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

LETTER TO JMG

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,1,2 and patients reported by Lygonis3 and those by us4 were only familial isolated congenital anosmia. Other cases of familial congenital anosmia had some additional manifestations5–9 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,1 or aplasia of the olfactory bulbs, sulci, and tract.1 Diagnosis of isolated congenital anosmia is made by one or more of history, physical examinations, 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.4 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 the present 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 the present study with written informed consent. Of the 54 members, seven were diagnosed as having isolated congenital anosmia (fig 1). By clinical history, physical examination, and smelling testing by intravenous injection of combined vitamins (AlaminTM, Takeda Pharmaceutical Co Ltd, Japan), the disease was confirmed in each affected member. All affected people had a history of never being able to smell, and had never had hypogonadism signs, nasal polyposis, rhinoscleroma, or any underlying infections or other neurological disorders. Clinical manifestations of deceased persons (I-1 in family 1, and I-2, I-10, and II-13 in family 2) were presumed by their relatives’ recollection.

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, GenescanTM and GenotyperTM (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 (fig 1).

Two point linkage analysis was performed under an assumption that isolated congenital anosmia in the two families is inherited in 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

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 θ=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.
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.
Isolated anosmia maps to 18p11.23-q12.2

Family 2

I

II

III

IV

www.jmedgenet.com
isolation 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 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 D18S452 and D18S457 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 GNAL (or the G protein ζ subunit, G (olf)) gene (guanine nucleotide binding protein, ζ 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 Gnai 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

### Table 1

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lod score at 0.00</th>
<th>Lod score at 0.05</th>
<th>Lod score at 0.10</th>
<th>Lod score at 0.15</th>
<th>Lod score at 0.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>D18S452</td>
<td>4.94</td>
<td>2.29</td>
<td>0.21</td>
<td>0.25</td>
<td>0.44</td>
</tr>
<tr>
<td>D18S471</td>
<td>6.33</td>
<td>3.51</td>
<td>1.83</td>
<td>1.94</td>
<td>0.42</td>
</tr>
<tr>
<td>D18S452</td>
<td>9.52</td>
<td>4.74</td>
<td>1.09</td>
<td>0.24</td>
<td>0.35</td>
</tr>
<tr>
<td>D18S1163</td>
<td>3.17</td>
<td>2.56</td>
<td>2.93</td>
<td>2.61</td>
<td>2.22</td>
</tr>
<tr>
<td>D18S464</td>
<td>0.11</td>
<td>0.54</td>
<td>0.79</td>
<td>0.98</td>
<td>0.43</td>
</tr>
<tr>
<td>D18S1152</td>
<td>0.98</td>
<td>0.55</td>
<td>0.85</td>
<td>0.97</td>
<td>0.69</td>
</tr>
<tr>
<td>D18S1150</td>
<td>3.91</td>
<td>3.63</td>
<td>3.31</td>
<td>2.95</td>
<td>2.55</td>
</tr>
<tr>
<td>D18S53</td>
<td>3.03</td>
<td>3.07</td>
<td>2.65</td>
<td>2.37</td>
<td>2.94</td>
</tr>
<tr>
<td>D18S453</td>
<td>3.75</td>
<td>3.75</td>
<td>3.67</td>
<td>3.32</td>
<td>3.04</td>
</tr>
<tr>
<td>D18S571</td>
<td>3.07</td>
<td>3.99</td>
<td>3.58</td>
<td>3.15</td>
<td>2.84</td>
</tr>
<tr>
<td>D18S1104</td>
<td>1.59</td>
<td>1.59</td>
<td>1.51</td>
<td>1.41</td>
<td>1.21</td>
</tr>
<tr>
<td>D18S1101</td>
<td>3.06</td>
<td>4.06</td>
<td>3.78</td>
<td>3.44</td>
<td>3.06</td>
</tr>
<tr>
<td>D18S1107</td>
<td>4.55</td>
<td>4.54</td>
<td>4.19</td>
<td>3.76</td>
<td>3.25</td>
</tr>
<tr>
<td>D18S1108</td>
<td>5.14</td>
<td>5.11</td>
<td>4.73</td>
<td>4.29</td>
<td>3.81</td>
</tr>
<tr>
<td>D18S480</td>
<td>1.38</td>
<td>1.38</td>
<td>1.31</td>
<td>1.24</td>
<td>1.12</td>
</tr>
<tr>
<td>D18S66</td>
<td>2.42</td>
<td>4.42</td>
<td>4.02</td>
<td>3.62</td>
<td>3.16</td>
</tr>
<tr>
<td>D18S463</td>
<td>3.29</td>
<td>3.28</td>
<td>3.01</td>
<td>2.70</td>
<td>2.32</td>
</tr>
<tr>
<td>D18S556</td>
<td>3.28</td>
<td>2.62</td>
<td>2.41</td>
<td>2.02</td>
<td>1.80</td>
</tr>
<tr>
<td>D18S1133</td>
<td>3.47</td>
<td>3.45</td>
<td>3.31</td>
<td>3.03</td>
<td>2.68</td>
</tr>
<tr>
<td>D18S1102</td>
<td>3.28</td>
<td>3.28</td>
<td>3.02</td>
<td>2.68</td>
<td>2.31</td>
</tr>
<tr>
<td>D18S1139</td>
<td>3.16</td>
<td>3.16</td>
<td>3.07</td>
<td>2.78</td>
<td>2.63</td>
</tr>
<tr>
<td>D18S57</td>
<td>3.61</td>
<td>3.61</td>
<td>3.42</td>
<td>3.27</td>
<td>2.79</td>
</tr>
<tr>
<td>D18S1128</td>
<td>1.01</td>
<td>1.03</td>
<td>1.11</td>
<td>1.16</td>
<td>1.03</td>
</tr>
<tr>
<td>D18S475</td>
<td>3.71</td>
<td>3.71</td>
<td>3.51</td>
<td>3.27</td>
<td>2.84</td>
</tr>
<tr>
<td>D18S1157</td>
<td>4.45</td>
<td>4.45</td>
<td>4.16</td>
<td>3.62</td>
<td>3.25</td>
</tr>
</tbody>
</table>

