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Gene defect in hypodontia: exclusion of MSX1 and MSX2 as candidate genes

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Abstract Hypodontia, congenital lack of one or a few teeth, is an autosomally inherited dominant trait. Homeobox genes MSX1 and MSX2 are expressed in presumptive dental tissues at the stage of initiation of tooth development. Recently, tooth development was shown to be inhibited in transgenic mice lacking a functional Msx1 gene. Here, we studied the relationship of the MSX1 and MSX2 genes to familial hypodontia in five Finnish families with a total of 20 affected individuals, by linkage analysis. The pairwise lod-scores regarding the intragenic microsatellites in the MSX1 and MSX2 genes at a recombination fraction of 0.0 were –3.1 and –3.0, respectively, thus excluding these genes as causative loci for hypodontia in these families.

Introduction

Many studies have shown that hypodontia, congenital lack of teeth, is genetically controlled. Hypodontia of one or a few teeth appears as a common trait in permanent dentitions of European and Asian populations (Grahnen 1956; Alvesalo and Portin 1969; see also Burzynski and Escobar 1983). The teeth most often affected are lateral incisors and second premolars. Consequently, we call this trait incisor and premolar hypodontia. The prevalence of this type of hypodontia is 5%–10% among European and Asian populations. In some cases, it expresses itself as peg-shaped lateral incisors, thus demonstrating variability in the expression of the trait. On the other hand, the term oligodontia commonly refers to the congenital absence of more than six teeth (Hobkirk and Brook 1980; Schalk-van der Weide 1992).

Segregation analyses in many family studies have established that incisor and premolar hypodontia is inherited via an autosomal dominant gene demonstrating incomplete penetrance (Grahnen 1956; Alvesalo and Portin 1969; Svinhufvud et al. 1988; Burzynski and Escobar 1983). Burzynski and Escobar (1983) calculated the penetrance to be 86% from Grahnen's data (1956). However, speculations of a polygenic model of inheritance also appear in the literature (Suarez and Spence 1974; Peck et al. 1993). As there are slightly more affected females in most of the studied populations, sex-influenced inheritance has also been considered.

In addition to premolar and incisor hypodontia and oligodontia, defects in tooth number are seen in numerous syndromes (see Gorlin et al. 1990). Altogether, 49 genetic conditions are listed in the OMIM (TM) database (Johns Hopkins University, Baltimore, Md.). In EDA (hypohidrotic ectodermal dysplasia), a recessively inherited X-linked condition, complete agenesis of teeth can be seen. The gene defect in this condition has been localised to Xq13-21 (Kere et al. 1993). In trisomy 21, missing teeth have been reported in 21%–47% of patients, with the absence of deciduous lateral incisors in 12%–17% of cases (Cohen and Cohen 1971).

So far, the genes causing hypodontia, oligodontia or different human systemic syndromes with defects in tooth number are not known. However, tooth development in mouse embryos was recently shown to require a functional Msx1 gene (earlier Hox-7). In transgenic mice carrying a knockout mutation of the Msx1 gene, tooth development was inhibited (Satokata and Maas 1994). Ivens et al. (1990) have demonstrated that the human MSX1 gene is deleted in some patients with Wolf-Hirschhorn syndrome, which is characterized by mental retardation, heart defects and facial clefting. Severe hypodontia of the permanent dentition of patients with Wolf-Hirschhorn syndrome was reported by Burgersdijk and Tan (1978).

MSX1 and the closely related MSX2 (earlier Hox-8) are homeobox-containing transcription factors. A muta-

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tion in the human MSX2 gene was recently identified as the cause of the Boston-type familial craniosynostosis (Jabs et al. 1993). Homeobox genes, in general, are believed to regulate developmental patterning by controlling the expression of other genes. MSX1 and MSX2 have previously been proposed to be important for tooth development based on the expression patterns of their murine homologues during early tooth morphogenesis (MacKenzie et al. 1991, 1992). They show close correlations with dental patterning and hence are good candidate genes for incisor and premolar hypodontia, which can be regarded as a patterning defect.

