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PHENOTYPIC AND GENOTYPIC FEATURES OF FAMILIAL HYPODONTIA

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Academic Dissertation

to be publicly discussed with the permission of the Faculty of Medicine of the University of Helsinki in the Main Auditorium of the Institute of Dentistry on 19 October, 2001, at 12 noon.

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To Lauri, Eero, and Elisa

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals. In addition, some unpublished data are presented.

I Arte S, Nieminen P, Apajalahti S, Haavikko K, Thesleff I, Pirinen S. Characteristics of incisor-premolar hypodontia in families. J Dent Res 80:1445-1450, 2001.

II Nieminen P, Arte S, Pirinen S, Peltonen L, Thesleff I. Gene defect in hypodontia: exclusion of *MSX1* and *MSX2* as candidate genes. Hum Genet 96:305-308, 1995.

III Arte S, Nieminen P, Pirinen S, Thesleff I, Peltonen L. Gene defect in hypodontia: exclusion of *EGF*, *EGFR*, and *FGF-3* as candidate genes. J Dent Res 75:1346-1352, 1996.

IV Nieminen P, Arte S, Tanner D, Paulin L, Alaluusua S, Thesleff I, Pirinen S. Identification of a nonsense mutation in the *PAX9* gene in molar oligodontia. Eur J Hum Genet 9:743-746, 2001.

ABBREVIATIONS

BMP	bone morphogenetic protein
DLX	homeobox transcription factor, homolog of *Drosophila distal-less* gene
cM	centimorgan
DNA	deoxyribonucleic acid
ED	ectodermal dysplasia
EDA	hypohidrotic ectodermal dysplasia
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
GLI	transcription factor, homolog of *Drosophila* segment polarity gene
INHBA	inhibinβ-A
IPH	incisor-premolar hypodontia
kb	kilobase
LEF	lymphoid enhancer-binding factor
Lod	logarithm of odds
MSX	homeobox transcription factor, homolog of *Drosophila* muscle segment gene
mRNA	messenger ribonucleic acid
OMIM	Online Mendelian Inheritance in Man
PAX	paired-box transcription factor, homolog of *Drosophila* paired box gene
PCR	polymerase chain reaction
p	short arm of chromosome
TGF	transforming growth factor
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
q	long arm of chromosome
θ	theta, recombination fraction

ABSTRACT

The congenital lack of teeth has interested dentists for a long time, but genetic studies using modern DNA techniques have not been published before the last decade.
Studies of odontogenesis at the molecular level, mostly using mouse teeth as models, have indicated that the development of teeth is under strict genetic control, which determines the position, number, size, and shape of teeth. More than 200 genes have so far been identified which are expressed during tooth development, and mutations in several of these genes cause arrested tooth development in mice.

In this work the segregation and phenotype of hypodontia and associated dental anomalies were analyzed in 214 family members in three generations of 11 families. These families were also participating in the genetic linkage study on incisor-premolar hypodontia (IPH). The analysis confirmed the autosomal dominant transmission with reduced penetrance of IPH. The prevalence of hypodontia and/or peg-shaped teeth was over 40% in first- and second-degree relatives and 18% in first cousins of the probands. The results supported the findings that ectopic canines, rotation of premolars, and taurodontism are related to hypodontia. Four of nine noted obligate carriers of a hypodontia gene – with no missing teeth themselves - had minor dental anomalies. These anomalies were observed at higher than normal frequency also in relatives of the probands affected with hypodontia.

Linkage analysis is the first step in the localization of human disease genes. In this study linkage analysis was used to search for the gene locus causing incisor-premolar hypodontia. First, candidate genes including *MSX1*, *MSX2*, *EGF*, *EGFR*, and *FGF-3* were studied, but no evidence of linkage or association to hypodontia could be found. Instead, in many families recombinations were found with respect to these genes. In the second stage, a genome-wide scan was carried out, revealing several regions with positive lod scores, but no significant evidence for confirming linkage in these families.

In a family with oligodontia involving permanent molars together with upper lateral incisors and premolars, a nonsense mutation was identified in the *PAX9* gene. The A340T transversion creates a stop codon at lysine 114, and truncates the coded PAX9 protein at the end of the DNA-binding paired-box. All affected members were heterozygous for the mutation. In another family with molar tooth agenesis this mutation in the *PAX9* gene was absent.

It is evident that both hypodontia and oligodontia are genetically heterogenous traits. Combining clinical and molecular genetic studies, classification of the differing forms of these traits will become more exact.

INTRODUCTION

Congenital lack of one or more teeth is a common anomaly in man. Lack of one or a few permanent teeth, hypodontia, without any systemic disorders is the mildest and most common phenotype. Second premolars and upper lateral incisors are the teeth most frequently affected. The same teeth are also most often lacking in the more severe phenotype, oligodontia.

Both environmental and genetic factors can cause failure of tooth development. Children treated for malignant diseases at tooth-developing ages show a high frequency of missing teeth. Irradiation produces more severe effects than chemotherapeutic agents (Maguire et al.,1987; Näsman et al.,1997).

Numerous different genes have been implicated in tooth development by gene expression and experimental studies in the mouse, and in theory, any of these genes may cause tooth agenesis (for review, Thesleff, 2000). Family studies show that, as an isolated form, both hypodontia and oligodontia are inherited as an autosomal dominant trait with incomplete penetrance and variable expression (Grahnen, 1956; Burzynski and Escobar, 1983). Sex-linked and polygenic or multifactorial models of inheritance have also been suggested (Suarez and Spence, 1974; Chosack et al., 1975; Brook, 1984; Peck et al., 1993). An autosomal recessive model was shown in one family (Ahmad et al., 1998). Variability in expression includes the number and region of missing teeth, and various other dental features associated with the trait.

The obscure mechanisms underlying congenital lack of teeth and the differing results of genetic studies have drawn attention to the phenotypic and genotypic variation in this phenomenon. Thus, the original purpose of this study was to further define the phenotype and to map the gene locus responsible for incisor-premolar hypodontia. During the course of this study, mutations in two transcription factors, *MSX1* and *PAX9*, have been identified in three families with oligodontia (Vastardis et al., 1996; Stockton et al., 2000; van den Boogaard et al., 2000). A mutation screening of the *PAX9* gene, was therefore performed in two Finnish families with oligodontia.

REVIEW OF THE LITERATURE

1. CONGENITALLY MISSING TEETH

1.1. Definition, diagnosis, and terminology

Congenital lack of a tooth results from disturbances during the early stages of tooth development. A tooth is defined to be congenitally missing if it has not erupted in the oral cavity and is not visible in a radiograph. All primary teeth have erupted by the age of 3 and all permanent teeth except the third molars between the ages of 12 and 14. Therefore, 3- to 4-year-old children are suitable for diagnosis of congenitally missing primary teeth by clinical examination, and 12- to 14-year-old children, for diagnosis of permanent teeth, excluding the third molars. Radiographic diagnosis can be made at younger age depending on tooth group. The use of panoramic radiography is recommended, together with clinical examination in detecting or confirming dental development (Pirinen and Thesleff, 1995).

All **primary teeth** and the crypts of first permanent molars are visible by radiograph at birth. The crowns of first premolars, second premolars, and second permanent molars start to mineralize near the second birthday, and all **permanent tooth** crowns except the third molars have begun their mineralization by the age of six. The formation of third molars shows very large variation. Usually at the age of 8 to 10 years, the first signs of the third molars appear on a radiograph but occasionally, very late appearance (age 14 to 18) occurs (Pirinen and Thesleff, 1995). The formation of dentition continues many years, and differences exist in mineralization stages among children depending on race, on gender, and even on family and on the individual. Especially second premolars may show late onset of mineralization, and give a false-positive diagnosis of hypodontia in radiographs. Therefore, diagnosis of tooth agenesis in the permanent dentition should be made after the age of 6 (Pirinen and Thesleff, 1995) excluding third molars, and after 10 years of age if third molars are also studied.

Hypodontia is the term most frequently used when describing the phenomenon of congenitally missing teeth in general. Many other terms appear in the literature to describe a reduction in number of teeth: oligodontia, anodontia, aplasia of teeth, congenitally missing teeth, absence of teeth, agenesis of teeth, and lack of teeth.

Hypodontia and oligodontia are classified as **isolated or nonsyndromic hypodontia/oligodontia** and **syndromic hypodontia/oligodontia** or **hypodontia/ oligodontia associated with syndromes**.

The term **hypodontia** is used in a narrow sense when the number of missing teeth is one or a few. **Oligodontia** is defined as missing a large number of teeth. **Anodontia** is an extreme case, denoting complete absence of teeth. There is no clear definition in the literature concerning the limits of these classes. In the recent years, however, the following definitions have been used:

Hypodontia:1 to 6 teeth missing (excluding the third molars)

Oligodontia: more than six teeth missing (excluding the third molars)

Anodontia: complete absence of teeth.

Incisors and premolars are the most frequently missing teeth. Therefore **incisor-premolar hypodontia (IPH)** is the term that we have used to describe this form of the anomaly.

1.2. Etiology

Many theories of the etiology of tooth agenesis have been suggested in the literature, especially before the intense genetic studies of the present day, and obviously both genetic and environmental factors may contribute (Grahnen, 1956; Schalk-van der Weide, 1992; for review, Jorgenson, 1980; Vastardis, 2000).

1.2.1. Environmental factors

In principle, many environmental factors may cause arrested tooth development. Different kinds of trauma in the dental region such as fractures, surgical procedures on the jaws, and extraction of the preceding primary tooth are mentioned in the literature (for review, Grahnen, 1956; Schalk-van der Weide, 1992).

Developing teeth are irreversibly affected by multiagent chemotherapy and radiation therapy, and effects depend on age of patient and dosage (Näsman et al., 1997). Children after treatment for malignant disease at an early age show arrested root development with short V-shaped roots, roots with premature apical closure, enamel hypoplasia, microdontia, and hypodontia. Irradiation produces more severe effects than those caused by chemotherapeutic agents (Maguire et al.,1987; Näsman et al.,1997).

Congenitally missing teeth have been reported in children whose mothers had used Thalidomide^{R} (N-phthaloylglutamimide) during pregnancy (Axrup et al., 1966). No definite etiologic relationship has been found between hypodontia and systemic diseases or endocrine disturbances (for review, Grahnen, 1956; Schalk- van der Weide, 1992).

A relationship has been proposed between the function of peripheral nerves and tooth agenesis (Kjaer et al., 1994). This report focused on an etiological explanation of hypodontia based on disturbances in nerve tissue, oral mucosa, and supporting tissues, all interacting in tooth development.

1.2.2. Genetic factors

Although tooth agenesis is occasionally caused by environmental factors, in the majority of cases hypodontia has a genetic basis. In hypodontia, the criteria for a genetic disease are fulfilled: It is more common among individuals related to hypodontia patients than in population in general (Burzynsky and Escobar, 1983; Brook, 1984). The classic family study of Grahnen in Sweden of a total of 685 family members of 171 probands affected with hypodontia (Grahnen, 1956) showed that hypodontia in the permanent dentition is primarily determined by genetic factors. Differences in frequency of hypodontia exist between races, no environmental etiology is apparent in individuals with the disease, and greater concordance of hypodontia appears in identical than in non-identical twins (Markovic, 1982; Kotsomitis et al., 1996).

In familial hypodontia, the type of inheritance in the majority of families seems to be autosomal dominant with incomplete penetrance and variable expressivity. In addition, peg-shaped upper lateral incisors are considered to be a modified manifestation of the same genotypes as hypodontia (Grahnen, 1956; Alvesalo and Portin, 1969). Sex-linked inheritance patterns and a polygenic or multifactorial model of inheritance have also been suggested (Suarez and Spence, 1974; Chosack et al., 1975; Brook, 1984; Peck et al., 1993), and an autosomal recessive model in one family (Ahmad et al., 1998). Female predominance has been reported (Bergström, 1977; Wisth et al., 1974; Stamatiou and Symons, 1991; Kotsomitis et al., 1996), but in most studies the difference does not reach statistical significance (Grahnen, 1956; Haavikko, 1971; Rolling, 1980).

The most direct evidence for a genetic basis is that during the course of this study, two mutated genes causing an autosomal dominant form of human nonsyndromic tooth agenesis were identified (Vastardis et al., 1996; Van den Boogaard et al., 2000). A missense mutation was found by the Vastardis group in the homeodomain of *MSX1* gene in chromosome 4 (4p16) in all affected members of a family with missing second premolars and third molars as a prominent feature. Some affected individuals also lacked their maxillary first premolars, mandibular first molars, one or both upper lateral incisors, or a single lower central incisor. All affected individuals were reported to have had normal primary dentitions. In a Dutch family, however, a nonsense mutation in the *MSX1* gene was associated with tooth agenesis and various combinations of cleft lip and/or palate (Van den Boogaard et al., 2000). Another report has excluded *MSX1* as the gene responsible for tooth agenesis (Scarel et al., 2000). A frameshift mutation in another transcription factor gene, *PAX9*, in chromosome 14 (14q21-q13) was identified in a family with autosomal dominant

oligodontia (Stockton et al., 2000). The point mutation, insertion of a guanine, causes a frameshift and premature termination of the protein (Stockton et al., 2000). The affected individuals lack most permanent molars. Some individuals also were missing their maxillary and/or mandibular second premolars as well as mandibular central incisors. The primary dentition had been normal. *MSX1* and *PAX9* are transcription factors which, before being associated with human tooth agenesis, had been shown to regulate early tooth morphogenesis in the mouse. They are expressed in dental mesenchyme after initiation of tooth development in response to epithelial signals (for review, Thesleff, 2000). Inactivation of *Msx1* and *Pax9* genes in the mouse causes arrested development of teeth at the bud stage and malformations of palate, limb, and pharyngeal pouch derivatives, whereas heterozygous mice develop normal teeth (Satokata and Maas, 1994; Peters et al., 1998). In humans, inactivation of one copy of the gene causes dental defects (Vastardis et al., 1996; Stockton et al., 2000) or dental defects and clefting in the case of *MSX1* nonsense mutation (Van den Boogaard et al., 2000).

