LETTER TO JMG

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

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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 Lygonis[3] and those by us[4] were only familial isolated congenital anosmia. Other cases of familial congenital anosmia had some additional manifestations[5–9] 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,[2] 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 Lygonis[3] 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.

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 $\theta = 0.00$) 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.

MATERIALS AND METHODS

Families and patients

Two unrelated Iranian families with isolated congenital anosmia (families 1 and 2), reported previously,[4] 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 (Alinamin™, 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, 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 (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

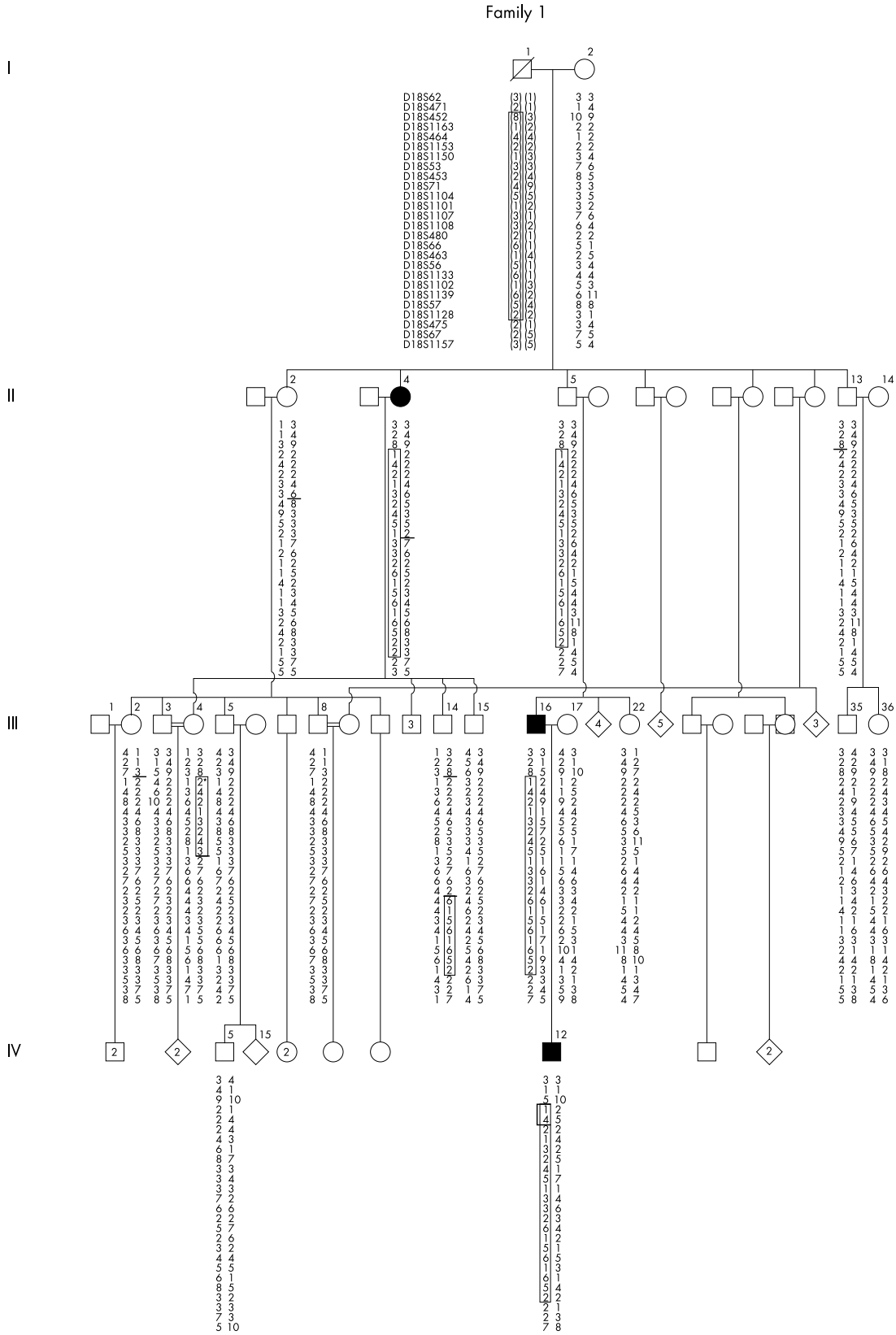

Figure 1 Pedigrees of families 1 and 2 with haplotypes at marker loci on chromosome 18p11.23-q12.2. Solid squares and circles show individuals with isolated congenital anosmia. Numbers in open boxes show a possible disease associated haplotype. Heavy short lines indicate definite recombination sites, 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.