Penetrance (p) of 0.8 was used for both families.
the better understanding of the development of the olfactory system.

ACKNOWLEDGEMENTS

We are indebted to the family members for their participation in this research. We also thank Dr Arthur L Beaudet at Baylor College of Medicine for his critical review of the manuscript and Miss Naoko Yanai at the Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences for her great assistance.

Authors’ affiliations

M Ghadami, A Kinoshita, K Komatsu, N Niikawa, K Yoshiura, Department of Human Genetics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

M Ghadami, Department of Medical Genetics

P Pasalar, Department of Biochemistry, Faculty of Medicine; Tehran University of Medical Sciences

M T Najafi, K Majidzadeh-A, Iranian Academic Centre for Education, Culture and Research, Tehran Medical Sciences Branch

S Morovvati, E Damavandi, Baghiyatollah University of Medical Sciences, Tehran, Iran

G Nishimura, Tokyo Metropolitan Kiyose Children’s Hospital, Tokyo, Japan

M Ghadami, N Niikawa, K Yoshiura, CREST, Japan Science and Technology Agency, Kawaguchi, Japan

NN was supported in part by a Grant-in-Aid for Scientific Research (Category S, No 13854024) from the Ministry of Education, Sports, Culture, Science, and Technology of Japan, and by CREST from the Japan Science and Technology Agency (JST).

Conflicts of interest: none declared.

Correspondence to: M Ghadami, MD, PhD, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza Rm T613, Houston Tx 77030, USA; mghadami@bcm.tmc.edu, or (cc) mghadami@yahoo.com

REFERENCES


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

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

*J Med Genet* 2004 41: 299-303
doi: 10.1136/jmg.2003.015313

Updated information and services can be found at:
http://jmg.bmj.com/content/41/4/299

These include:

**References**

This article cites 14 articles, 2 of which you can access for free at:
http://jmg.bmj.com/content/41/4/299#BIBL

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**

Articles on similar topics can be found in the following collections

- Clinical diagnostic tests (356)
- Surgery (105)
- Surgical diagnostic tests (105)
- Dermatology (240)
- Ethics (220)
- Molecular genetics (1254)
- TB and other respiratory infections (24)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/