In addition, several gene defects have been identified which cause syndromes with hypodontia or oligodontia (see section on syndromic hypodontia).

2. CONGENITALLY MISSING TEETH IN PRIMARY DENTITION

Variation in the number of teeth is not as common in the primary as in the permanent dentition, and no significant difference exists in prevalence of hypodontia by gender (Grahnen and Granath, 1961; Ravn, 1971; Järvinen and Lehtinen, 1981). The prevalence varies from 0.4% to 0.9% in the European population (Grahnen and Granath, 1961; Ravn, 1971; Järvinen and Lehtinen, 1981; Magnusson, 1984; Carvalho, 1998), and is reported to be higher, 2.4%, in Japan (Yonezu et al., 1997) whereas in New Zealand the prevalence of 0.4% corresponds to that of Europeans (Whittington, 1996). Table 1. Mostly one (55% of the children, according to Daugaard-Jensen et al., 1997) or two teeth are missing, and the majority of cases represent unilateral hypodontia.

In the primary dentition the incisor region seems to be affected most often (Grahnen and Granath, 1961; Järvinen and Lehtinen, 1981; Magnusson, 1984; Daugaard-Jensen et al., 1997; Yonezu et al., 1997). In European studies the upper primary lateral incisors are the most frequently missing (Järvinen and Lehtinen, 1981; Daugaard-Jensen et al, 1997), whereas the lower lateral incisors are affected most often in the Japanese (Yonezu et al., 1997). Peg-shaped teeth are seen also in the primary dentition; this Japanese study reported a frequency of 0.55% .

Table 1. Prevalence of hypodontia in the primary dentition in various countries

Author	Year	Country	sample size	prevalence	tooth most frequently missing
Grahnen and Granath	1961	Sweden	1173	0.4%	upper lateral incisor
Ravn	1971	Denmark	4564	0.6%	upper lateral incisor
Brook	1974	Britain	741	0.3%	-
Järvinen and Lehtinen	1981	Finland	1141	0.9%	upper lateral incisor
Magnusson	1984	Iceland	927	0.5%	upper lateral incisor
Whittington and Durward	1996	New Zealand	1680	0.4%	upper lateral incisor
Yonezu et al.	1997	Japan	2733	2.4%	lower lateral incisor
Carvalho et al.	1998	Belgium	750	0.4%	upper lateral incisor

In Scandinavian studies, absence of a primary canine in the mandible was reported (Grahnen and Granath, 1961; Ravn, 1971). In a sample of Danish children (N=193) with hypodontia in the primary dentition, agenesis of first or second primary molar or primary canines was seen, but only very rarely (Daugaard-Jensen et al., 1997). A strong correlation exists between hypodontia in the primary and permanent dentitions. Children with hypodontia in the primary dentition nearly always show hypodontia of the successors. Some syndromes, especially syndromes with ectodermal dysplasia, show tooth agenesis also in the primary dentition (see section on syndromic hypodontia).

3. NONSYNDROMIC HYPODONTIA IN PERMANENT DENTITION

3.1.Prevalence

Congenital lack of one or a few permanent teeth without any systemic disorders is a common dental anomaly. Numerous studies have appeared on the prevalence of hypodontia in different countries, showing some variation in populations, on continents and among races. The early reports give lower frequencies, ranging from 2.8% in the USA (Byrd, 1943) to 3.4% in Switzerland (Dolder, 1936). The prevalence of hypodontia seems to be lower in North America (3.5%-3.7%) (Muller et al., 1970) than in European countries (6-8%) (Grahnen, 1956; Haavikko, 1971). However, in a Canadian study the frequency of hypodontia was 7.4% (Thompson and Popovich, 1974). In Australia (6.3%) (Lynham, 1990) and Japan (6.6%) (Niswander and Sujaku, 1963) the frequency of hypodontia corresponds to the values for Caucasians in Europe. There is only a little difference in frequency of hypodontia between white and black students in the USA (Muller et al., 1970) (Table 2).

Table 2. Prevalence of tooth agenesis (third molars excluded) in the permanent dentition in various countries

Author	Year	Country	Sample size	prevalence females	prevalence males	prevalence total
Dolder	1936	Switzerland	10000	?	?	3.4%
Grahnen	1956	Sweden	1006	5.7%	6.4%	6.1%
Niswander and Sujaku	1963	Japan	4150	9.2%	5.8%	6.6%
Hunstadbraten	1964	Norway	548	11.6%	8.5%	10 %
Muller et al. (white students)	1970	USA	13459	4.1%	2.9%	3.5%
Muller et al. (black students)	1970	USA	1481	4.1%	3.2%	3.7%
Haavikko	1971	Finland	1041	9.5%	6.5%	8.0%
Thompson and Popovich	1974	Canada	1191	8.9%	6.0%	7.4%
Brook	1974	Britain	1115	5.7%*	3.1%	4.4%
Wisth, Thunold and Böe	1974	Norway	813	8.1%	5.6%	6.6%
Bergström	1977	Sweden	2589	9.3%*	5.6%	7.4%
Magnusson	1977	Iceland	1116	8.9%	6.7%	7.9%
Rolling	1980	Denmark	3325	7.8%	7.7%	7.8%
Davis	1987	China (Hong Kong)	1093	7.7%	6.1%	6.9%
Lynham	1990	Australia	662	-	6.3%	6.3%
Aasheim and Ögaard	1993	Norway	1953	7.2%	5.8%	6.5%

* gender difference statistically significant

The reported frequency of agenesis of the third molar(s) is higher, varying from 9% to more than 30% (Grahnen, 1956; Haavikko, 1971). In a Finnish study (Haavikko, 1971), one or more third molars were missing in 21% of individuals with no significant gender difference. Furthermore, 71% of the individuals with hypodontia of some other teeth also lacked their third molar(s).

Most authors report a small but not significant predominance of hypodontia in females (Muller, 1970; Haavikko, 1971; Thompson and Popovich, 1974; Magnusson, 1977; Rolling, 1980; Davis, 1987). Statistically significant differences were calculated in some studies (Brook, 1974; Bergström, 1977).

Family studies have shown the frequency of hypodontia and peg-shaped lateral incisor(s) in parents and sibs of the probands to be significantly higher than in the general population (Grahnen, 1956; Chosack et al., 1975; Brook, 1984). Further, more relatives of the probands with oligodontia (6 or more teeth missing) had tooth agenesis than did probands with hypodontia (Brook, 1984). Hypodontia was diagnosed in a Swedish study in 41% of the parents and 26% of the sibs (Grahnen, 1956).

3.2. Characteristics

The mandibular second premolar is, in most studies, the most frequently missing tooth (excluding third molars), followed by a maxillary lateral incisor or second premolar (Grahnen, 1956; Haavikko, 1971; Thompson and Popovich, 1974). In a Finnish study, 42% of the missing teeth were lower second premolars, 29% upper

second premolars, 19% upper lateral incisors, 4% lower first premolars, 3% lower central incisors, and 1% lower lateral incisors. Hypodontia of second molars and lower canines were rare, 0.7% of the missing teeth (Haavikko, 1971). In an American study, an upper lateral incisor was the most frequently missing in the individuals with agenesis of one or two teeth, while in those who lacked more than two teeth, the second premolar was most commonly missing (Muller et al., 1970). Absence of maxillary central incisors, maxillary and mandibular first molars and canines seems to be very rare. No clear difference in congenitally missing teeth has been found between the maxilla and the mandible (Grahnen, 1956; Haavikko, 1971; Bergström, 1977). Unilateral hypodontia is common, with no significant difference between the left and right sides of the jaws (Magnusson, 1977; Lai and Seow, 1989). Predominance of hypodontia on the left side has been reported in some Scandinavian studies (Grahnen, 1956; Haavikko, 1972; Wisth et al., 1974; Bergström, 1977).

Most individuals with hypodontia lack only one or two permanent teeth (Grahnen, 1956; Muller, 1970; Haavikko, 1971; Thompson and Popovich, 1974; Bergström, 1977). Panoramic radiographs of three individuals with hypodontia and a photograph of a peg-shaped upper lateral incisor are in Figure 1.

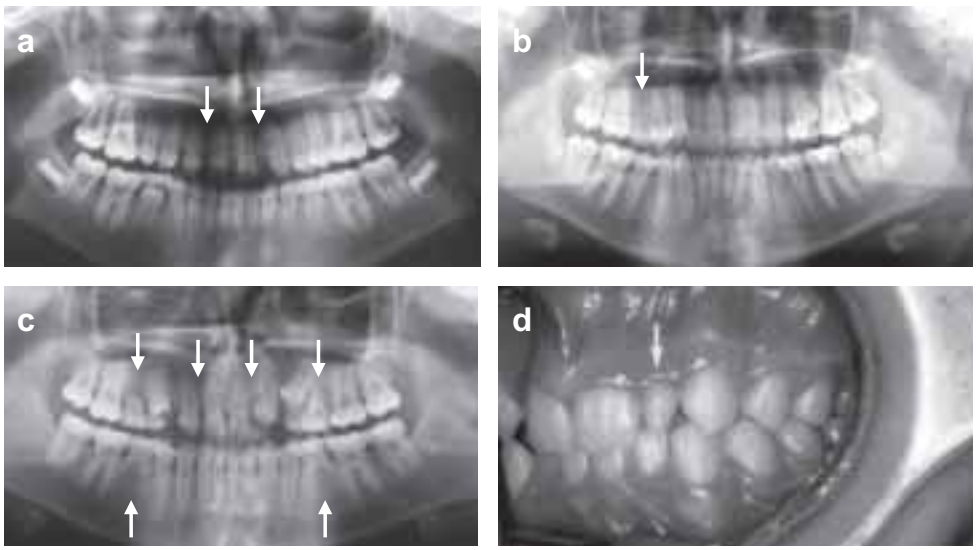

Fig. 1. Hypodontia of permanent upper lateral incisors (a). Upper second premolar (b). Upper second premolars, lateral incisors, and lower second premolars (c). Peg-shaped upper lateral incisor (d). Missing or peg-shaped teeth indicated by arrows.

4. NONSYNDROMIC OLIGODONTIA IN PERMANENT DENTITION

Oligodontia, congenital lack of more than six permanent teeth, has a prevalence of 0.08% in a Dutch study (Schalk-van der Weide, 1992), and 0.16% in a Danish study (Rolling and Poulsen, 2001). A panoramic radiograph of an individual with oligodontia is Figure 2. The difference in the frequency of oligodontia between males and females is not statistically significant, nor is the difference in distribution of missing teeth over maxilla/mandible and left/right sides (Schalk-van der Weide, 1992; Rolling and Poulsen, 2001). However, combining data from 6 studies, females show a higher frequency than males (Rolling and Poulsen, 2001).Two of every three congenitally missing teeth in oligodontia are second premolars or upper lateral incisors (Rolling and Poulsen, 2001). Oligodontia, like hypodontia, is seen as an isolated trait or as a part of a syndrome. Isolated oligodontia is inherited in an autosomal dominant form with reduced penetrance. Oligodontia and hypodontia have similar associated anomalies with a tendency toward delayed tooth formation, reduced size of teeth, and taurodontism. In a Dutch study of patients with oligodontia, 28.9% showed taurodontism of one or two mandibular first molars, while 9.9% of the control subjects had taurodontism (Schalk-van der Weide, 1992).

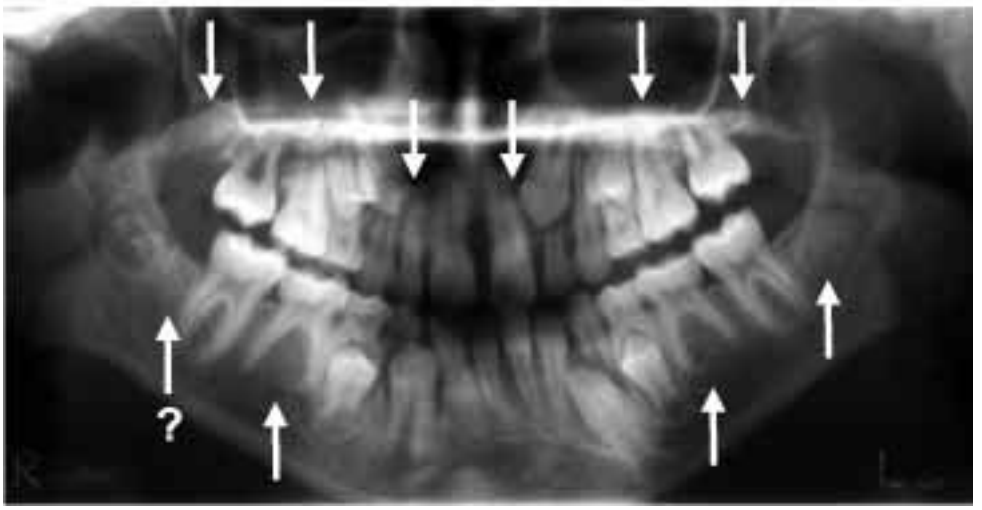

Fig. 2. Congenitally missing upper second molars, second premolars, lateral incisors, lower second molar(s), and second premolars in a patient with oligodontia. Missing teeth indicated by arrows.

5. ANODONTIA

Congenital lack of all teeth without associated abnormalities is extremely rare. Some case reports of anodontia have suggested autosomal recessive inheritance (OMIM (TM) database; Gorlin et al., 1980). Anodontia occurs as an extreme dental phenotype in ectodermal dysplasia syndromes.

