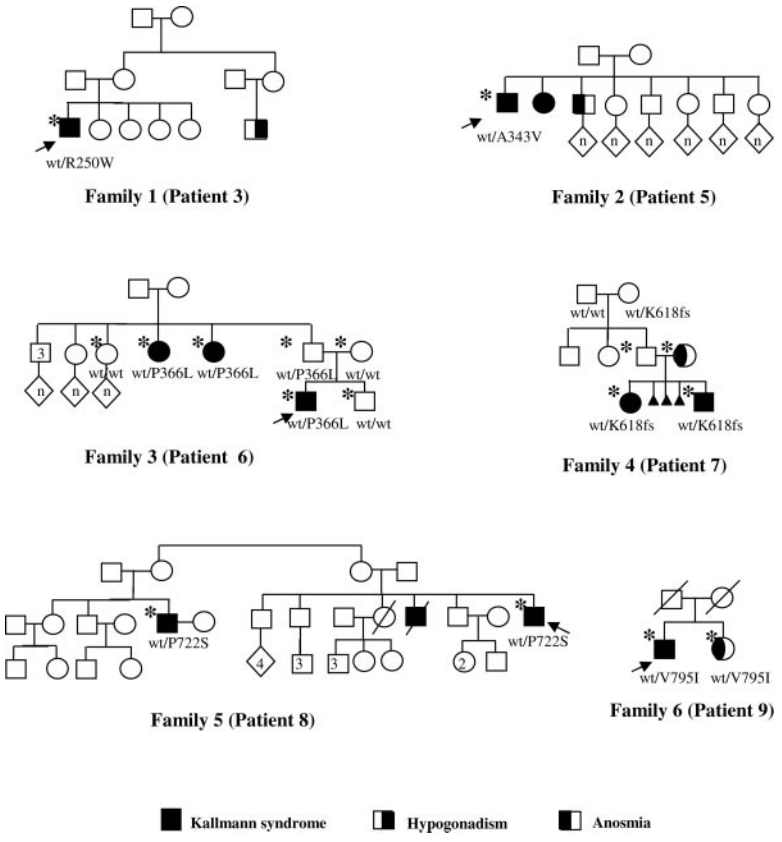

FIG. 4. Pedigrees of six families with Kallmann syndrome due to *FGFR1* mutations. Pedigrees 3 and 4 are consistent with an autosomal dominant pattern of inheritance with incomplete penetrance in the first family. In the other families, the mode of inheritance could not be determined by visual inspection. The proband is identified by the *arrow*. *Squares* denote male subjects, *circles* female subjects, *triangles* spontaneous abortion, *lines through symbols* deceased individual, and *diamonds* sex unknown. The *numbers inside symbols* indicate multiple individuals and "n" indicates unknown number of multiple individuals. All subjects signed with *asterisks* were evaluated by objective olfactory test. Cardinal clinical features of Kallmann syndrome are described in the key.

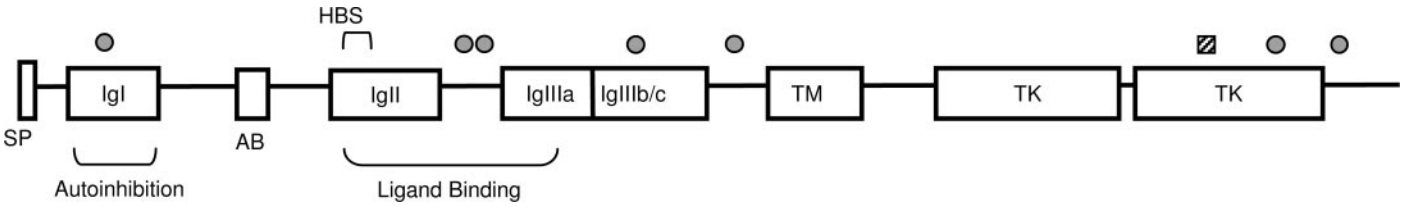

FIG. 5. Structure of FGFR1 containing the functional domains and the distribution of the novel *FGFR1* mutations. All mutations were identified in heterozygous state. *Gray circle*, Missense mutations; ▨, frameshift mutations; SP, signal peptid; AB, acidic box; HBS, heparin binding site; TM, transmembrane domain.

