

Loss-of-function mutations in *FGFR1* cause autosomal dominant Kallmann syndrome

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We took advantage of overlapping interstitial deletions at chromosome 8p11–p12 in two individuals with contiguous gene syndromes and defined an interval of roughly 540 kb associated with a dominant form of Kallmann syndrome, *KAL2*. We establish here that loss-of-function mutations in *FGFR1* underlie *KAL2* whereas a gain-of-function mutation in *FGFR1* has been shown to cause a form of craniosynostosis. Moreover, we suggest that the *KAL1* gene product, the extracellular matrix protein anosmin-1, is involved in FGF signaling and propose that the gender difference in anosmin-1 dosage (because *KAL1* partially escapes X inactivation) explains the higher prevalence of the disease in males.

Kallmann syndrome involves hypogonadotropic hypogonadism and anosmia, a deficiency of the sense of smell. Anosmia is related to the absence or hypoplasia of the olfactory bulbs and tracts. Hypogonadism is due to deficiency in gonadotropin-releasing hormone (GnRH) and probably results from a failure of embryonic migration of neurons that synthesize GnRH. These cells normally migrate from the olfactory epithelium to the forebrain along the olfactory nerve pathway¹. Familial cases of Kallmann syndrome have been reported^{2–4}, which suggested X-chromosome linked, autosomal dominant, or, less often, recessive modes of inheritance of the disease. Two intriguing points have been noted: sporadic cases are much more numerous than familial cases, and the disease has a much higher prevalence in males (1:10,000) than in females (the sex ratio has been estimated at 5:1). The gene underlying the X-linked form of the disease (*KAL1*) has been identified¹. But in many families with only males affected and presumably maternal transmission of the disease, no mutation has been found in the *KAL1* coding region. Moreover, a molecular analysis of the gene in sporadic male cases rarely identified a mutation (ref. 4 and C.D., unpublished results). This suggests that mutations in the X-linked gene *KAL1* do not account for the higher prevalence of the disease in males. Because the incomplete penetrance of the disease^{2,3} and the infertility in affected individuals impede linkage analysis, the genes underlying the autosomal forms of Kallmann syndrome have so far escaped identification.

By segregation analysis of polymorphic markers combined with fluorescence *in situ* hybridization (FISH) analysis, we determined that two *de novo* deletions of roughly 10.7 Mb and 11.6 Mb at chromosome 8p11.2–p12 in two individuals⁵ who are affected by different contiguous gene

syndromes that both include Kallmann syndrome overlap by approximately 540 kb (see Supplementary Notes 1 and 2 and Supplementary Fig. 1 online). Three genes have been reported in this interval; from telomere to centromere, they are *WHSC1L1* (OMIM 607083), *FGFR1* (OMIM 136350) and the first two exons of *TAC1* (OMIM 605301). *FGFR1* encodes the fibroblast growth factor receptor 1 and was considered the best candidate to underlie Kallmann syndrome because of a possible functional interaction with the *KAL1* gene product.

Southern-blot analysis of 43 individuals with familial or sporadic Kallmann syndrome using two genomic probes encompassing exons 4–5 and 16–18 of *FGFR1* did not show a deletion of the gene in any of them (data not shown). We then determined the nucleotide sequence of the 18 coding exons and splice sites of *FGFR1* in 129 unrelated individuals with Kallmann syndrome (91 males and 38

females). In four familial cases (Fig. 1) and eight sporadic cases, we detected heterozygous mutations of *FGFR1*: one nonsense mutation, two frameshift mutations, two donor splice-site mutations and seven missense mutations. In addition, we found that one individual, who was born to consanguineous parents and is affected by Kallmann syndrome with cleft palate, agenesis of the corpus callosum, unilateral hearing loss and fusion of the fourth and fifth metacarpal bones, was homozygous with respect to a deleterious missense mutation (Table 1 and Supplementary Fig. 2 online). From these results, we conclude that *FGFR1* underlies one autosomal dominant form of Kallmann syndrome (*KAL2*). Moreover, cleft palate or lip and dental agenesis, two anomalies that are occasionally associated with Kallmann syndrome^{1–3} and were present in five individuals with mutations in *FGFR1* (Table 1), can now be ascribed to this genetic form of the disease. We also found that bimanual synkinesia, identified in one family with *KAL2* (Table 1), can be observed in autosomal Kallmann syndrome, whereas it had thus far been considered to be specific to the X-linked form of the disease.