6. HYPODONTIA ASSOCIATED WITH CLEFTING AND SYNDROMES

Dental manifestations are seen in several syndromes together with malformations of other organs. For instance, 150 syndromes with hypodontia are included in British dysmorphology database (Baraitser and Winter, 1999). Some best known of these syndromes are described in this section.

6.1. Isolated cleft lip/ palate

Hypodontia is a very common dental anomaly in patients with oral and facial clefts. The prevalence of hypodontia increases with cleft severity, and varies between populations. Prevalence of hypodontia ranges from 10% to 68% in different cleft types in Finland, being 10% in cleft lip, 33% in cleft palate, 49% in unilateral, and 68% in bilateral cleft lip and palate groups, and even higher in twins with clefts (Ranta,1986; Laatikainen and Ranta, 1994). The upper lateral incisor is the most frequently affected tooth in the cleft area both in primary and permanent dentitions. Hypodontia is more common than in the normal population also outside the cleft region, where the upper and lower second premolars are most frequently missing. A higher incidence of hypodontia in the maxilla has been reported, and has been suggested to be a result of the same factors as for the cleft (Ranta and Tulensalo, 1988). If the permanent lateral incisor is present on the cleft side it usually shows abnormalities in size and shape. In addition, the dimensions of other teeth are smaller, and timing of tooth formation and eruption in cleft children is delayed (Ranta, 1986).

6.2. Pierre Robin sequence

In Pierre Robin sequence with cleft palate, micrognathia, and glossoptosis, a 50% prevalence of hypodontia, excluding the third molars, has been reported. Hypodontia in the mandible is more frequent in Pierre Robin patients than in that of the cleft patients (Ranta, 1986).

6.3. Van der Woude syndrome

Even higher prevalence of hypodontia (69%) has been shown in patients with the autosomal dominant Van der Woude syndrome associated with cleft lip and/or palate and pits of the lower lip. Genetic heterogeneity in Van der Woude syndrome has been proven; It has been mapped to 1q32-41 in some families, but the same locus is excluded in other families (Wong, 2000).

6.4. *MSX1* mutation

The *MSX1* mutation is associated with clefting and hypodontia. A large Dutch family with tooth agenesis and various combinations of cleft lip and palate showed a nonsense mutation in exon 1 of *MSX1* in chromosome 4 (Van den Boogaard et al., 2000). The mutation (Ser104stop) was found to be heterozygous in all affected family members. Both mandibular and maxillary second premolars were missing in most affected individuals, but in addition, third molars, upper lateral incisors, lower central incisors, first premolars, and second molars were missing in some individuals. The phenotype of the Dutch family corresponds to that of the *Msx1*-mutant mouse (Satokata and Maas, 1994), but is more severe than the phenotype of the previously reported *MSX1* missense mutation family (Arg239Pro), which showed oligodontia without clefts (Vastardis et al., 1996).

6.5. Ectodermal dysplasias (EDs)

The term Ectodermal Dysplasia (ED) covers a heterogenous group of conditions affecting ectodermal organs including hair, teeth, nails, and glands. A condition characterized by ectodermal signs only is called pure ED. A condition with ectodermal symptoms associated with other malformations is called ED/malformation syndrome or ED syndrome. More than 150 EDs have been described and classified into 11 clinical subgroups (Pinheiro and Freire-Maia, 1994; OMIM (TM) database), with variation in mode of inheritance as well as genetic heterogeneity demonstrated. EDs include X-linked, autosomal dominant, and autosomal recessive forms.

6.5.1. Hypohidrotic ectodermal dysplasia (EDA or HED)

Hypohidrotic ectodermal dysplasia, EDA, the most common and best-known ED, is usually inherited as an X-linked semidominant trait, although rarer autosomal dominant and recessive forms exist. The defective gene behind X-chromosomal EDA has been identified (Xq12-q13.1) (Kere et al., 1996; Monreal et al., 1998). The protein product, ectodysplasin, is a novel member of the tumor necrosis factor (TNF) family and functions as a signaling molecule during epithelial morphogenesis

(Mikkola et al., 1999; Thesleff, 2000). Affected males show severe oligodontia or anodontia, and abnormalities in tooth shapes. Anomalies are seen in both primary and permanent dentitions. Frontal bossing and nasal and maxillary hypoplasia, and sparse or absent scalp hair contribute to the typical appearance of EDA patients. Due to absence of sweat glands these patients suffer from hypohidrosis. Female EDA carriers have variable, milder phenotypic expressions depending on the consequences of X-chromosome inactivation (Cambiaghi et al., 2000). They have hypodontia or oligodontia, and also abnormally shaped teeth. All carrier mothers (N=5) of X-linked EDA patients in a Finnish study lacked more than four permanent teeth, and one mother had had hypodontia in her primary dentition. In addition, three of five mothers had peg-shaped teeth (Söderholm and Kaitila, 1985).

Another gene mutation, which was found to cause both autosomal dominant and recessive forms of EDA, was identified in chromosome 2 (2q11-q13); it encodes a TNF receptor (TNFR) called Edar (Monreal et al., 1999). In autosomal recessive EDA, heterozygous carriers show no features of the disorder, unlike the X-linked EDA, in which female carriers show mild manifestations (Cambiaghi et al., 2000).

6.5.2. Ectrodactyly-ectodermal dysplasia-clefting syndrome (EEC)

The characteristics of EEC syndrome are ectrodactyly of the hands and feet, ectodermal dysplasia, and cleft lip and/or palate (Gorlin et al., 1990). Features of ectodermal dysplasia in 77% of the patients include sparse hair, dystrophic nails, hypopigmentation or pigmented nevi of the skin, and abnormal dentition (Roelfsema and Cobben, 1996). Congenitally missing permanent teeth and conical teeth are common. Missing of maxillary first primary molars has been reported (Gorlin et al.,1990). Cleft lip and/or palate is seen in 68% of the patients (Roelfsema and Cobben, 1996).

Buss et al. (1995) reported dental features of 24 patients with EEC syndrome: The permanent dentitions of all patients were affected with oligodontia and microdontia. The teeth were not as strongly conical as in cases of X-linked EDA, being more often straight-edged with gaps; taurodontism was also common. The number of teeth was normal in the primary dentitions, but with abnormal morphology of the tooth crowns.

Microcephaly and mental retardation have been reported in about 10% of the patients (Gorlin et al., 1990). In addition, anomalies of the lacrimal ducts, urogenital defects, and conductive hearing loss have been reported (Roelfsema and Cobben, 1996).

Autosomal dominant transmission with reduced penetrance and variable expression is shown in EEC, with proven genetic heterogeneity. EEC1 syndrome is associated with chromosome 7 (7q11.2-q21.3) (Qumsiyeh, 1992). Linkage of EEC2 to a locus

on the chromosome 19 pericentromeric region has been reported in a Dutch kindred (O´Quinn et al., 1998). The third form, EEC3, is caused by mutations in the transcription factor gene p63 in chromosome 3q27 where another EEC-like disorders, limb-mammary syndrome and ADULT syndrome (acro-dermato-ungual-lacrimal-tooth syndrome) have been mapped (Propping et al., 2000). Several different mutations of gene p63 have been revealed in EEC families (Celli et al., 1999).

6.5.3. Cleft lip/palate-ectodermal dysplasia syndrome (CLPED1)

Cleft lip and/or cleft palate together with ectodermal dysplasia (CLPED1) has been reported in clinical conditions called Zlotogora-Ogur syndrome (Zlotogora, 1994), and the Margarita Island form of ectodermal dysplasia (Bustos et al., 1991). The gene mutated in these autosomal recessive syndromes is identified as PVRL1, located at the chromosomal region 11q23-q24 (Suzuki et al., 2000). PVRL1 encodes nectin-1, a cell adhesion molecule. In both syndromes, the patients have scanty eyebrows and eyelashes, sparse, short and dry scalp hair, syndactyly of the fingers and toes, cleft lip/palate, nail dysplasia, and hypodontia. Hypodontia affects mainly the upper lateral incisors, and in addition, changes in size and shape of tooth crowns (Bustos et al., 1991). Mental retardation is present in Zlotogora-Ogur syndrome but is absent from Margarita Island ectodermal dysplasia (Zlotogora, 1994).

6.5.4. Incontinentia pigmenti (IP, Bloch-Sulzberger syndrome)

Incontinentia Pigmenti (IP) is a rare multisystem disorder classified as an ectodermal dysplasia with variable abnormalities of the skin, hair, nails, teeth, eyes, and central nervous system. The skin of IP patients shows vesicular, verrucous, and pigmented macular lesions. In addition, the patients have dental, ocular, central nervous system, and structural anomalies (Gorlin et al., 1990). IP is an X-linked dominant disorder and has been shown to be due to mutations in IKK-gamma (NEMO) gene located in Xq28 (Smahi et al., 2000). The affected individuals are mostly females (97% of the patients). It is assumed that males with the mutation usually do not survive through gestation (Macey-Dare and Goodman, 1999).

In studies on dental anomalies, over 90% of the patients have hypodontia, mostly classified as severe (6 or more teeth missing), microdontia (generalized microdontia or peg-shaped teeth), macrodontia (extra cusps in the posterior teeth), delayed eruption of permanent teeth, or taurodontism (Gorlin et al., 1990; Macey-Dare and Goodman, 1999). Incidence of congenitally missing teeth has been reported to be as high as 43%; in addition, 30% of the patients have conical teeth (Macey-Dare and Goodman, 1999). Tooth anomalies are seen both in primary and permanent dentitions, but the permanent dentition is usually more severely affected.

6.5.5. Hypohidrotic ectodermal dysplasia and immune deficiency (HED-ID)

A novel form of EDA, together with immunodeficiency, segregates as an X-linked recessive trait (Zonana et al., 2000; Döffinger et al., 2001). Clinical findings: hypohidrosis and abnormal dentition, are similar to those in other forms of EDA. Tooth agenesis occurs both in primary and in permanent dentitions. Hypodontia, oligodontia, and conical teeth are the forms of tooth manifestation. Affected males manifest dysgammaglobulinemia and suffer significant mortality from infections. Female carriers show no clinical signs of immunodeficiency but have other manifestations including hypodontia and conical teeth. Mutations in the IKK-gamma (NEMO) gene have been found in HED-ID patients, and thus HED-ID is allelic to incontinentia pigmenti. IKKγ is required for activation of NFκB, a transcription factor transducing TNF signaling. NFκB was recently shown also to transduce signaling via ectodysplasin/ Edar, which presumably explains the phenotypic similarities between EDA and HED-ID (Kumar et al., 2001; Koppinen et al., submitted).

6.5.6. Oral-facial-digital syndrome type 1 (OFD1)

Oral-facial-digital syndromes are a heterogenous group of developmental disorders of which at least nine forms have been described (Ferrante et al., 2001). Oral-facial-digital syndrome type 1 is characterized by malformations of the face, oral cavity, and digits. Typical characteristics include facial asymmetry, hypertelorism, micrognathia, broadened nasal bridge, and facial milia (Ferrante et al., 2001). Median pseudoclefting of the upper lip has been reported in 45%, palatal clefts in over 80%, clefts of the tongue in 30%. In addition, they have supernumerary frenulae in the oral cavity and alveolar ridges may be thickened (Gorlin et al., 1990; Ferrante et al., 2001). Hypodontia typically affects mandibular lateral incisors in about 50% of these patients (Gorlin et al., 1990). Hypodontia of lower lateral incisors is associated with the fibrous bands in this region. Oral-facial-digital syndrome type 1 is transmitted as an X-linked dominant condition affecting females and causing mortality in males. The gene responsible for this syndrome is CXORF5 in chromosome Xp22.3-22.2; many different mutations have been found in these patients (Ferrante et al., 2001).

6.5.7. Witkop tooth-nail syndrome

Dysplasia of nails together with hypodontia was first described by Witkop (1965). This syndrome is inherited in an autosomal dominant manner. Fingernails and especially toenails are dysplastic in childhood. Mandibular incisors, second molars, and maxillary canines are the teeth most often missing or having conical crowns. Some patients show hypodontia or a conical form of primary teeth. A nonsense mutation in the homeodomain of *MSX1* has been shown to cause Witkop syndrome in a three-

generation family (Jumlongras et al., 2001). The predominant missing teeth were premolars, first molars, and third molars in this family. In a few cases, incisors or canines were also absent. Permanent teeth showed reduced mesiodistal dimensions and shorter root lengths than normal teeth. Primary teeth were normal in size, shape, and number in all patients except one individual with fused mandibular primary central and lateral incisor (Jumlongras et al., 2001).

6.5.8. Fried syndrome

Agenesis of primary incisors or their conical form together with thin hair and nails has been described by Fried in children born to consanguineus parents (1977), who suggested autosomal recessive inheritance of this trait.

6.5.9. Böök syndrome (PHC)

The features of Böök syndrome include premolar agenesis, hyperhidrosis of the hands and feet, and early graying of the hair (canities prematura). Early, diffuse whitening of the hair may appear even in childhood. Hypodontia in the Böök syndrome affects the premolar region, with one or more premolars missing. The syndrome has an autosomal dominant inheritance with high or complete penetrance; the gene defect is unknown (Gorlin et al., 1990; Böök, 1950).

6.5.10. Hair-nail-skin-teeth dysplasias

A large number of rare disorders involving dysplasia of hair, nails, skin, and teeth have been described (Gorlin et al., 1990). The features of these dysplasias overlap the ectodermal dysplasias, and both autosomal recessive and autosomal dominant inheritance patterns have been reported. Oligodontia and/or microdontia, peg-shaped teeth, and enamel hypoplasias of the teeth are the typical dental manifestations (Gorlin et al., 1990).