Family 2

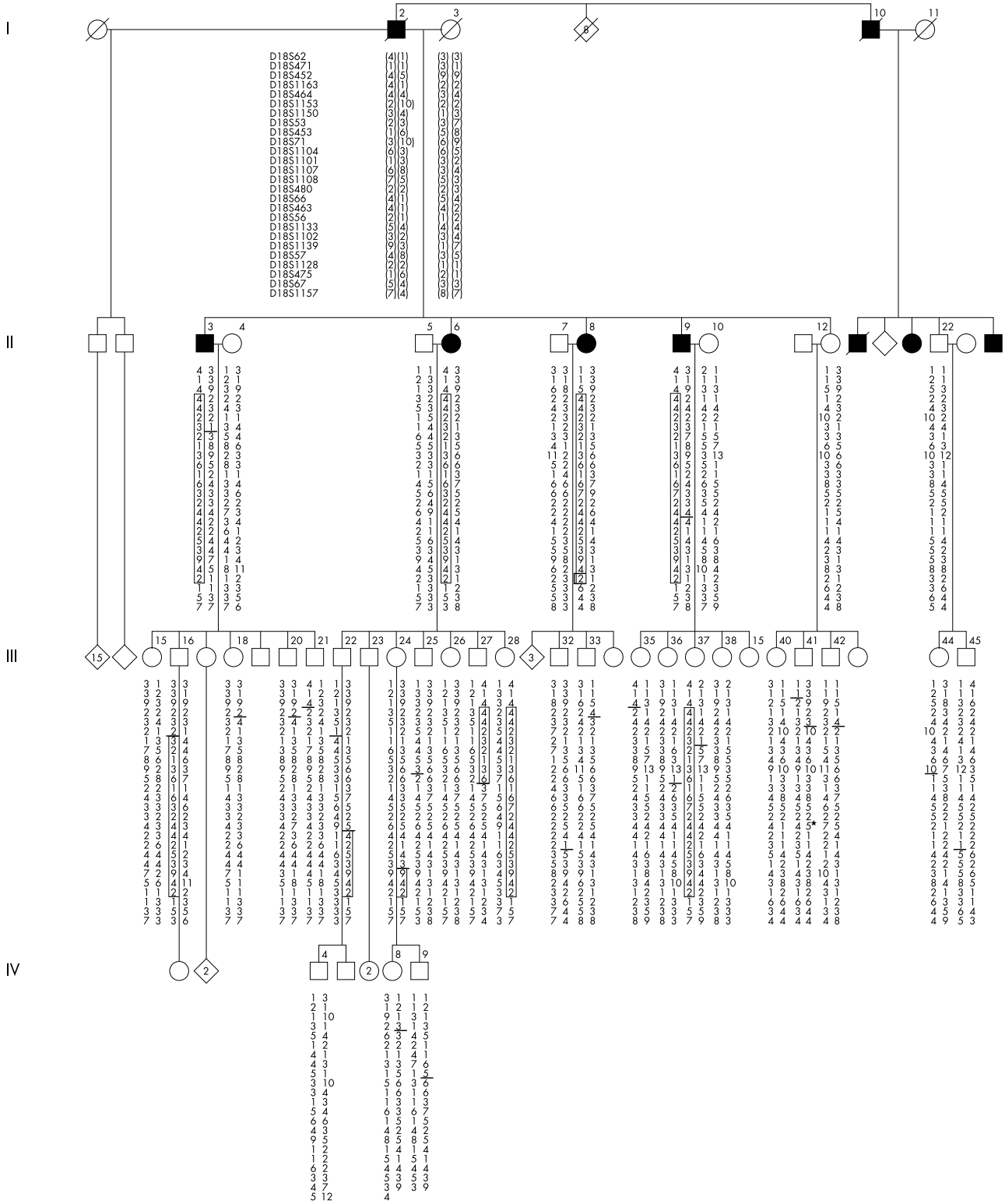
I
II
III
IV
D18S62
D18S471
D18S452
D18S1163
D18S464
D18S1153
D18S1150
D18S53
D18S453
D18S71
D18S1104
D18S1101
D18S1107
D18S1108
D18S480
D18S66
D18S463
D18S56
D18S1133
D18S1102
D18S1139
D18S57
D18S1128
D18S475
D18S67
D18S1157