We have started a search for the gene causing incisor and premolar hypodontia. Using amplifiable markers and linkage analysis in five three-generation families expressing a uniform phenotype and arising from the genetically isolated Finnish population, we aim to screen the human genome for the incisor and premolar hypodontia gene locus.

Materials and methods

Five Finnish families with autosomal dominant transmission of hypodontia were selected for the study. The patients affected with hypodontia were orthodontic patients at the Institute of Dentistry, University of Helsinki, Finland. The diagnosis of incisor and premolar hypodontia in the propositi was based on congenital absence of one to four teeth or the presence of one or more peg-shaped incisors (Table 1). All members of the families were studied clinically. Panoramic tomograms were taken in order to observe the possible absence of third molars, ectopic canines, peg-shaped lateral incisors or otherwise abnormally formed teeth. All these features were considered indicative of hypodontia. Histories of hypodontia of deciduous dentition were assessed by interviews. In some cases, information of hypodontia and X-ray films were obtained from documents of the respective dentists of the patients.

Venous blood samples, 10–20 ml, were obtained and high molecular weight DNA was extracted from leucocytes according to the method of Vandenplas et al. (1984) with minor modifications, from 42 members of the families, 20 being affected with hypodontia. Polymerase chain reaction (PCR) primers for the analysis of the microsatellite in the MSX1 gene were obtained from the Department of Clinical Genetics of University Hospital, Uppsala, Sweden. The MSX2 primers were a gift from Dr. Ethylin Wang Jabs (Johns Hopkins University School of Medicine, Baltimore, Md.). Primers were 5′-end-labelled with ^{32}P-phosphate by T4 kinase (Pharmacia, Sweden) in buffer provided by the manufacturer of the enzyme and containing 0.4 μCi γ-^{32}P-ATP (Amersham). PCR reactions were carried out in a Perkin Elmer Cetus Thermal Cycler 480 (Norwalk, Conn.) in a total volume of 13 μl containing 12 ng chromosomal DNA, 3 pmol each of the primers, 3 nmol each of the deoxynucleotides, 0.25 U *Taq* polymerase (Amplitaq, Perkin Elmer Cetus, Calif.) and 1.5 m*M* $MgCl_2$ in a buffer recommended by Weissenbach et al. (1992). PCR products were denatured in 50% formamide and 10 m*M* EDTA, pH 8.0, and analysed in a standard 5% sequencing gel with 7.5 *M* urea (Sambrook et al. 1989) in an IBI gel apparatus. The alleles were detected by autoradiography with Kodak films.

The pairwise lod scores were estimated by the Mlink option of the Linkage package (Version 5.1; Lathrop et al. 1984). The hypodontia locus was modelled as an autosomal dominant two-allele system with the gene frequency of the hypodontia allele set at 8%. The penetrance of hypodontia of 86% calculated by Burzynski and Escobar (1983) was in good agreement with our family data. Equal frequencies were given to each allele of the microsatellites in the MSX1 and MSX2 genes. Simulation with the Slink program (Ott 1989; Weeks et al. 1990) showed that the five families were informative enough to reveal significant evidence for linkage of hypodontia to markers with four or more alleles.

Table 1 Hypodontia status of the family members. Missing teeth are depicted according to FDI's two-digit system (Keiser-Nielsen 1971). Peg-shaped incisors are indicated by *'peg'* plus tooth *'number'* (– No defects, *nd* not determined)