6.6. Rieger syndrome

Rieger syndrome is an autosomal dominant disorder with malformations of the anterior chamber of the eye, umbilical anomalies, and hypodontia. The maxillary primary and permanent incisors and second premolars are the most commonly missing. Peg-shaped incisors have also been reported. Hypodontia in the anterior region of the maxilla results in underdevelopment of the premaxillary area (Gorlin et al., 1990; OMIM (TM)database). Rieger syndome has proven to be genetically heterogenous, caused by mutations in a homeobox transcription factor gene, *PITX2* in 4q25-q26 (Semina et al., 1996). Another locus for Rieger syndrome has been

identified on 13q14 by linkage analysis (Phillips et al., 1996), but the gene has not yet been found.

6.7. Holoprosencephaly

Holoprosencephaly is a rare malformation sequence in which the basic feature is impaired midline cleavage of the embryonic forebrain (Gorlin et al., 1990). It is an etiologically heterogenous condition; teratogenic and genetic factors may both be responsible. The phenotype varies widely; the facial dysmorphism includes cyclopia, hypertelorism, single nostril or flat nose, cleft lip, and hypodontia. A single maxillary central incisor can be seen as the mildest phenotype of holoprosencephaly. In familial cases, an autosomal dominant inheritance with reduced penetrance has been observed (Odent et al., 1998). At least 12 different loci have been associated with holoprosencephaly and several distinct genes identified (Wallis and Muenke, 2000).

6.8. Down syndrome (trisomy 21)

Down syndrome, the most common chromosomal abnormality in man, is caused by trisomy of all or a critical portion of chromosome 21 (21q22.3). The birth prevalence of trisomy 21 syndrome is 1/650 live births, with the risk of having a child with Down syndrome increasing with maternal age (Gorlin et al., 1990; OMIM (TM) database). Down syndrome is characterized by a combination of phenotypic features that includes typical dysmorphic features and mental retardation. Congenital malformations of the heart (30-40% of the patients) and gastrointestinal tract are common. Congenital absence of teeth has been reported in 23 to 47% (Gorlin et al, 1990). Lateral maxillary incisors, lower incisors, second premolars, and third molars are the most commonly missing. One or both primary upper lateral incisors are missing in more than 10% of the patients, and peg-shaped maxillary lateral incisors are seen in 10% (Gorlin et al., 1990). Shapira et al. (2000) studied hypodontia and other dental anomalies in a sample of 34 individuals with Down syndrome in Israel. In this group, 74% of the individuals lacked one or more third molars, and 60% lacked at least one other tooth. In addition, 25% of the individuals had small or peg-shaped upper lateral incisor(s). With third molars excluded, teeth were missing in 59% of the total sample (Shapira et al., 2000).

6.9. Wolf-Hirschhorn syndrome (deletion 4p)

Wolf-Hirschhorn syndrome is a malformation syndrome caused by deletions of the distal short arm of chromosome 4 (4p16.3), with variations in both the size of the deletions and position of the breakpoints. It has been suggested that the critical region is approximately 165kb long (Wright et al., 1997). This region has been

sequenced intensively during the search for the genes causing Huntington disease, achondroplasia, and other skeletal dysplasias, and is found to be a gene-dense region; the *MSX1* gene is located nearby. Wolf-Hirschhorn syndrome is characterized by severe growth and psychomotor retardation, microcephaly, and striking facial features, and closure defects: cleft lip or palate, coloboma of the eye, and cardiac septal defects. About one-third of the patients have an isolated cleft palate, another third high arched palate with micrognathia, and 10% have cleft lip and palate. Agenesis of many permanent teeth has been suggested to belong to the oral manifestations of this syndrome (Burgersdijk and Tan, 1978).

6.10. Kabuki syndrome

The features of Kabuki syndrome (KS) include characteristic facial dysmorphic features, skeletal abnormalities, dermatoglyphic abnormalities, mild to moderate mental deficiency, and postnatal growth retardation. The etiology of KS is unknown (Mhanni et al., 1999). Agenesis of upper and lower permanent incisors or premolars, conical incisors, and ectopic upper first molars has been reported (Mhanni et al., 1999).

6.11. Diastrophic dysplasia (DTD)

Diastrophic dysplasia is a recessively inherited osteochondrodysplasia belonging to the group of disorders called the ”Finnish Disease Heritage.” Abnormalities in DTD seem to be restricted mainly to cartilage and bone. The main features include short-limbed short stature, generalized joint dysplasia, and spinal deformities. Mutations in the sulphate transporter gene *DTDST* in the long arm of chromosome 5 result in impaired sulphate uptake of the cells and reduced sulphation of the extracellular matrix macromolecules, particularly the proteoglycans. One-third of DTD patients have hypodontia in their permanent dentition; the lower second premolar, upper lateral incisor, and upper second premolar being the teeth most commonly missing. In addition, tooth crown sizes may be reduced. Cleft palate or submucous cleft palate is seen in 30% and 26% of the patients (Karlstedt et al., 1996).

6.12. Hemifacial microsomia

Hemifacial microsomia is a condition affecting primarily aural, oral, and mandibular development. The phenotype varies from mild to severe, it is usually limited to one side, but bilateral involvement also occurs (Gorlin et al., 1990). The etiology of hemifacial microsomia is unknown, with both environmental and genetic factors proposed; 20% of the patients show marked facial asymmetry, but mild asymmetry is evident in 65% (Gorlin et al., 1990). Asymmetry results from hypoplasia or aplasia

of the mandibular condyle and ramus. The maxillary, temporal, and malar bones may also be reduced in size. Anomalies of the heart, kidney, lung, and eye have been reported. These patients have unilateral microtia, and preauricular tags of skin and cartilage are common, and supernumerary ear tags may occur anywhere from the tragus to the angle of the mouth (Gorlin et al., 1990). The prevalence of hypodontia in hemifacial microsomia patients has been reported to be 25 to 27% (Farias and Vargervik, 1988; Maruko et al., 2001) and absence of one tooth is most common. There is an increase in prevalence of missing teeth with increasing severity of the mandibular deformity. The tooth most commonly missing is the mandibular second premolar, followed by the maxillary second molar, mandibular second molar, mandibular lateral incisor, maxillary second premolar, and maxillary lateral incisor in the study of Maruko et al. (2001).

6.13. Recessive incisor hypodontia (RIH)

A specific form of hypodontia with an autosomal recessive mode of inheritance characterized by missing primary and permanent incisors and an increased inclination to eczema and asthma has been found in Finland (Pirinen et al., 2001) and apparently also in other countries (Fried, 1977; Akyuz and Atasu 1993; Lyngstadaas et al., 1996). RIH patients lack several lower incisors and upper permanent lateral incisors. In addition, some other permanent teeth can be missing. Half the patients have a corresponding primary tooth either missing or peg-shaped. Taurodontism of the molars is noted in more than half the patients. A large proportion of the patients (62%) report allergies manifesting as atopic skin (52%), and asthma (43%). Minor dental anomalies are seen in their parents and siblings in the form of missing and/or peg-shaped upper lateral incisors and missing third molar(s). The proportion of atopic diseases, both in the patients and their family members exceeds reported population prevalences (Anonymous 1998). In a Finnish study, pedigrees of 31 families have been traced back at least five generations, and in two families, the parents of the proband had a common ancestor six and seven generations back, which supports the hypothesis of autosomal recessive inheritance (Pirinen et al., 2001).

7. ASSOCIATED DENTAL ANOMALIES

In general, two anomalies are considered associated if, in a sample of subjects selected according to one anomaly, the prevalence of the other anomaly is significantly higher

than in the general population or in a control sample. Several dental anomalies have been reported together with congenitally missing teeth.

7.1. Delayed formation and eruption of teeth

Delayed formation and eruption of premolars and molars were found in children with agenesis of the lower third molar(s) or third molar(s) together with some other teeth (Garn et al., 1961). In children missing 6 to 7 teeth including the third molar(s), a mean of 1.8 years delay for boys and 2.0 years for girls in relation to chronological age has been reported by Rune and Sarnäs (1974). No significant pattern of developmental timing in tooth formation could be ascertained in relation to sex, age, or number and distribution of missing teeth. However, a tendency to retardation was found in teeth contralateral to the missing teeth (Rune and Sarnäs, 1974). In oligodontia patients (more than 6 teeth missing excluding the third molars), great individual variation in tooth formation has been noticed (Schalk-van der Weide, 1992). Some patients showed severely delayed tooth formation, whereas others showed normal timing; this delay was more obvious in males than females (Schalk-van der Weide, 1992).

7.2. Reduction in tooth size and form

Reduction in the mesiodistal dimensions of tooth crowns has been reported in individuals with hypodontia (Grahnen,1956; Garn and Lewis, 1970). Tooth-number reduction was associated with **crown-size reduction**, so that the more teeth were missing the greater the possibility of clinically apparent microdontia in the same individual and the more reduction measured in remaining tooth crowns (Garn and Lewis, 1970; Brook, 1984).

A relationship between tooth agenesis and molar crown morphology has also been demonstrated. Third molar agenesis was associated with **reductions in the cusp number** of the molars (Garn et al., 1966).

A most striking example of crown-size reduction associated wih hypodontia is a **mesiodistally reduced** or **peg-shaped upper lateral incisor**. Baccetti (1998) showed significant reciprocal associations between agenesis of second premolars and reduced upper lateral incisors. The group with agenesis of second premolars showed a higher prevalence of small maxillary lateral incisors than did the control group and, conversely, the group with small maxillary laterals showed a higher prevalence of aplasia of second premolars than did their control group. Peg-shaped upper lateral incisors were found in 5.5% of the family members of the probands with hypodontia compared with the frequency of 1.7% in the population (Grahnen, 1956). Alvesalo and Portin (1969), studying the frequency and inheritance pattern of missing,

peg-shaped, and strongly mesio-distally reduced upper lateral incisors in families, suggested that absence and peg-shaping of upper lateral incisors are different expressions of one dominant autosomal gene with reduced penetrance.

7.3. Malposition of teeth

7.3.1. Ectopic maxillary canines

Ectopic maxillary canines occur in about 2% of the Caucasian population (Thilander and Jakobsson,1968; Shah et al., 1978; Ericson and Kurol, 1986). Becker et al. (1981) and Brin et al. (1986) reported that displaced canines and missing or peg-shaped upper lateral incisors appeared simultaneously. A study of orthodontic patients with at least one palatal canine showed that, in a high percentage of cases, the lateral incisors adjacent to these canines were missing (Zilberman et al., 1990). In this study, 46% of the probands with palatal canines had an anomalous lateral incisor; 5% of the parents and 11% of the siblings also had palatal canines and anomalous lateral incisors, in 31% and 28%, respectively. Palatal canine displacement showed significant reciprocal associations with small size of maxillary lateral incisors and absence of second premolars in a study of a population with no orthodontia (Baccetti, 1998). The frequencies were also significantly higher than for the control group.

Ectopic eruption of maxillary canines occurred at a higher than normal frequency in children with such dental anomalies as infraocclusion of the primary molars, ectopic eruption of maxillary first molars, and agenesis of the premolars (Bjerklin et al., 1992). Svinhufvud et al. (1988) studying tooth malpositions and their association with hypodontia in four large Finnish kindreds, demonstrated an association of palatal and labial canine malpositions with hypodontia (Svinhufvud et al., 1988).

Ectopic permanent canines were shown to associate with hypodontia in another Finnish study (Pirinen et al., 1996). The frequency of hypodontia was analyzed in 106 patients treated for ectopic canines and their family members: 36% of the patients and 20% of the first-degree relatives were missing some permanent teeth.

Peck et al. (1996, 1998) reported significantly elevated hypodontia frequencies in individuals with either maxillary canine-first premolar transposition, palatal displacement of the maxillary canine, or mandibular lateral incisor-canine transposition.

7.3.2. Ectopic eruption of other teeth

Ectopic eruption of the first permanent molar(s) showed a significant association with agenesis of second premolars and reduced maxillary lateral incisors, the most common manifestations of hypodontia (Baccetti, 1998). Malpositions of the upper lateral incisors, lower canines, and second premolars have also been noticed to occur

more often than in the general population in a Finnish family study (Svinhufvud et al., 1988).

7.4. Infraposition of primary molar(s)

A reciprocal association exists between infraocclusion of primary molars and aplasia of premolars (Bjerklin et al., 1992; Baccetti, 1998). In 18% to 22% of the subjects, aplasia of the second premolars was associated with infraocclusion of the first primary molars (Baccetti, 1998), whereas the population prevalence of infraocclusion is 10% (Bjerklin et al., 1992).

7.5. Short roots of teeth

Tooth agenesis has appeared in 46% of individuals with short roots of some permanent teeth, with maxillary central incisors and premolars the most frequently affected teeth in this condition, called short root anomaly (Lind, 1972; Apajalahti et al., 1999). The missing teeth were mostly the same as shown in hypodontia: upper lateral incisors and second premolars (Apajalahti et al., 1999).

7.6. Taurodontism

Investigations of patients with hypodontia and their siblings have revealed an association of taurodontism with hypodontia (Stenvik et.al.,1972; Seow and Lai, 1989) as well as with oligodontia (Schalk-van der Weide, 1993). Seow and Lai (1989) reported taurodontism of the lower molars in 35% of individuals with hypodontia. Taurodontism of the lower first molar(s) were seen in a Dutch study in 29% of oligodontia patients, compared with 10% of the control group (Schalk-van der Weide, 1993).

7.7. Rotation of premolars and/or maxillary lateral incisors

The prevalence of tooth rotation, together with agenesis of nonadjacent teeth, was studied by Baccetti (1998) in a sample of 1620 subjects and in a control group of 1000 individuals. The occurrence of tooth rotation in association with agenesis of nonadjacent teeth was significantly higher than in the control group for all the categories of tooth rotation. This study concluded that rotation of premolars is significantly associated with congenitally missing upper lateral incisors. Significant associations also appeared between unilateral agenesis of upper lateral incisors and rotation of of the lateral incisor on the other side of the dental arch, and between unilateral agenesis of premolars and rotation of premolars on the other side of the arch (Baccetti, 1998).