FGF signaling is involved in a variety of developmental processes including the formation, growth and shaping of tissues and organs. Extracellular interaction between FGF, the FGF receptor and heparan sulfate proteoglycans (HSPGs) is required for receptor dimerization and resulting autophosphorylation of several tyrosine residues in the intracellular domain. The phosphotyrosines either stimulate protein tyrosine kinase activity of the receptor or serve as docking sites for downstream signaling molecules. A

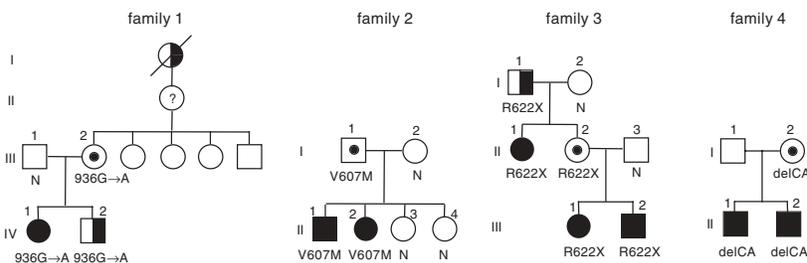


Fig. 1 Segregation of the mutations in *FGFR1* in four families affected with Kallmann syndrome. Filled symbols indicate clinically affected individuals with both hypogonadism and anosmia. Half-filled symbols indicate individuals with anosmia only. Open symbols containing a black dot indicate unaffected carriers of the mutation. N indicates absence of the mutation. In family 1, individual IV2 (who was 16 years old) underwent spontaneous puberty but suffers from anosmia; individuals III2, IV1 and IV2 have multiple dental agenesis. In family 2, individuals II1 and II2 both have bimanual synkinesia. In family 3, individual III1 has a cleft lip and individual III2 has both a cleft lip and a cleft palate. Notably, non-penetrance of the disease in some mutation carriers can simulate recessive transmission of Kallmann syndrome in certain families, for example, families 1, 2 and 4. Diagonal line indicates deceased individual; question mark indicates unknown clinical status.

Table 1 • Mutations in *FGFR1* associated with Kallmann syndrome

Mutation	Exon/Intron	Kallmann syndrome associated symptoms
G97D	exon 3	
Y99C	exon 3	
303–304insCC	exon 3	
A167S	exon 5	cleft palate, corpus callosum agenesis, unilateral hearing loss, fusion of the fourth and fifth metacarpal bones
C277Y	exon 7	
936G→A	exon 7 (donor splice site)	multiple dental agenesis
V607M	exon 13	bimanual synkinesia
R622X	exon 14	cleft lip or palate
1970–1971delCA	exon 14	
W666R	exon 15	cleft palate
IVS15+1G→A	intron 15 (donor splice site)	
M719R	exon 16	
P772S	exon 18	cleft palate, unilateral absence of nasal cartilage, iris coloboma

All the mutations were present in the heterozygous state except for A167S, which was detected in an individual born of consanguineous parents. Four of the mutations were found in individuals with familial cases of the disease (Fig. 1). The mutation 936G→A was found in two affected siblings (male and female) and their unaffected mother; all three had seven to eight teeth missing. The presumed pathogenic effect of this synonymous substitution on splicing has not been formally shown. The amino-acid substitution V607M was present in two affected siblings (male and female) and their unaffected father; both affected individuals had mirror movements of the hands (bimanual synkinesia). The mutation R622X was detected in four affected individuals in one family, two of whom also had cleft palate or cleft lip. Finally, the mutation 1970–1971delCA was found in two affected brothers and was also present in their unaffected mother. The amino-acid substitutions G97D, Y99C, A167S, C277Y, W666R, M719R and P772S and the mutations 303–304insCC and IVS15+1G→A were found in sporadic cases (seven males and two females). The amino-acid substitution C277Y was also present in the individual's unaffected mother. In addition, one female (with the amino-acid substitution W666R) and one male (with the amino-acid substitution P772S) had undergone surgery for a cleft palate in infancy, and the male also had absence of the nasal cartilage on the right and iris coloboma. All missense mutations were absent in 100 chromosomes from unrelated Caucasian individuals. Four of the amino-acid substitutions (V607M, W666R, M719R, P772S) affect residues that are located in the intracellular part of the receptor, whereas the other four (G97D, Y99C, A167S, C277Y) affect extracellular residues (see Supplementary Fig. 2 online). The amino-acid substitution Y99C creates a novel cysteine residue close to Cys101, which is expected to disrupt the normal disulfide bond of the first Ig-like domain; notably, this mutation is the equivalent of the Y105C substitution in *FGFR2* that has been found in two individuals with craniosynostosis. The amino-acid substitution A167S (in the second Ig-like domain) alters a residue that has been shown to interact with FGF2 (ref. 15) and is located in the domain of interaction with HSPGs. The amino-acid substitution C277Y affects one of the two cysteines that form the disulfide bond of the third Ig-like domain; notably, a missense mutation in *FGFR2* that affects the corresponding cysteine residue (C278F) causes craniosynostosis.