Family 1:		II/1	–
I/1	nd	II/2	31
I/2	nd	II/3	–
II/1	15	III/1	peg 12
II/2	–	III/2	–
II/3	–		
II/4	35	Family 4:	
II/5	35	I/1	nd
III/1	25, 35, 45	I/2	–
III/2	35	II/1	–
III/3	45	II/2	12, peg 22
III/4	–	II/3	nd
		II/4	15, 35
Family 2:		II/5	22, peg 12
I/1	nd	III/1	–
I/2	nd	III/2	–
II/1	–	III/3	15, 35, 45
II/2	–	III/4	–
II/3	31, 41	III/5	–
III/1	–	III/6	–
III/2	–		
III/3	peg 12, peg 22	Family 5:	
III/4	–	I/1	peg 22
IV/1	–	I/2	–
IV/2	12, 22	II/3	15, 25
IV/3	12, 22	II/4	–
		III/5	15, 25
Family 3:		III/6	25
I/1	nd	III/7	–
I/2	–		

Results and discussion

In this study, we report evidence against linkage of incisor and premolar hypodontia with the MSX1 and MSX2 genes. The linkage was studied in five Finnish families where hypodontia was inherited as an autosomal dominant trait exhibiting incomplete penetrance. The alleles of the microsatellites in the MSX1 and MSX2 genes in the five pedigrees are shown in Fig. 1. Both microsatellites were intragenic, i.e. they resided in the immediate vicinity of the coding regions. One of the microsatellites is located in the 3′-flanking region of MSX1 gene in 4p16 (Padanilam et al. 1992), with the other lying in the intron of the MSX2 gene in 5q34-35 (Jabs et al. 1993). Pairwise analyses of the allelic data in these families resulted in negative lod scores of less than –2 with recombination fractions 0.0 and 0.01 (Table 2). There was no evidence for locus heterogeneity among the families studied. The negative lod scores can be taken as evidence against linkage of hy-

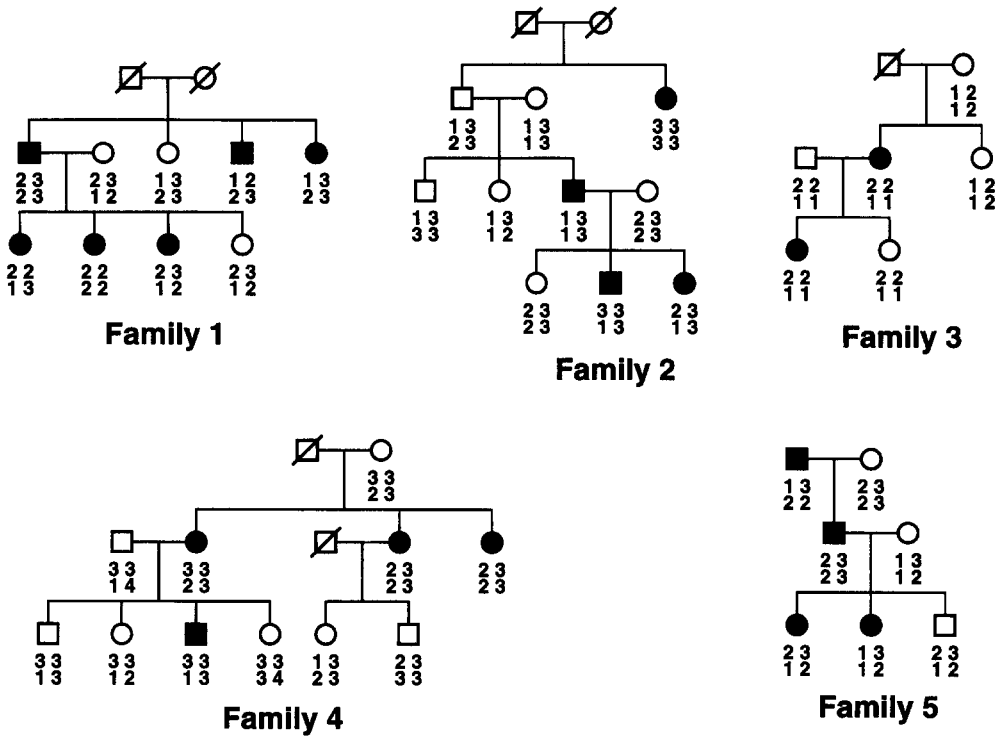

Fig. 1 Hypodontia pedigrees. The allelic data of the microsatellites on the MSX1 *(upper numbers)* and MSX2 *(lower numbers)* loci analysed by PCR is presented *below* the symbols