7.8. Enamel hypoplasia, hypocalcification

Ahmad et al. (1998) reported a recessively inherited hypodontia, in a large family, mapped to chromosome 16. Affected individuals had associated dental anomalies such as enamel hypoplasia, hypocalcification, and dentinogenesis imperfecta (Ahmad et al., 1998). Baccetti (1998) included enamel hypoplasia in seven types of dental anomalies, the associations of which were investigated in an untreated orthodontic population. In this study, the group with enamel hypoplasia presented significant associations with agenesis of the second premolars, small size of the upper lateral incisors, infraocclusion of the primary molars, and palatal displacement of the upper canines.

8. TOOTH DEVELOPMENT

8.1. Initiation and morphogenesis

Teeth develop from the oral ectoderm and from the underlying neural crest-derived mesenchymal cells, which have migrated from the cranial neural crest to the facial processes. The first sign of tooth development is thickening of the oral epithelium, which bud to the underlying neural crest-derived mesenchyme (Koch and Thesleff, 2001). This is accompanied by condensation of the mesenchymal cells around the bud. The cap stage of development is reached after rapid growth and folding of the epithelium, allowing the formation of the mesenchymal dental papilla, giving rise to tooth pulp and the odontoblasts, and to the dental follicle. The follicle gives rise to the cementoblasts, which deposit dental cementum as well as giving rise to the periodontal membrane, which connect the roots of the teeth to the alveolar bone. The dental epithelium forms the enamel knot, which functions as a control center in the formation of the cusps. During the following bell stage, the cusp pattern is established, and the morphology of the tooth crown is determined. The mesenchymal odontoblasts and the epithelial ameloblasts differentiate, and the deposition of dentin and enamel begins. Mineralization begins at the cusp tips and proceeds in a cervical direction. Root development follows the crown formation, and Hertwig´s epithelial root sheath determines the form of the roots. Interactions between epithelial and underlying mesenchymal tissues regulate the advancing tooth development (Pirinen and Thesleff, 1995; Koch and Thesleff, 2001). A schematic presentation of tooth morphogenesis is in Figure 3.

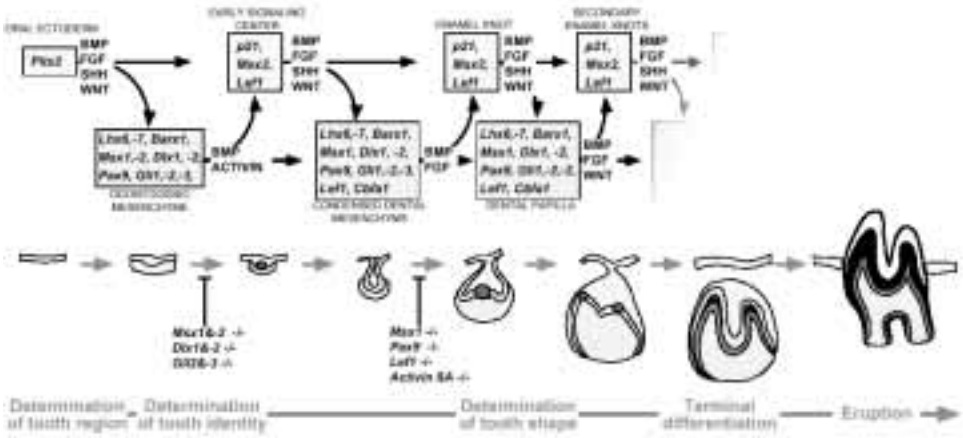

Fig. 3. Schematic presentation of tooth morphogenesis and current knowledge of signals and molecular events mediating communication between epithelial and mesenchymal components of developing tooth germ. The molecular cascades are shown above and corresponding morphological stages below. By courtesy of Jukka Jernvall and Irma Thesleff.

8.2. Tooth families and the development of dentition

8.2.1. Evolution

Features of dental-like tissues have been found in small structural units known as odontodes or denticles in fossils from over a half billion years ago. Teeth are found only in vertebrates, and their evolution is believed to be associated with the appearance of the neural crest (for review, Smith and Hall, 1993; Weiss et al., 1998; Thesleff and Sharpe, 1997). A characteristic feature of the evolution of dentition has been the transition from homodonty to heterodonty. In homodonty, teeth show some differences in size and shape but the number of teeth is quite variable. In addition, numerous bones of the oral cavity and pharynx can bear teeth. In heterodonty, regional structural differentiation among teeth is more qualitative. In many mammalian species, heterodonty involves spatulate incisors, and conical canines, followed by oval-to square premolars and molars. A major feature is the distinctive pattern of cusps and ridges typical for the crowns of the teeth (for review, Weiss et al., 1998). The developmental anatomy of tooth morphogenesis has been conserved to a high degree, and the morphological steps of early tooth development are very similar in all vertebrates studied (for review, Thesleff and Sharpe, 1997; Weiss et al., 1998). There are, however, a variety of modifications in the ways in which the dentitions are organized and the ways in which they function in different animals. The numbers of

teeth vary, and the teeth express differing forms with specialized functions (for review, Thesleff and Sharpe, 1997). The dentition of modern man, 32 permanent teeth (two incisors, one canine, two premolars, and three molars) is a result of changes in tooth number during evolution relative to the ancestral dental formula of three incisors, one canine, four premolars, and three molars (for review, Weiss et al., 1998).

8.2.2. Tooth families

Teeth are grouped into families according to their specific locations in the jaws. In mammals, the differences between tooth families correspond to the typical shape categories, as incisors, canine, premolars, and molars in man. While shape differences between tooth families are typical, the teeth in the same family resemble each other, and differences are typically only quantitative. Each tooth group forms from one epithelial thickening, the dental lamina, and development starts with the most anterior tooth and proceeds posteriorly (for review, Thesleff and Sharpe, 1997).

While knowledge of the processes in the development of individual teeth has expanded, much less is known about the control of dental patterning: the location, number, and differing morphology of the teeth (for review, Weiss et al, 1998; Tucker and Sharpe, 1999; Jernvall and Thesleff, 2000). The maxilla and mandible develop differently, but it is suggested that both jaws may be patterned by a consistent process that occurs before neural crest migration happens (for review, Weiss et al.,1998).

Two different theories have been suggested for the mechanisms of segmental tooth patterning and regulation of shape differences between the teeth. The field theory proposes that concentrations of chemical morphogens regulate the different types of teeth (Butler, 1939; 1995). The clone theory suggests that stem cells giving rise to different tooth families differ initially from each other (Osborn, 1978), and that the neural crest cells have their positional identity before their final migration into the region of tooth development.

Sharpe (1995) proposed that tooth shape and position are specified by combinatorial activities of different homeobox genes expressed in neural crest-derived jaw mesenchyme. For instance, several homeobox genes are expressed in specific spatial patterns in the jaws before initiation of tooth development. These genes are often expressed also during later stages of tooth development. Some findings in mutant mice support this suggestion. The *Dlx1/Dlx2* double-mutant knockout mouse lacks maxillary molars, whereas development of mandibular molars and all incisors is normal (Qiu et al., 1997). The single-mutant mouse has normal teeth. *Barx1* is expressed in the molar region but is almost completely absent from the incisor region (Tissier-Seta et al., 1995).

8.2.3. Chronology of the development of human dentition

The initiation of tooth development happens in 5- to 6-week-old human embryos. Calcification begins during 14 to 18 weeks, and the crowns of all 20 **primary teeth** are half-way mineralized at birth. Root formation is completed between 1.5 and 3 years (Koch and Thesleff, 2001). Central upper and lower incisors are the first developing teeth, followed by the first molars, lateral incisors, canines, and second molars (Pirinen and Thesleff, 1995). The lower incisor is the first tooth erupting, and mean eruption time is close to 7 months of age. Other primary teeth erupt during the following 20 to 22 months (Nyström, 1977; Pirinen and Thesleff, 1995).

Development of the **permanent teeth** also begins prenatally, when the dental lamina proliferates lingually and distally to the germs of the primary teeth, giving rise to 32 permanent tooth germs. The cusps of the first permanent molars have started mineralization at birth. At 2 to 3 months of age, the lower incisors, upper central incisors, and upper and lower canines start to calcify. Calcification of the upper lateral incisors begins close to the first birthday. Between the second and third years of life, the first and second premolars and second molars start to calcify. The crowns of the permanent teeth (except third molars) are generally completed between 5 and 7 years of age. Root development takes about 6 to 7 years. The third molars show a very large variation in development. They start to mineralize between the eight and eleventh year of life (Koch and Thesleff, 2001). However, sometimes the third molar appear very late, at 14 to 18 years of age (Pirinen and Thesleff, 1995). A sex-difference has been observed in tooth development, with girls are average half a year ahead of boys.

The lower incisors or the first molars are the first permanent teeth to emerge, with the mean age for their eruption being 6 to 7 years. The second premolars erupt at the age of 11 to 12, and the second molars at 11.5 to 12.4, as last permanent teeth excluding third molars. The mean age of eruption for third molars is around 21 years in males and 23 in females (Pirinen and Thesleff, 1995).

8.3. Signaling networks in tooth development

Developmentally, a tooth is a typical example of an epithelial-mesenchymal organ. A complex molecular network operates during tooth formation. Studies of odontogenesis at the molecular level, mostly using mouse teeth as models, have indicated that the development of teeth is under strict genetic control which determines the position, number, size, and shape of different teeth (for review, Thesleff and Nieminen, 1996; Tucker et al., 1998; Tucker and Sharpe, 1999; Jernvall and Thesleff, 2000). More than 200 genes are included at present in the graphic database illustrating the gene

expression patterns during tooth development (Nieminen et al., 2001). Tooth development is initiated by signals from the epithelial dental lamina to the mesenchyme, and thereafter the mesenchyme regulates epithelial morphogenesis. Complex interactions between the epithelium and mesenchyme are responsible for regulation during all stages of development. In the cap stage the enamel knot is functioning as an important signaling center regulating tooth shape.

Figure 3. summarizes current knowledge of the signals and molecular events mediating communication between epithelial and mesenchymal components of the developing tooth germ.

8.3.1. Signals

Several different families of signal molecules, also called growth factors and their receptors, function reciprocally between the epithelium and mesenchyme during tooth development. The families most studied are the transforming growth factor β (TGFβ), which includes bone morphogenetic proteins (BMPs), the fibroblast growth factor (FGF), the epidermal growth factor (EGF), and the hedgehog (Hh) and wingless (Wnt) families (for review, Thesleff, 2000). One or more members of all of these five families have been detected in developing teeth and also in other organs.

Enamel knots, transient clusters of dental epithelial cells, act as signaling centers for cusp development (Jernvall, 1995). It has been proposed that the enamel knot determines the site of the first cusp of a tooth and regulates the formation of other cusps in the molars. There is an enamel knot also in the incisors during their cap stage, and in the molars new enamel knots appear at the sites of new cusps (Jernvall, 1995). The primary enamel knot, at the tip of the epithelial bud, expresses many signaling molecules, including Fgf4, Fgf3, Fgf9, Bmp2, 4, 7, Shh, Wnt 10a, and 10b, and it is suggested to regulate the formation of epithelial folding in the cap stage. The secondary enamel knots at the bell stage of tooth development, at the tip of developing cusps, are suggested to determine the cusp position and size, resulting in a functional tooth shape (Jernvall, 1995; Jernvall and Thesleff, 2000). Fgf4 is the signal specifically located in the secondary enamel knots, but other signals like Shh and Bmp4 are also expressed.

8.3.2 Transcription factors

Transcription factors are proteins that regulate the transcription of genes in the nucleus, and are grouped according to those regions in the molecules that mediate their binding to DNA (for review, Thesleff, 1998). Homeobox-containing transcription factors are a specific group of transcription factors which have been called master regulatory

genes. They contain the homeobox, a 180-base-pair sequence (containing three α-helical regions) which turns in the peptide chain between these regions (for review, Thesleff 1995; see also Rasko and Downes, 1995). The special feature of these homeobox-containing genes is that they appear to act as position-specific regulators during embryogenesis, and their own transcription is regulated according to their order in the genome. Mutations in these genes cause cells to misunderstand their position and to form organs normally emerging from other regions. A striking example of the function of these genes has been shown in the fruit fly, *Drosophila*; mutation in the gene Antennapedia causes fruitflies to grow legs on their heads, where antennae ought to be. Homeotic genes were first detected in *Drosophila*. They are arranged in clusters in both the *Drosophila* and mammalian genome. Antero-posterior patterning of embryos also in mammals, including man, is regulated by a specific set of homeobox-containing genes. Four clusters have been identified in the human genome, each with numerous homeobox genes (Hox-clusters in chromosomes 2, 7, 12, and 17). Hox genes are closely similar in their sequence in different animals, demonstrating their strong evolutionary conservation (for review, Thesleff, 1998).

No expression of Hox cluster genes has been shown anterior to the second branchial arch, and thus they do not regulate face and tooth development. However, several other homeobox-containing genes are important for craniofacial development and that of different organs. Tooth development is regulated by many homeobox genes including *Msx1*, *Msx2*, *Dlx1*, *Dlx2*, and *Barx1*, in addition to important transcription factors in other groups. These include the paired box gene *Pax9*, and *Lef1*, a HMG-group transcription factor (for review, Thesleff, 1998; Peters and Balling, 1999).

