Isolated congenital anosmia locus maps to 18p11.23-q12.2

M Ghadami, S Morovvati, K Majidzadeh-A, E Damavandi, G Nishimura, A Kinoshita, P Pasalar, K Komatsu, M T Najafi, N Niikawa, K Yoshiura

Isolated congenital anosmia (MIM 107200) is a very rare condition characterised by a complete smelling defect that is present from birth in otherwise normal subjects. To our knowledge, nine sporadic cases of isolated congenital anosmia have been known,¹² and patients reported by Lygonis³ and those by us⁴ were only familial isolated congenital anosmia. Other cases of familial congenital anosmia had some additional manifestations⁴⁺ or had Kallmann syndrome. The defective smelling in isolated congenital anosmia may be attributed to the absence of olfactory function—that is, either replacement of the olfactory epithelium by respiratory epithelium,³ or aplasia of the olfactory bulbs, sulci, and tract.¹ Diagnosis of isolated congenital anosmia is made by one or more of history, physical examination, a standardised smelling test, computed tomography, magnetic resonance imaging, and biopsy of the nasal mucous tissue. Patients with isolated congenital anosmia had been unable to smell as back as they could remember, and had no history of other causes of anosmia, such as significant head trauma, neoplasm involving the olfactory system, or upper respiratory infection leading to damage of the olfactory epithelium. Physical examinations are useful to exclude an association of anosmia with other symptoms and to exclude secondary anosmia. A standardised smelling test confirms complete olfactory dysfunction. Computed tomography and magnetic resonance imaging may disclose other anomalies of the central nervous system, and biopsy may show abnormal replacement of the olfactory epithelium. An autosomal dominant mode of inheritance was suggested in a family reported by Lygonis³ and two families by us.¹ However, nothing has been known for the disease gene localisation. Here we report the result of a genome-wide linkage analysis of the two unrelated Iranian families.

MATERIALS AND METHODS

Families and patients

Two unrelated Iranian families with isolated congenital anosmia (families 1 and 2), reported previously,⁷ were analysed in this study. Family 1 consisted of three affected members in three generations, and family 2 contained nine patients in two generations. A total of 54 members of the two families agreed to participate in this study and seven of them had isolated congenital anosmia.

Genotyping, linkage analysis, and sequencing

Genomic DNA was extracted from whole blood of the 54 family members, and PCR was performed using standard methods. The amplified samples were genotyped at 400 microsatellite marker loci with an average distance of 10 cm in the genome (Linkage Mapping Set version 2 (LMS-MD10, Applied Biosystems, Foster City, CA) on a DNA sequencer (Model 377, Applied Biosystems), and marker alleles were assigned using software, Genescan™ and Genotyper™ (Applied Biosystems). When data assigned to a chromosomal region were obtained, additional markers around the critical region were used to confirm a linkage and narrow the region.

Two point linkage analysis was performed under an assumption that isolated congenital anosmia in the two families is inherited as an autosomal dominant fashion with incomplete penetrance. Lod scores were calculated separately in each family, at the same penetrance, using the computer program MLINK of FASTLINK version 4.1P.¹⁰⁻¹² Allele frequencies at the 400 marker loci were set as 1/N (where N is present from birth in otherwise normal subjects. To our knowledge, nine sporadic cases of isolated congenital anosmia have been known,¹² and patients reported by Lygonis³ and those by us⁴ were only familial isolated congenital anosmia. Other cases of familial congenital anosmia had some additional manifestations⁴⁺ or had Kallmann syndrome. The defective smelling in isolated congenital anosmia may be attributed to the absence of olfactory function—that is, either replacement of the olfactory epithelium by respiratory epithelium,³ or aplasia of the olfactory bulbs, sulci, and tract.¹ Diagnosis of isolated congenital anosmia is made by one or more of history, physical examination, a standardised smelling test, computed tomography, magnetic resonance imaging, and biopsy of the nasal mucous tissue. Patients with isolated congenital anosmia had been unable to smell as back as they could remember, and had no history of other causes of anosmia, such as significant head trauma, neoplasm involving the olfactory system, or upper respiratory infection leading to damage of the olfactory epithelium. Physical examinations are useful to exclude an association of anosmia with other symptoms and to exclude secondary anosmia. A standardised smelling test confirms complete olfactory dysfunction. Computed tomography and magnetic resonance imaging may disclose other anomalies of the central nervous system, and biopsy may show abnormal replacement of the olfactory epithelium. An autosomal dominant mode of inheritance was suggested in a family reported by Lygonis³ and two families by us.¹ However, nothing has been known for the disease gene localisation. Here we report the result of a genome-wide linkage analysis of the two unrelated Iranian families.