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

	Lod score at θ					
Locus	0.00	0.001	0.05	0.10	0.15	0.20
D18S62	−4.94	−2.29	−0.21	0.25	0.44	0.51
D18S471	−6.33	−4.51	−1.83	−1.24	−0.74	−0.42
D18S452	−9.52	−4.74	−1.09	−0.24	0.21	0.35
D18S1163	3.17	3.16	2.93	2.61	2.22	1.77
D18S464	−0.11	−0.09	−0.01	0.01	0.08	0.14
D18S1153	0.98	0.97	0.85	0.79	0.69	0.63
D18S1150	3.91	3.91	3.63	3.31	2.95	2.55
D18S53	3.03	3.01	2.65	2.37	2.28	1.94
D18S453	3.75	3.75	3.57	3.32	3.01	2.63
D18S71	3.99	3.99	3.81	3.53	3.21	2.81
D18S1104	1.59	1.59	1.51	1.41	1.26	1.11
D18S1101	4.06	4.06	3.78	3.44	3.06	2.64
D18S1107	4.55	4.54	4.19	3.76	3.32	2.83
D18S1108	5.14	5.11	4.73	4.29	3.81	3.29
D18S480	1.38	1.38	1.31	1.24	1.12	1.07
D18S66	4.43	4.42	4.02	3.62	3.16	2.69
D18S463	3.29	3.28	3.01	2.70	2.32	2.01
D18S56	2.63	2.62	2.41	2.02	1.80	1.57
D18S1133	3.47	3.45	3.31	3.03	2.68	2.44
D18S1102	3.28	3.28	3.02	2.68	2.31	2.01
D18S1139	3.16	3.16	3.07	2.78	2.63	2.16
D18S57	3.61	3.61	3.42	3.27	2.79	2.52
D18S1128	1.01	1.03	1.11	1.16	1.23	1.03
D18S475	−3.71	0.15	1.73	1.78	1.63	1.42
D18S67	−2.24	−0.06	1.46	1.65	1.65	1.41
D18S1157	−4.45	−0.64	1.26	1.42	1.34	1.27

Penetrance (*p*) of 0.8 was used for both families.

N is the observed number of alleles), while those at seven loci (*D18S452*, *D18S53*, *D18S66*, *D18S1108*, *D18S56*, *D18S1133*, and *D18S1128*) around the critical region were calculated among 40 chromosomes from unrelated healthy Iranian individuals.

Eight genes or expressed sequence tags of interest; *GNAL*, *VAPA*, *PTPRM*, *PTPN2*, *CABYR*, *RNMT*, *CDH2*, and *NOL4*, were analysed for their sequences, to see whether affected members in the families had mutations in them. All their exons and exon-intron boundaries were amplified by PCR. PCR products were sequenced directly using BigDye™ Terminator ver. 3.0 Cycle Sequencing kit (Perkin-Elmer, Foster, CA, USA), and samples were run on an automated sequencer model 3100 (Applied Biosystems).