The homeobox genes *MSX1* and *MSX2*, earlier called *HOX7* and *HOX8*, are related to the *Drosophila* muscle-segment homeobox gene (msh). Mouse *Msx1* and *Msx2* are expressed in many tissues including teeth and bone during organogenesis and have been shown to associate with epithelial-mesenchymal interactions, being targets of *Bmp* and *Fgf* signaling (Satokata and Maas, 1994). The expression of *Msx1* is very intense in the dental mesenchyme throughout tooth morphogenesis (for review, Thesleff, 1995). *Msx2* is expressed both in mesenchymal and in epithelial cells including the enamel knot, which suggest a role in cusp development (for review, Tucker and Sharpe 1999; Thesleff, 2000). Mouse *Msx2* is also present in and adjacent to the calvarial sutures (Rice, 1999), and mutated *MSX2* on chromosome 5 (5q34-35) causes the Boston-type craniosynostosis in man (Jabs et al., 1993). Loss-of-function deficiency of *Msx2* causes defects in skull ossification and persistent calvarial foramen (Satokata et al., 2000). These mice also have defects in ectodermal organs, including teeth with enamel defects.

PAX9 is a member of the Pax gene family characterized by the presence of the paired domain which is a DNA-binding motif. Nine different *PAX* genes have been isolated in man including *PAX9* on chromosome 14 (14q12-q13). *Pax* genes function as key regulators of organogenesis at various sites during embryonic development. It is suggested that these *Pax* genes determine the time and place of organ initiation or morphogenesis (for review, Dahl et al., 1997). Interestingly, expression of *Pax9* has been shown to specifically mark the mesenchyme at the prospective sites of all teeth prior to any morphological manifestations (for review Peters and Balling, 1999).

9. TRANSGENIC MICE WITH TOOTH AGENESIS

Transgenic mice are intensively used for studies of mechanisms that regulate development and as models of human diseases. In transgenic animals individual genes can be misexpressed in defined tissues or their function can be disrupted. Mutations in several different genes lead to arrested tooth development in mice. All these genes participate in signaling networks in tooth formation (for review, Kettunen 1999; Thesleff, 2000).

Msx1 was the first gene shown to be essential for development of teeth in the mouse, when *Msx1*-deficient mice were demonstrated to lack all teeth (Satokata and Maas, 1994). Later, it was shown that tooth development does not proceed beyond the bud stage in mice lacking transcription factors *Lef1* or *Pax9* (van Genderen et al., 1994; Peters et al., 1998). *Msx1* is expressed in the dental mesenchyme, and its deletion results in inhibition of the expression of *Bmp4* and *Fgf3*, which act as reciprocal signals to the epithelium (for review, Thesleff, 2000). Recently, it has been shown that adding BMP4 to cultures of *Msx1* mutant tooth germs can restore their development (Bei et al., 2000).

Arrested tooth development at the lamina stage is seen in mice with double mutants of *Msx1/Msx2*, *Dlx1/Dlx2* and *Gli2/ Gli3* (Maas and Bei, 1997; Thomas et al., 1997; Hardcastle et al., 1998). Inactivation of *Pitx2* and *p63* in mice causes total agenesis of teeth, whereas heterozygous mice are normal (Lin et al., 1999; Mills et al., 1999).

In disruption of the gene encoding activin-binding protein follistatin, development of incisors is inhibited, whereas molars reach the bell stage, but look abnormal (Matzuk et al.,1995). Targeted inactivation of activinβA, normally expressed in dental mesenchyme, causes failure of the development of incisors and mandibular molars, but maxillary molars remain normal (Ferguson et al., 1998).

Some teeth are missing or their size is abnormal in the spontaneous mouse mutant *Tabby* with its ectodysplasin deficiency (Srivastava et al, 1997; Mikkola et al.,

1999; Pispa et al., 1999). Ectodysplasin, a novel tumor necrosis factor (TNF), is one protein required for epithelial morphogenesis. The mutant mouse *downless* (dl) has an identical phenotype with the *Tabby* mouse. Cloning of the gene responsible for the downless mouse revealed a novel TNF receptor, edar (Headon and Overbeek, 1999). Both ectodysplasin and edar were recently shown to mediate interactions within the epithelium (Laurikkala et al., 2001). Thus, identical phenotypes may be caused by mutations in two genes in the same pathway.

10. METHODS FOR IDENTIFYING GENES BEHIND HUMAN DISEASES

If genetic factors are assumed to cause a human disease, there are, in principle, two basic approaches - functional or positional cloning - for the identification of the causative gene. Cloning can be performed by **functional cloning**, in which no information as to the chromosomal location of the disease gene is required. In functional cloning, identification of the gene is based on knowledge of the gene product (Collins, 1995). This information may include the basic biochemical defect of the disease, the amino acid sequence of the protein, or the possibility of using information from an animal model of that disease. If a molecular candidate for the gene mutated in a disease or the chromosomal location of the disease can be proposed or an animal model can show possible candidate genes, a method can be used called the **candidate gene approach**. In this approach, several polymorphic markers either within the potential gene or in the flanking region are chosen for analyses and tested for linkage with the disease in affected families. If only the phenotype of the disease gene is known, the gene has to be sought on the basis of its chromosomal location. This method is called **positional cloning** (Collins,1995). The first step in positional cloning is assignment of the chromosomal locus, often by linkage analysis.This analysis is based on the segregation of the markers and the disease in families. When the chromosomal region has been identified, the genes of this region are subjected to mutation detection by sequencing or other methods.

10.1. Mapping of a disease gene

10.1.1. General principles

Gene mapping refers to the process where a causative gene is localized in the genome by comparing the inheritance of a disease and genetic marker loci in families. The first step is to examine a family sample where the disease of interest

segregates. The phenotypes of family members must be defined as precisely as possible and the mode of inheritance determined. The genotypes of family members are determined with respect to genetic markers covering the human genome, and segregation of the markers with the disease is analyzed. If two gene loci, a marker and a disease, are close each other, they tend to segregate together. The closer they are on the same chromosome, the greater the chance of their staying together at meiosis, and co-segregation happens more often than expected by chance. Evidence for linkage is tested by different statistical methods.

10.1.2. Linkage analysis

Linkage analysis is the method most often used in mapping studies. In linkage analysis, the co-segregation of a disease and markers in a family are followed and measured. Linkage analysis relies on the ability to detect recombination events between the disease and the marker locus during meiosis. Traditionally, linkage analysis has been used for studying Mendelian traits, but at present it is used also in complex diseases (Öhman, 2001). If two loci are close to each other on the same chromosome, the chance of recombination between them at meiosis is low, and they are said to be linked.

Statistical analysis is necessary to calculate the probability of linkage in a pedigree. The lod score method is based on calculating the overall likelihood of two alternative assumptions: that the two loci are linked at the given recombination fraction, L(θ), or are not linked but are inherited independently, L(1/2). In this case a recombination is seen in 50% of meiosis. The probability of linkage is expressed as a **lod score**, logarithm (to the base 10) of the odds ratio (Morton, 1955; Ott, 1974). The lod score is estimated at different θ values, usually ranging from 0 to 0.4. A lod score of 3 (odds ratio of 1000:1) or greater is regarded as significant, and evidence for linkage at a given recombination fraction is assumed. A lod score less than –2, on the other hand, indicates exclusion of linkage. A single pedigree does not, however, usually contain enough information to reach a decisive score. It is therefore necessary to combine data from several pedigrees. A lod score can be summed from several families to give a final lod score. More statistical power can be obtained from multipoint analysis, which utilizes segregation of haplotypes of several markers. In linkage analysis, the specification of inheritance model, allele frequencies, and penetrance values increases the power of the analysis and may eliminate false-positive results. Probability methods and computer programs have been developed to make it possible to collect and count data from different families and from many marker loci. The most commonly used program package for linkage analysis has been LINKAGE (Lathrop and Lalouel, 1984; Lathrop et al., 1984, 1986). Several new algorithms and programs have been developed

to overcome limitations of computer power and to provide simulation and non-parametric methods.

10.1.3. Genetic markers and the polymerase chain reaction (PCR)

Second-generation markers, the **microsatellites** or short tandem repeat markers (STR), have aided considerably in the mapping of genes by linkage analysis (Miesfeld et al., 1981). The advantage of microsatellites is their high rate of polymorphism, as well as their dense distribution throughout the genome. Microsatellites and the method called the **polymerase chain reaction (PCR)** have led to the present technique of detecting length polymorphisms in microsatellite regions. With PCR, alleles of a microsatellite are amplified a million-fold, and the products are separated by polyacrylamide gel electrophoresis (Saiki et al., 1988; Weber and May, 1989). Recently, fluorescence-based semiautomated genotyping of the marker loci has become more popular in genetic mapping (Reed et. al., 1994).

The Human Genome Project, established in the 1980s, is an international project established to acquire fundamental information on human genetics and the role of various genes important in development and diseases (Strachan and Read, 2000). This system has created numerous microsatellite maps with thousands of markers from all chromosomes (Weissenbach et al., 1992; Guapay et al., 1994; Murray et al., 1994; Dib et al., 1996). The final task has been to obtain the ultimate physical map, the complete sequence of the human genome. This year, 2001, the results of genome sequencing cover about 94% of the human genome. There appear to be from 30 000 to 40 000 protein-coding genes in the human genome – only about twice as many as in the fruit fly. However, these genes are more complex, with more alternative splicing generating a large number of protein products (Lander et al., 2001). The project interacts with research on mapping and identifying human disease genes and studying genetic variation (Strachan and Read, 2000). Data on mapping and sequencing are entered into large electronic databases freely accessible through the Internet.

AIMS OF THE PRESENT STUDY

The aim of the present study was to investigate the phenotypic and genotypic features of familial tooth agenesis. Families of eleven probands with the common mild form of incisor-premolar hypodontia were studied. In addition, mutation analysis of the *PAX9* gene was carried out in two families with oligodontia.

The specific aims were:

- to analyze the inheritance and phenotype of familial hypodontia
- to study the association of dental anomalies with hypodontia
- to search by linkage analysis for the genetic locus causing hypodontia
- to find a putative mutation in the *PAX9* gene in two families with oligodontia

SUBJECTS AND METHODS

1. SUBJECTS

1.1. Families with hypodontia (I, II, III)

The criterion for selecting persons with hypodontia (probands) was the congenital absence of one to six permanent teeth or the presence of peg-shaped permanent upper lateral incisors. The probands were nonsyndromic patients at the Department of Pedodontics and Orthodontics, Institute of Dentistry, University of Helsinki. The probands and/or their parents were interviewed about occurrence of hypodontia in their families. Families with more than one individual with hypodontia were first contacted to learn about the aims of the study. All family members voluntarily participating in the study were invited for an examination. The study was approved by the Ethics Committee of the Institute of Dentistry, University of Helsinki.

The study population comprised 11 probands with hypodontia, four males and seven females (aged 10-36 years), and their family members, totaling 214 individuals in 11 Finnish families (I). The spouses of the family members were also interviewed and examined. Information concerning general health was obtained by interview. A clinical dental examination of the probands and their family members was performed by three orthodontists (S.A., S.P., I.T.). In addition to hypodontia, other visible dental abnormalities were recorded. Panoramic radiographs were taken if this was necessary for the diagnosis of hypodontia. In some cases, information on hypodontia was confirmed from documents and radiographs in dental files.

Pedigrees of the families were constructed, and individuals with congenitally missing teeth or peg-shaped/strongly mesio-distally reduced upper lateral incisor(s) were recorded as affected (Figure 4). The prevalences of different dental anomalies were calculated separately for family members who were first- and second-degree relatives, first cousins, and more remotely related relatives of the probands. In addition, obligate carriers of the hypodontia gene who did not display tooth agenesis or peg-shaped teeth were identified from the pedigrees and analyzed separately for dental features.

The clinical study (I) included the whole sample of 11 families. The molecular genetic exclusion studies (II, III) included 5 (II) and 7 (III) families. In a genome-wide scan, a sample of 4 families was chosen, including families 1, 2, 6, and 9. Simulation with the Slink program (Ott, 1989; Weeks et al., 1990) confirmed that, assuming genetic homogeneity, the families were sufficiently informative to reveal significant evidence for linkage. Family 10 was excluded from the linkage studies because both

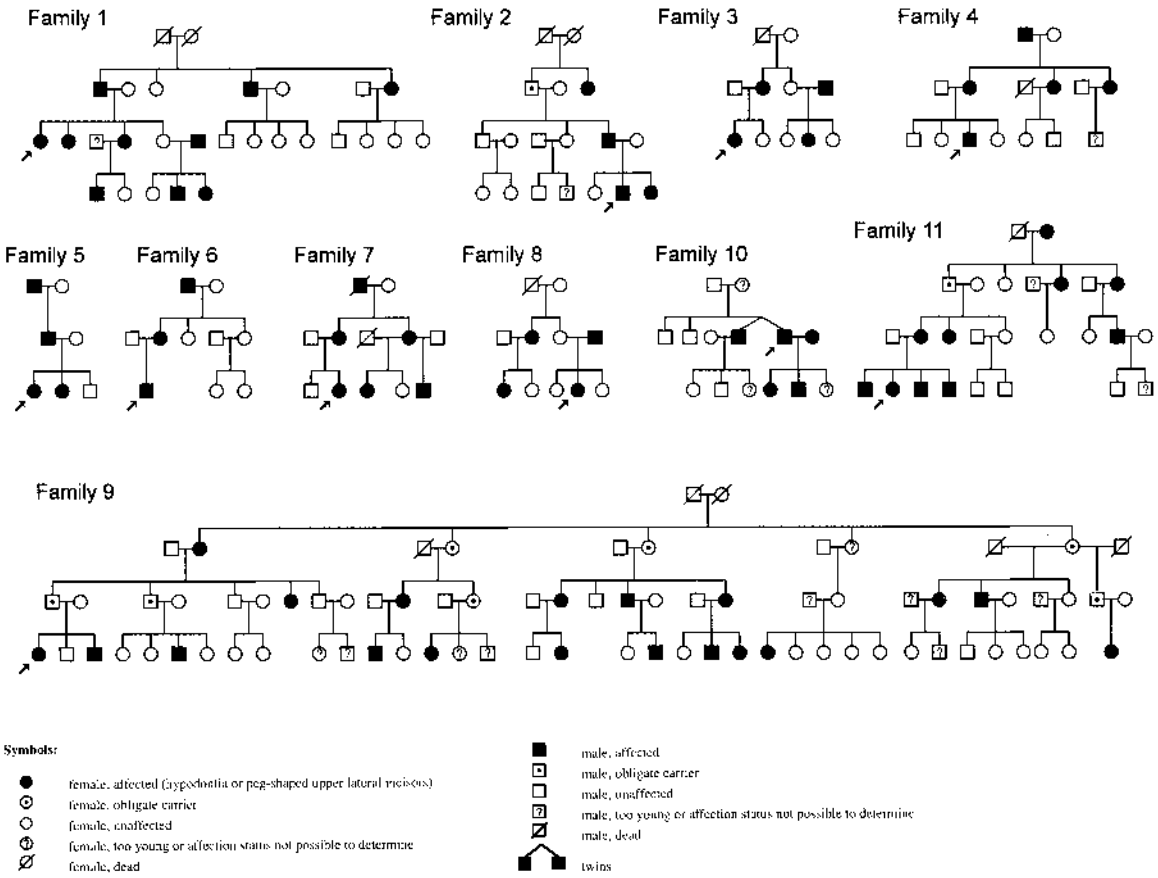

Fig. 4. Pedigrees of 11 hypodontia families. An arrow indicates a proband.

the mother and the father in the second generation had hypodontia, and family 11 because the proband had a cleft of the soft palate. Families 1 and 2, as well as 3 and 8 overlap, but they have been separated into smaller families in the linkage studies.