Table 2 Pairwise lod-scores of hypodontia to the intragenic microsatellite markers in the MSX1 and MSX2 genes

MSX1							
Theta	0.000	0.010	0.050	0.100	0.200	0.300	0.400
Family 1	− 0.737	− 0.568	− 0.257	− 0.106	− 0.010	0.003	0.000
Family 2	− 0.189	− 0.177	− 0.140	− 0.112	− 0.074	− 0.039	− 0.011
Family 3	− 0.136	− 0.130	− 0.107	− 0.083	− 0.045	− 0.019	− 0.005
Family 4	− 0.357	− 0.317	− 0.197	− 0.100	0.003	0.041	0.037
Family 5	− 1.728	− 1.058	− 0.473	− 0.227	− 0.027	0.041	0.044
Total	− 3.147	− 2.250	− 1.174	− 0.628	− 0.152	0.027	0.065
MSX2							
Theta	0.000	0.010	0.050	0.100	0.200	0.300	0.400
Family 1	− 1.120	− 1.065	− 0.836	− 0.595	− 0.281	− 0.111	− 0.026
Family 2	− 0.408	− 0.396	− 0.363	− 0.348	− 0.342	− 0.297	− 0.173
Family 3	− 0.136	− 0.130	− 0.107	− 0.083	− 0.045	− 0.019	− 0.005
Family 4	− 1.260	− 1.130	− 0.780	− 0.517	− 0.228	− 0.086	− 0.019
Family 5	− 0.074	− 0.052	0.016	0.071	0.122	0.121	0.079
Total	− 2.999	− 2.773	− 2.071	− 1.472	− 0.773	− 0.392	− 0.144

podontia to these genes in our family material. Hence, the mutation in incisor and premolar hypodontia apparently involves another gene or genes.

The reported expression patterns of Msx1 and Msx2 suggest important roles in the initiation and early morphogenesis of teeth (MacKenzie et al. 1991, 1992). In particular, the recent finding that tooth development is inhibited in Msx1-negative transgenic mice directly implicates this gene in tooth development (Satokata and Maas 1994). Although a defect in MSX1 is not involved in incisor and premolar hypodontia, it is conceivable that a mutation of the gene may cause other types of hypodontia in humans. In addition to anodontia, the Msx1-knockout mice exhibited other abnormalities in the craniofacial area, including cleft palate. This implies that, in humans, a mutation in MSX1 may be involved in cases where hypodontia is associated with cleft palate (Ranta 1986) or with other craniofacial malformations.

Recently, molecules in different categories have been localized in developing teeth and associated with early tooth morphogenesis (cf. Thesleff et al. 1990; Vainio et al. 1993). The molecular regulation of tooth development has also been studied experimentally and regulatory functions have been proposed for many genes (Kronmiller et al. 1991; Vaahtokari et al. 1991; Vainio and Thesleff 1992; Vainio et al. 1993; Jernvall et al. 1994). In principle, a mutation in any one of these molecules could result in failure of tooth development.

In addition to MSX1 and MSX2, other homeobox-containing transcription factors, including the DLX genes, show clear associations with the initiation and patterning of tooth development (Dollé et al. 1992). Especially good candidate genes for hypodontia are those that code for growth and differentiation factors that mediate signalling during the sequential and reciprocal epithelial-mesenchymal interactions that govern tooth morphogenesis (Thesleff and Vaahtokari 1992; Thesleff et al. 1994). In particular, BMP-2 and BMP-4, which are members of the TGFβ superfamily and which act as morphogens in various animal species, were recently implicated in the regulation of early tooth morphogenesis (Vainio et al. 1993). Growth factors in the EGF and FGF families have also been proposed to play important functions during tooth morphogenesis (Wilkinson et al. 1989; Kronmiller et al. 1991; Jernvall et al. 1994).

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