RESULTS

The genome-wide linkage analysis first ruled out the loci for Kallmann syndrome types 1 and 2 (Xp22.3 for KAL1 and 8p11.2-p11.1 for KAL2) from the candidacy for the anosmia locus in the two families. The other locus, KAL3, was also ruled out normally because of its X-linked inheritance. A high two point lod score of 3.03 was obtained at the *D18S53* locus on chromosome 18. Extensive linkage analysis to define the critical region using 52 additional markers flanking *D18S53* finally reached the maximum lod score of 5.14 (1.85 for family 1, and 3.29 for family 2) at *D18S1108*, when penetrance was set at 0.8 for both families, and the recombination fraction to be 0.00 (table 1). Haplotype analysis demonstrated that affected members in each family each had a common haplotype including alleles at 35 marker loci between *D18S452* and *D18S475* (fig 1). A recombination between *D18S452* and *D18S1163* or between *D18S464* and *D18S1153* was observed in a patient (IV-12) of family 1 (fig 1). However, as only two markers have been known between *D18S452* and *D18S1153*, more precise identification of recombination sites was not possible in this patient. Two recombination events were observed in a patient (II-8) of family 2, one between *D18S452* and *D18S1163*, and the other between *D18S57* (or *D18S1128*) and *D18S475*. These findings indicated that the isolated congenital anosmia locus lies in a 45.9 cM segment between *D18S452* and *D18S475* 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 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,[4] 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 *D18S47* 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 31.2 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 α subunit, G (olf)) gene (guanine nucleotide binding protein, α 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 *Gnal* are anosmic, showing reduced electrophysiological response of the primary olfactory sensory neurones to odours.[13] 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 *NOL.4*, all located within the critical region.

The sense of smell is one of the first developed special sense organs in animals[14] and plays a role in feeding, differentiation of a parent from others, and in reproductive behaviour.[15] Male mice do not show mating behaviours or sexual development when their olfactory bulbs are removed shortly after birth.[16 17] 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

the better understanding of the development of the olfactory system.

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REFERENCES

1 **Assouline S**, Shevell MI, Zatorre R-J, Jones-Gotman M, Schloss MD, Oudjhane K. Children who can't smell the coffee: isolated congenital anosmia. *J Child Neurol* 1998;**13**:168–72.
2 **Jafek B-W**, Gordon A-S, Moran D-T, Eller PM. Congenital anosmia. *Ear Nose Throat J* 1990;**69**:331–7.
3 **Lygonis C-S**. Familial absence of olfaction. *Hereditas* 1969;**61**:413–5.
4 **Ghadami M**, Majidzadeh-A K, Morovvati S, Damavandi E, Nishimura G, Kinoshita A, Najafi M-T, Komatsu K, Niikawa N, Yoshiura K. Familial isolated congenital anosmia with morphologically normal olfactory bulb in two unrelated Iranian families: a new clinical entity? *Am J Med Genet* (in press).
5 **Glaser O**. Hereditary deficiencies in the sense of smell. *Science* 1918;**48**:647–8.
6 **Mainland R-C**. Absence of olfactory sensation. *J Hered* 1945;**36**:143–4.
7 **Joyner R-E**. Olfactory acuity in an industrial population. *J Occup Med* 1963;**5**:37–42.
8 **Yamamoto K**, Ito K, Yamaguchi M. A family showing smell disturbances and tremor (text in Japanese). *Jpn J Hum Genet* 1966;**11**:36–8.
9 **Singh N**, Grewal MS, Austin J-H. Familial anosmia. *Arch Neurol* 1970;**22**:40–4.
10 **Lathrop G-M**, Lalouel J-M, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci U S A* 1984;**81**:3443–6.
11 **Cottingham RW Jr**, Idury RM, Schaffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;**53**:252–63.
12 **Schaffer A-A**, Gupta S-K, Shriram K, Cottingham R-W. Avoiding recomputation in linkage analysis. *Hum Hered* 1994;**44**:225–37.
13 **Belluscio L**, Gold G-H, Nemes A, Axel R. Mice deficient in G(olf) are anosmic. *Neuron* 1998;**20**:69–81.
14 **Paparella MM**, Shumrick DA, Gluckman JL, Meyerhoff WL. Physiology of the nose and paranasal sinus. In: Meyerhoff WL, Schaefer SD, eds. *Contributions to otolaryngology*, 3rd Ed. St Louis, MO: Mosby, 1998;**1**(2):315–32.
15 **Alberts J-R**. Producing and interpreting experimental olfactory deficits. *Physiol Behav* 1974;**12**:657–70.
16 **Rowe F-A**, Edwards D-A. Olfactory bulb removal: influence on the mating behavior of male mice. *Physiol Behave* 1972;**8**:37.
17 **Rowe F-A**, Smith W-E. Effects of peripherally induced anosmia on mating behavior of male mice. *Psychon Sci* 1972;**27**:33.