1.2. Families with oligodontia (IV)

We studied the association of the *PAX9* gene mutation with oligodontia in 2 two-generation families with 3 and 2 affected individuals, respectively (IV). The proband of family 1 was originally referred for treatment to the Pediatric Dental Department of the Hospital for Children and Adolescents, Helsinki University Central Hospital. The proband of family 2 was referred for consultation and treatment to the Department of Pedodontics and Orthodontics, Institute of Dentistry, University of Helsinki.

The families were informed of the genetic study, and family members consented to participate. The study was approved by the Ethics Committee of the Institute of Dentistry, University of Helsinki.

2. METHODS

2.1. Diagnosis of congenitally missing teeth

2.1.1. Studies I, II, and III

Both a clinical and a radiographic examination were performed for diagnosing hypodontia. The third molars were excluded because reliable retrospective data on them was incomplete. The presence of peg-shaped or strongly mesio-distally reduced upper lateral incisor(s) was also diagnosed as hypodontia. In addition, retrospective dental data were collected by questions, and from dental files. Data included the recording of missing teeth, extractions of teeth, ectopic teeth, orthodontic treatment, and hypodontia of primary dentition. Children under 6 (N=10) were excluded from the study because hypodontia cannot be reliably diagnosed at a young age.

2.1.2. Study IV

The diagnosis of missing teeth was based on both clinical and radiographic examinations. Retrospective dental information was collected from dental files. The youngest child was 6 years old. Agenesis of third molars was not possible to diagnose in the proband and in two brothers of the proband in family 1 because of their young ages.

2.2. Penetrance (I)

The penetrance of hypodontia in the pedigrees was calculated with the maximum likelihood method as described by Mather (1957).

2.3. Dental age (I)

Dental age was assessed from the developing dentition as seen in the panoramic radiographs of 6 probands (3 females, 3 males) and 32 relatives (17 females, 15 males) by a computer-aided method (Software Publishing Corporation, Mountain View, CA, USA) with norms corrected for Finnish children (Kataja et al., 1989). If the index tooth was missing, a contralateral tooth was used. In 3 children, the second premolar was missing bilaterally, and the value of one stage lower than for the first premolar was used.

2.4. Invaginations (I)

Invaginations in permanent upper lateral incisors were recorded clinically or from panoramic radiographs. The lingual pit (foramen cecum), fillings at the location for invagination, and teeth which showed radiographically distinct enamel notching were recorded as invaginations. The study population was comprised 9 probands and 98 relatives. Individuals with missing upper lateral incisors were excluded. Of the relatives, 33 were first- and 15 second-degree relatives, 16 were cousins, and 34 were more remote relatives.

2.5. Taurodontism (I)

Taurodontism of the first and second permanent molars was determined from panoramic radiographs of 11 probands and 96 family members, the latter composed of 31 first- and 13 second-degree relatives, 24 first cousins, and 28 more remotely related individuals. Persons with large restorations or extractions of molars were excluded from the study. The determination was made only on teeth with at least half of the root developed. Pyramidal or single-rooted molars were excluded. The method was that described by Shifman and Chanannel (1978), and modified by Tulensalo et al. (1989). Three vertical measurements were made directly in the panoramic radiographs with a ruler by one of the authors (S.A.). Measurements were recorded to the nearest 0.5 mm. Measure 1 was the vertical height of the pulp chamber: the distance between the lowest point of the roof of the pulp chamber and the highest point of the floor of the pulp chamber. Measure 2 was the distance between the lowest point of the roof of the pulp chamber and the apex of the longest root. These measurements were used to calculate the taurodontic index (TI): TI = (measure1/ measure 2) x 100. Measure 3 was the distance between the baseline connecting the two cementoenamel junctions and the highest point of the floor of the pulp chamber (Blumberg et al., 1971).

Taurodontism was diagnosed in those molars in which the TI was above 20 or the variable 3 exceeded 3.5 mm. The value of measure 3 was used in teeth with incomplete development of the roots. All measurements were performed by the same author (S.A.) and recorded to the nearest 0.5 mm.

2.6. Reliability of measurements

To compare assessment reliability for taurodontism, 144 molars in 20 radiographs were measured twice with a 2-week interval. One molar, 1/144 (0.7%), was classified differently in the two measurements, leading to a different diagnosis of taurodontism in 1 individual (1/20) and to an intraexaminer reliability of 95%.

2.7. Controls

We used the population prevalences of dental anomalies in the literature as controls (I). In addition, 2 distinct control groups were used (Table 3). The frequency of invagination(s) in the upper lateral incisor(s) of the study group was compared with that of an unselected control sample which comprised 170 children with late mixed or early permanent dentition attending an annual dental examination (91 females, 79

Table 3. Prevalences of dental anomalies in control groups or published population data

Dental anomaly	Prevalence (%)	Control sample	Reference
Hypodontia	8		Haavikko, 1971
Peg-shaped lateral incisor	1.7		Grahnen, 1956
Ectopic upper canine(s) -palatal or labial	1-2		Thilander and Jakobson, 1968
Rotation of premolar(s)	3-5		Baccetti, 1998
Invagination(s)	42	170 unselected children	examined for this study
Taurodontism	22	841 panoramic radiographs of unselected Finnish sample, collected 1965-1970 (Haavikko, 1971)	Taurodontism assessed for this study

males, age-range 12-14 years). All were clinically examined (S.A.) at the Department of Pedodontics and Orthodontics.

The control group for taurodontism included 841 panoramic radiographs (389 females, 452 males, age-range 6-21 years), the sample was collected from 1965 to 1970 (Haavikko, 1971). The anomalies were analyzed separately in controls with and without hypodontia.

2.8. DNA analysis

2.8.1. DNA extraction (II, III, IV)

Venous blood samples, 10 to 20 ml, were taken for DNA analyses from all available family members. DNA was extracted from leukocytes according to standard procedures (Vandenplas et al., 1984).

2.8.2. Genotyping

The polymerase chain reaction (PCR) method with amplifiable microsatellite markers was used for assessing the genotypes of the individuals (II, III). The second stage of linkage studies used a genome-wide search with a selection of microsatellites having a maximum genetic distance of 15cM for genotyping the members of four families.

PCR primers were obtained from Genethon, France, or from the Department of Clinical Genetics, University Hospital of Uppsala, Sweden, or they were synthesized in the Laboratory of Human Molecular Genetics of the National Public Health Institute, Finland. In addition, primers for the MSX2 microsatellite were a gift from Dr Ethylin Wang Jabs (Johns Hopkins University School of Medicine, MD, USA). Microsatellite polymorphism was analyzed by radioactive or fluorescence-based detection methods. When radioactive detection of PCR products was used, one of the primers was 5‘- end–labeled with ^{32}P-phosphate with T4 kinase (Pharmacia, Sweden) in a buffer provided by the manufacturer of the enzyme, containing 0.3 μC of μ-^{32}P-ATP (Amersham, UK). PCR reactions were done in a Perkin-Elmer Cetus Thermal Cycler 480 (Norwalk, CT, USA) or DNA Engine (MJ Research, MA, USA) in a total volume of 13μL containing 12ng of chromosomal DNA, 3pmol of each of the primers, 3 nmol of each of the deoxynucleotides, 0.25 U of enzyme, and 1.5nM $MgCl_2$ in a buffer described by Weissenbach et al. (1992). Either Taq-polymerase (Amplitaq, Perkin Elmer Cetus, Ca) or Dynazyme II (Finnzymes, Finland) were used. PCR products were made 50% of formamide and 10 mM of EDTA, pH 8.0, and separated in a 5% sequencing gel with 7.5 M urea in an IBI gel apparatus or in an ABI 377 sequencer (Perkin Elmer Biosystems, CA). ^{32}P-labeled alleles were detected by autoradiography with Kodak films exposed for 15 to 20 hours and analyzed m anually. Fluorescent alleles were detected with Genescan software and identified with Genotyper software (Perkin Elmer Biosystems).

2.8.3. Genetic maps

For the genome-wide scan of incisor-premolar hypodontia locus, markers were selected to provide a density of at least one marker per 15cM interval from published genetic maps of Genethon (Dib et al., 1996; ftp://. Genethon.fr.pub/Gmap/Nature-1995/), Cooperative Human Linkage Center (CHLC; Murray et al., 1994; http://

lpg.nci.nih.gov/CHLC/), and Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics/). In the multipoint analyses, genetic locations were based primarily on the Marshfield maps.

2.8.4. Sequencing of the *PAX9* gene

Four sets of primers were used to amplify the coding region of the *PAX9* gene (IV). Amplification was performed in 100µl with DNA concentrations of 1.5ng/µl, 1.5mM $MgCl_2$ and 1 mU Dynazyme™ EXT polymerase (Finnzymes). The primers and PCR conditions were as follows, with numbering indicating the position of the first base of the primer in the coding sequence: exon 2, forward primer 1 AGGCAGCTGTCCCAAGCAGCG (exon start–58), reverse primer 1 GGAGGGCACATTGTACTTGTCGC (357), annealing T 64°C, 32 cycles; exon 2, forward primer 2 ATCCGACCGTGTGACATCAGCC (109), reverse primer 2 GAGCCCCTACCTTGGTCGGTG (exon end +10), annealing T 64°C, 30 cycles; exon 3, forward primer GGGAGTAAAACTTCACCAGGC (exon start –197), reverse primer CCACCTGGCCTGACCCTC (exon end +28), annealing T 61°C, 32 cycles; exon 4, forward primer GGAGAGTAGAGTCAGAGCATTGCTG (exon start –121), reverse primer GAGACCTGGGAATTGGGGGA (stop +74), annealing T 61°C, 32 cycles. Primers for exons 2 and 4 were adapted from Stockton et al (2000). PCR products were purified by agarose gel electrophoresis and the Qiaquick gel extraction kit (Qiagen, Bothell, WA, USA). Both strands of the PCR products were sequenced with BigDye terminator chemistry and analyzed on an ABI 377XXL DNA sequencer (Applied Biosystems, Foster City, CA, USA).

2.9. Statistics

The significance of differences in prevalence of taurodontism and rotation of premolar(s) was tested in the study groups and in controls with the Chi-square test (I).

In the studies (II, III), the lod score method served for evaluation of the existence of linkage (Ott, 1974). The lod score was obtained by calculating the overall likelihood of the observed data with two alternative assumptions: that the loci are linked at a given genetic distance (recombination fraction θ) or that they are not linked. The logarithm to the base 10 of the ratio of these two likelihoods (the odds ratio) is the lod score. It has been agreed that a lod score exceeding 3 should be taken as statistically significant proof for linkage, and a lod score less than –2 indicates the exclusion of linkage.

The hypodontia locus was modeled as an autosomal dominant, two-allele system with a gene frequency of the hypodontia allele of 8%. A penetrance of hypodontia of

86% was used (Burzynski and Escobar, 1983), as this value was in good agreement with our family data. The informativeness of the family material was evaluated with the Slink software package, assuming an autosomal dominant model and gene homogeneity (Ott, 1989; Weeks et al., 1990). Allele frequencies for all markers were calculated from all known genotypes. The pairwise lod scores for different recombination fractions were estimated by the Mlink and Ilink options of the Linkage package, version 5.1 (Lathrop et al., 1984). In affecteds-only analyses, the phenotypes of the unaffected individuals were coded as unknowns. The Homog program (Ott, 1991) was used to estimate the lod scores under the assumption of non-allelic gene heterogeneity.

RESULTS AND DISCUSSION

1. HYPODONTIA IN FAMILIES

1.1. Characteristics of hypodontia in families (I)

Pedigrees of the 11 probands (Fig. 4) indicate an autosomal dominant mode of transmission, confirming previous findings (Grahnen, 1956). The calculated penetrance of hypodontia in all families was 97%, being higher than the penetrance (86%) in the data published by Grahnen (Burzynski and Escobar, 1983). The value of penetrance may vary between studies due to calculation method, sample, and diagnostic criteria. We collected these families by choosing probands with 1 to 6 teeth missing or peg-shaped in order to have a phenotypically uniform hypodontia sample of families.