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Key points

- Isolated congenital anosmia is a condition characterised by lifelong inability to smell in otherwise normal individuals.
- We performed a genome-wide linkage analysis of two unrelated Iranian families in which a total of 54 members were available for this study and seven of them had isolated congenital anosmia.
- In both families, the isolated congenital anosmia trait appeared to be inherited as an autosomal dominant fashion with incomplete penetrance.
- Two point linkage analysis revealed a maximum lod score of 5.14 (recombination fraction 0=0.00) at penetrance of 0.8 for the D18S1108 microsatellite marker locus.
- Haplotype analysis revealed that all the affected individuals shared each common haplotype for each family between D18S452 and D18S475 at the 18p11.23-q12.2 region.
- Although all exon and exon-intron boundaries of eight candidate genes, GNAL, VAPA, PTPRM, PTPN2, CABYR, RNMT, CDH2, and NOL4, mapped within the region, were sequenced, no mutations were found in any affected family members.

(G-1 in family 1, and I-2, I-10, and II-13 in family 2) were presumed by their relatives’ recollection.
Figure 1  Pedigrees of families 1 and 2 with haplotypes at marker loci on chromosome 18p11.23-q12.2. Solid squares and circles show individuals with isolated congenital anosmia. Numbers in open boxes show a possible disease associated haplotype. Heavy short lines indicate definite recombination sites, and heavy double short lines depict recombination sites that could have occurred on either side of the corresponding markers. Haplotypes of I-1 in family 1, and I-2 and I-3 in family 2 are deduced from those in their offspring. Asterisks indicate alleles showing novel microsatellite mutations.
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D18S452 recombinant events were observed in a patient (II-8) of recombination sites was not possible in this patient. Two D18S452 loci between each had a common haplotype including alleles at 35 marker analysis demonstrated that affected members in each family recombination fraction to be 0.00 (table 1). Haplotype penetrance was set at 0.8 for both families, and the for family 1, and 3.29 for family 2) at D18S1108 D18S53 ruled out normally because of its X-linked inheritance. A locus in the two families. The other locus, KAL3, was also Kallmann syndrome types 1 and 2 (Xp22.3 for KAL1 and 45.9 cM interval between D18S452 and D18S474 at 18p11.23-q12.2. The sequence analysis of the eight genes or expressed sequence tags revealed no mutation in any affected members of the two families.

**DISCUSSION**

Isolated congenital anosmia in our two families is compatible with an autosomal dominant pattern of inheritance with incomplete penetrance. Individuals II-5 in family 1, III-28 and III-37 in family 2 are definite non-penetrants, who inherited the disease-associated haplotype but had a normal sense of smell, as determined both by past medical history and a smelling test. The isolated congenital anosmia gene was transmitted by one of them (II-5 in family 1) to his affected son (III-16), but the other two non-penetrants did not have any children. Five unaffected members (III-4 and III-14 in family 1, and III-16, III-22, and III-27, in family 2) inherited a part of the critical haplotype (fig 1). However, it remains unknown who are non-penetrants or normal individuals, because none of them had any affected children. Inheritance patterns in family 2 appeared unusual; especially those in its third generation in which none of a total of 25 children of patients were affected (fig 1). This seemingly strange observation may be explained by incomplete penetrance, or just by chance as suggested in our previous report, or the existence of a modifier gene.

We have assigned the isolated congenital anosmia locus in the two families to the 45.9 cM interval between D18S542 and D18S474 at 18p11.23-q12.2. However, this interval is the maximum extent estimated under an assumption that all the seven unaffected members carrying the critical haplotype were non-penetrants or carriers. The interval might be reduced to 51.2 cM, if the recombination in IV-12 of family 1 had occurred between D18S464 and D18S1153 (fig 1). Among several genes and expressed sequence tags that are expressed in the central nervous system and have been assigned within the isolated congenital anosmia region, the GNL (or the G protein subunit, G (olf)) gene (guanine nucleotide binding protein, λ activating activity polypeptide, olfactory type) located between D18S53 and D18S71 is one of the most interesting candidate genes for isolated congenital anosmia, since mice homozygous for a null mutation in Gnl are anosmic, showing reduced electrophysiological response of the primary olfactory sensory neurones to odours. However, by our preliminary study of affected members of the two families, no mutation has been found in this gene, or in seven other genes, VAPA, PTPRM, PTPN2, CABYR, RNMT, CDH2, and NOL4, all located within the critical region.

The sense of smell is one of the first developed special sense organs in animals and plays a role in feeding, differentiation of a parent from others, and in reproductive behaviour. Male mice do not show mating behaviours or sexual development when their olfactory bulbs are removed shortly after birth. The role of the human olfactory system in reproduction and sexual development is supported by clinical features of Kallmann syndrome in which congenital anosmia is associated with hypogonadotropic hypogonadism. The existence of familial isolated congenital anosmia with a normal reproductive system, as has been shown in affected members of our families, may indicate the independent migration of the olfactory system from the LH-RH cells, and suggest that other factors may influence the relationship between the reproductive and olfactory systems. Isolation of the putative isolated congenital anosmia gene and its functional analysis will contribute to

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**Table 1** Two point lod scores of representative marker loci at 18p11.23-q12.2 in two families with isolated congenital anosmia

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lod score at $\theta$</th>
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<tr>
<td></td>
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<tr>
<td>D18S452</td>
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<tr>
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<tr>
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<tr>
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<tr>
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Penetrance ($p$) of 0.8 was used for both families.
the better understanding of the development of the olfactory system.

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Conflicts of interest: none declared.

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