dominant gain-of-function mutation of *FGFR1* underlies a form of craniosynostosis⁶. This mutation is believed to result in enhanced affinity for certain FGF ligands⁷. In contrast, *KAL2* results from loss-of-function mutations in *FGFR1*, as shown by the presence of a single allele in the individuals carrying a chromosomal deletion at 8p11.2. Moreover, some of the mutations associated with *KAL2* could hinder the formation of functional receptor dimers by a dominant negative effect. Whatever the molecular mechanism may be, our results indicate that olfactory bulb development in humans is crucially sensitive to reduced dosage of *FGFR1*. Dominant gain-of-function and loss-of-function mutations in *FGFR1* cause different developmental disorders: premature fusion of skull bone sutures (craniosynostosis) and failed morphogenesis of the olfactory bulbs (Kallmann syndrome), respectively. Given the phenotype associated with gain-of-function mutation of *FGFR1*, it is noteworthy that delayed closure of calvarial sutures has not been reported in Kallmann syndrome.

The implication of *FGFR1* and *KAL1* in the same developmental disease raises the possibility that the gene products functionally interact. *KAL1* encodes anosmin-1, a locally restricted protein of roughly 100 kDa in embryonic extracel-

lular matrices⁸. Several arguments suggest that anosmin-1 is involved in FGF signaling through *FGFR1*. First, anosmin-1 binds to HSPGs⁹, and HSPGs are important in the dimerization of the binary FGF–*FGFR* complex¹⁰. Also, the axon-branching phenotype in some neurons in *Caenorhabditis elegans* that mis- or overexpress the nematode gene *kall1* was no longer present in mutant worms lacking heparan 6O-sulfotransferase, an enzyme involved in HSPG biosynthesis^{11,12}. Second, *KAL1* and *FGFR1* are coexpressed at different sites during embryonic development. *KAL1* is expressed in the presumptive olfactory bulbs⁸, and *Fgfr1* expression in the rostral forebrain is required for initial olfactory bulb evagination in the mouse¹³. In addition, cleft palate or cleft lip was present in several individuals with *KAL2* (Table 1), and a high arched palate, which can be regarded as a mild anomaly of palatal fusion, is a common feature of *KAL1*. Finally, the bimanual synkinesia that we report in one family with *KAL2* has been observed in 75% of *KAL1* cases.

Regarding the low prevalence of the X-linked form of the disease⁴, the proposal that *KAL1* and *FGFR1* functionally interact provides a possible explanation for the higher prevalence of Kallmann syndrome in males. The explanation is based on the

assumption that the local concentration of anosmin-1 is important in FGF signaling and the fact that *KAL1* partially escapes X inactivation in females¹⁴. Accordingly, females are expected to synthesize a higher amount of anosmin-1 than do males; in some cases, this could be enough to maintain FGF signaling above the critical threshold in the context of *FGFR1* haploinsufficiency. Indeed, in four of five families in whom we were able to follow the transmission of the *FGFR1* mutation, the mutation was transmitted by the unaffected mother (Table 1). Moreover, the probable pseudoautosomal location of *Kal1* in rodents can account for the observation that male mice heterozygous with respect to a null allele at the *Fgfr1* locus are viable and fertile¹³.

Note: Supplementary information is available on the Nature Genetics website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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