anomalies are frequently described in Kallmann syndrome (35, 36). Recently, one family harboring a nonsense FGFR1 mutation in the TK domain, whose proband presented spontaneous recovery of hypogonadism after androgen replacement therapy, was reported (16). It is possible that other, as yet unidentified factors, such as epigenetic phenomena and/or modifier genes, may compensate the loss-of-function of *FGFR1* and/or *KAL1* and thus prevent full expression of the phenotype.

We also demonstrated the V795I missense mutation in the carboxyl-terminal tail of the FGFR1 in an anosmic woman who exhibited normal pubertal development with normal basal and GnRH-stimulated gonadotropin as well as estradiol levels. She is the sister of a male patient (case 9, family 6) with Kallmann syndrome. In addition, isolated anosmia and an *FGFR1* defect were also identified in another female from family 4. In this family, the frameshift mutation in the catalytic TK domain was found in the mother, who had isolated anosmia, and in her two children—a male propositus (patient 7) and his sister, both affected by Kallmann syndrome. These findings supported the evidence of isolated anosmia as a partial phenotype of *FGFR1* mutations in females (14).

Several nonreproductive phenotypes have been described in patients with Kallmann syndrome (9, 11, 15, 24). Some of these anomalies were largely attributed to the mutated *KAL1* gene, such as bimanual synkinesis. Here, we described the occurrence of bimanual synkinesis in one familial Kallmann syndrome patient (patient 8, Table 2) harboring the P722S mutation in the catalytic TK2 domain of FGFR1. This mutation was also found in his maternal first cousin, who had Kallmann syndrome, but not bimanual synkinesis. Bimanual synkinesis occurs in approximately 75% of patients carrying *KAL1* mutations, but it has rarely been reported in subjects with *FGFR1* mutations (18). To date, patient 8 represents the second reported case of *FGFR1* mutation with bimanual synkinesis. Interestingly, a different mutation (P722H) disturbing the same 722 codon, although combined with a second mutation (N724K) in the same allele, was described in a patient with Kallmann syndrome and dental agenesis, but not bimanual synkinesia. Structural and biochemical studies showed that this double mutation reduced the TK activity of FGFR1 (34).

The FGFR1 signaling is achieved by receptor conformational changes upon ligand binding, leading to dimerization and subsequent activation by autophosphorylation of TK intracellular domains (37). Heparin or heparin sulfate proteoglycan binding is essential for the dimerization and activation of the FGF-FGFR complex (38). However, the mechanisms and effects of loss-of-function FGFR1 mutations are poorly understood. Inactivating *FGFR1* mutations in the IgI might actually augment the autoinhibited state of FGFR1. This region exhibits lower affinity for FGF ligand and heparin but is capable of directly interacting with IgII and IgIII regions, occluding the FGF binding interface and keeping FGFR1 in a "closed" low-activity state (32). FGFR1 variants, located in IgII-IgIII linker and IgIII domain, can interfere with the receptor interaction with the ligands because these regions are predicted to play an important structural role in correct folding of the Ig loops and, consequently, with the ligand binding (39). Additionally, mutations in the transmembrane and juxtamembrane domains of the FGFR1 can affect the rotational positioning of receptor subunits, disrupting a process necessary for biological activity (40).

Recent studies showed that anosmin-1 acts as an FGFR1-specific modulator and coligand that physically interacts with the FGFR1-FGF-heparin sulfate proteoglycan complex and amplifies the resulting downstream signaling responses (17). It is plausible that factors related to anosmin-1 may represent an additional level of complexity in the network of molecules involved in regulation of FGFR signal transduction during development (17). In addition, several studies on the expression patterns of FGF ligands and receptors during central nervous system development indicate the critical role of FGF in the initial generation of neural tissue (19). This activity is also present in the rostral forebrain, directly affecting the olfactory bulb development, which could directly affect the GnRH neuronal migratory activity (41).

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