Missing teeth were: one or more premolars in 2 families, incisors in 3, and premolars and incisors in 6 families. There was no gender difference in the frequency of hypodontia: hypodontia occured in 34% of the females and 35% of the males. The prevalence of hypodontia (Table 4) in first- (39%) and second-degree relatives (36%) was 4.5 to 4.9 times the population prevalence of 8% (Haavikko, 1971). The prevalence of peg-shaped upper lateral incisor(s) for these 2 groups was 9% and 11%, respectively. Of first cousins, 12% showed hypodontia, but in more remote relatives the frequency was 34%. The combined prevalence of hypodontia and/or peg-shaped teeth was high in first- (43%) and second-degree relatives (42%) and 18% in cousins of the probands. The high frequency of missing teeth in second-degree and in more remotely related relatives is a sign of selection of this sample, but is partly explained by the high frequency of hypodontia in the population. In large kindreds, the likelihood of the mutated gene´s entering the family from more than one carrier is great.

The mean number of missing teeth was 2.3 in probands and 1.7 in relatives (Table 5) concurring with previous population-based studies on hypodontia (Haavikko, 1971; Bergström, 1977). Hypodontia in the maxilla was seen in 59% and in the mandible in 55% of family members. In 15% of cases, hypodontia occurred in both jaws (Table 5). Asymmetric hypodontia was found as frequently in both jaws, and both sides of the maxilla were equally affected, but the left side of the mandible was affected in 81% of unilateral cases. Hypodontia was more common in the maxillae of our probands. This may be explained by their being orthodontic patients, with 5 of them having agenesis or peg-shaped upper lateral incisors as one of the indications for treatment. In the relatives, hypodontia equally affected both the maxilla and the mandible, similar to previous studies (Grahnen 1956; Haavikko, 1971). Unilateral

Table 4. Frequency of hypodontia (excluding third molars) and peg-shaped upper lateral incisors in relatives of probands

	Individuals N	Individuals with hypodontia N (%)	Individuals with peg-shaped upper lateral incisor(s) N (%)	Individuals with hypodontia and/or peg-shaped upper lateral incisor(s) N (%)
fathers	11	3 (27)	1 (9)	4 (36)
mothers	11	5 (45)	1 (9)	5 (45)
brothers	11	4 (36)	2 (18)	5 (45)
sisters	11	4 (36)	0	4 (36)
children	2	2 (100)	0	2 (100)
Total no. of 1st-degree relatives	**46**	**18 (39)**	**4 (9)**	**20 (43)**
grandfathers	7	3 (43)	2 (29)	4 (57)
grandmothers	10	1 (10)	0	1 (10)
aunts	16	8 (50)	3 (19)	10 (63)
uncles	5	1 (20)	0	1 (20)
nephews, nieces	7	3 (43)	0	3 (43)
Total no. of 2nd-degree relatives	**45**	**16 (36)**	**5 (11)**	**19 (42)**
first cousins	33	4 (12)	2 (6)	6 (18)
other relatives	58	20 (34)	2 (3)	22 (38)
Total	**182**	**58 (32)**	**13 (7)**	**67 (37)**

Table 5. Distribution and inheritance of hypodontia in probands and their relatives

	probands female	probands male	probands totals	relatives total	probands and relatives total
individuals with hypodontia	6	4	10	58	68
with hypodontia or peg-shaped tooth	7	4	11	67	78
number of missing teeth	16	7	23	97	120
mean number of missing teeth	2.7	1.8	2.3	1.7	1.8
hypodontia in maxilla	7 (100%)	3 (75%)	10 (91%)	36 (54%)	46 (59%)
unilateral	4 (57%)	1 (33%)	5 (50%)	16 (44%)	21 (46%)
right	0	1 (100%)	1 (20%)	9 (56%)	10 (48%)
left	4 (100%)	0	4 (80%)	7 (44%)	11 (52%)
bilateral	3 (43%)	2 (67%)	5 (50%)	20 (56%)	25 (54%)
hypodontia in mandible	3 (43%)	2 (50%)	5 (45%)	38 (57%)	43 (55%)
unilateral	1 (33%)	1 (50%)	2 (40%)	24 (63%)	26 (60%)
right	0	0	0	5 (21 %)	5 (19 %)
left	1 (100%)	1 (100%)	2 (100%)	19 (79%)	21 (81%)
bilateral	2 (67%)	1 (50%)	3 (60%)	14 (37%)	17 (40%)
hypodontia in maxilla and mandible	3 (43%)	1 (25%)	4 (36%)	8 (12%)	12 (15%)
inheritance of hypodontia					
from mother	3 (43%)	3 (75%)	6 (55%)	24 (36%)	30 (38%)
from father	4 (57%)	1 (25%)	5 (45%)	24 (36%)	29 (37%)
unknown				19 (28%)	19 (24%)

Table 6. Number of congenitally missing and peg-shaped teeth in probands and their relatives

		Probands	1st-degree relatives	2nd-degree relatives	First cousins	Other relatives	Total (%)
		(N=10)	(N=18)	(N=16)	(N=4)	(N=20)	(N=68)
Missing teeth[a]	15	3	3	2	0	9	17 (14.2)
	14	0	0	0	0	1	1 (0.8)
	12	4	4	4	1	0	13 (10.8)
	22	3	2	2	1	0	8 (6.7)
	24	0	0	0	0	1	1 (0.8)
	25	5	3	3	0	8	19 (15.8)
	35	5	6	6	2	14	33 (27.5)
	31	0	2	1	0	1	4 (3.3)
	41	0	0	0	0	1	1 (0.8)
	45	3	7	4	3	6	23 (19.2)
	Total	**23**	**27**	**22**	**7**	**41**	**120**
	mean	2.3	1.5	1.4	1.8	2	1.8
		(N=2)	(N=4)	(N=5)	(N=2)	(N=2)	(N=15)
Peg-shaped teeth							
	peg 12	0	2	2	1	1	6
	peg 22	2	3	4	1	1	11
	Total	**2**	**5**	**6**	**2**	**2**	**17**

[a] FDI two-digit system (Keiser-Nielsen, 1971)

hypodontia was registered more often in the left side of the mandible, consistent with results of previous Scandinavian investigations (Grahnen, 1956; Haavikko, 1971; Wisth et al., 1974; Bergström, 1977), whereas no difference between the right and left quadrants has been observed in some studies (Magnusson, 1977; Lai and Seow, 1989).

The teeth most frequently missing (N=120) were lower second premolars (47%), upper second premolars (30%), and upper lateral incisors (17.5%). Further, lower central incisors and upper first premolars were 4.2% and 1.6% of the missing teeth (Table 6). The majority of studies have shown that the mandibular second premolar is the tooth most commonly missing, but some variability is present in the order of the other teeth. Similar missing teeth and nearly the same frequencies have been reported in a previous study on Finnish children (Haavikko, 1971), with the exception of a higher frequency of agenesis of upper first premolars (3.5%) and a lower frequency of lower central incisors (2.7%) in that study.

Hypodontia of the primary dentition was neither found or reported, but the youngest persons studied were 7-year-olds. Of 11 probands, 6 had inherited hypodontia from their mothers and 4 from their fathers. One father without hypodontia or peg-shaped teeth was identified as an obligate carrier. Moreover, hypodontia had an equal maternal (36%) and paternal inheritance (36%) among relatives (N=67). The inheritance pattern remained undetermined for 19 (28%) relatives (Table 5). The inheritance pattern is in agreement with an autosomal dominant trait.

The mean dental age of the probands and their relatives with developing dentition (N=38) showed a 0.3-year advance compared with population standards. The mean dental age of the probands (N=6) was 0.2 years ahead, but the relatives with hypodontia (N=10) showed a minus 0.2- year delay. The dental age of 2 boys with hypodontia fell between the –1SD and –2SD percentiles.

1.2. Other dental features in probands (I)

Other dental anomalies of the probands are shown in Table 7. Ectopic upper permanent canines were seen in 2 (18%) probands, one palatally and the other labially displaced. This prevalence is 9-10 times that of the normal population (Thilander and Jakobsson, 1968).

The frequency of rotated premolars (18%) exceeds by 3.5 times the population prevalence reported in an Italian study (Baccetti, 1998). Invaginations in upper lateral incisors were noted less frequently in the probands (22%) than in the total population control sample of 170 children (42%). Interestingly, the frequency of invaginations was also lower among hypodontia patients of the control sample.

Table 7. Dental anomalies in probands and their relatives

	Ectopic canine(s) N (%)	Palatal canine(s) N (%)	Labial canine(s) N (%)	Invagination(s) N (%)	Rotation of premolar(s) N (%)	P value[a]	Taurodontism N (%)	P value[a]
Probands	2/11 (18)	1/11 (9)	1/11 (9)	2/9 (22)	2/11 (18)	NS	7/11 (64)	0.003
First-degree relatives								
hypodontia+	3/20 (15)	2/20 (10)	1/20 (5)	4/15 (27)	3/20 (15)	NS	3/16 (19)	NS
hypodontia -	0/26	0/26	0/26	8/18 (44)	2/26 (8)		5/15 (33)	
Total	3/46 (7)	2/46 (4)	1/46 (4)	12/33 (36)	5/46 (11)	NS	8/31 (26)	NS
Second-degree relatives								
hypodontia +	0/19	0/19	0/19	2/6 (33)	0/19		0/4	
hypodontia -	0/26	0/26	0/26	5/9 (56)	3/26 (12)	NS	0/9	
Total	0/45	0/45	0/45	7/15 (47)	3/45 (7)	NS	0/13	
First cousins								
hypodontia+	0/6	0/6	0/6	1/4 (25)	1/6 (17)	NS	5/6 (83)	0.002
hypodontia-	1/27 (4)	1/27 (4)	0/27	3/12 (25)	1/27 (4)		5/18 (28)	
Total	1/33 (3)	1/33 (3)	0/33	4/16 (25)	2/33 (6)	NS	10/24 (42)	0.036
Other relatives								
hypodontia+	0/22	0/22	0/22	6/16 (38)	4/22 (18)	0.050	2/12 (17)	NS
hypodontia-	1/36 (3)	0/36	1/36 (3)	6/18 (33)	3/36 (8)		2/16 (13)	
Total	1/58 (2)	0/58	1/58 (2)	12/34 (35)	7/58 (12)	NS	4/28 (14)	NS
Total number of family members								
hypodontia+	5/78 (6)	3/78 (4)	2/78 (3)	15/50 (30)	10/78 (13)	0.059	17/49 (35)	0.048
hypodontia-	2/115 (2)	1/115 (1)	1/115 (1)	22/57 (38)	9/115 (8)		12/58 (21)	
Total	7/193 (4)	4/193 (2)	3/193 (2)	37/107 (35)	19/193 (9)	0.023	29/107 (27)	NS
Controls								
hypodontia+				3/14 (21)			36/87 (41)	
hypodontia-				68/156 (44)			145/754 (19)	
Total	1-2% (Thilander and Jakobsson, 1968)			71/170 (42)	3-5% (Baccetti, 1998)		181/841 (22)	

[a] Chi-square test
NS, not significant, p>0.05

Taurodontism was a common anomaly in the molars of our probands; 7 probands (64%) had taurodontism of at least one molar. This is nearly 3 times the prevalence of taurodontism in the total control sample and is statistically significant (p=0.003).

One female proband had been operated on for a cleft of the soft palate. Small dimensions of the lower first premolars and one upper second premolar were noted in a male proband with hypodontia of 3 second premolars. The affected mother of this proband had small lower first premolars, and an affected aunt had a small upper second molar.

1.3. Other dental features in first- and second-degree relatives (I)

Dental anomalies are shown in Table 7. Ectopic upper permanent canines were seen in 3 (7%) first-degree relatives (N=46), all of these individuals with hypodontia. This is 3.5 times the population prevalence. Two of these ectopic canines were palatally and one labially displaced. No ectopic canines were noted in second-degree relatives (N=45).

The frequency of rotated premolars in first- (11%) and second-degree relatives (7%) exceeded by 2 to 3 times that of the population (3-5%) (Baccetti, 1998). As in probands, invaginations were seen less frequently in first- and second-degree relatives with hypodontia than among unselected controls.

The frequency of taurodontism (26%) in first-degree relatives (N=31) was similar to that in the control sample (22%), but never found in second-degree relatives (N=13). Taurodontism was assessed more frequently in upper molars, with second molars affected more often than first molars. Most taurodontic molars showed hypotaurodontism, with hypertaurodontism not observed.

In addition to the aforementioned family 4 with small premolar dimensions, small premolars were seen in 4 females: a cousin (7), a relative with hypodontia (9), an aunt (6), and a relative without hypodontia (11).

An unaffected sister of one proband (8) had fourth molars in the maxilla. A brother of a female proband with missing second premolars in the left side of the jaws (11) had had fused primary teeth in the region of the upper lateral incisor.

1.4. Other dental features in first cousins and more remotely related individuals (I)

One (3%) of the cousins without hypodontia had a palatally displaced canine. A labial canine was noted in another remote relative without hypodontia (2%), which corresponds to the population prevalence (Table 7).

Rotated premolars were noted in 2 first cousins (6%), one with hypodontia and the other without, which is similar to the population prevalence of 3 to 5%. The frequency of rotated premolars in remote relatives (12%) was 2-4 times the population prevalence. Rotated premolars were noted more frequently in individuals with hypodontia both among first cousins and more remote relatives.

The frequency of invagination(s) in upper lateral incisor(s) was 25% in cousins (N=16) and 35% in more remote relatives (N=34). No differences were present between individuals with and without hypodontia.

Taurodontism was assessed in 10 of 24 cousins(42%), which is 1.9 times the control prevalence. Cousins with hypodontia had a taurodontism frequency of 83%, which differed significantly from the population control prevalence. The frequency of taurodontism in the more remote relatives corresponded with that of the controls.

1.5. Dentition in obligate carriers (I)

Nine obligate carriers were identified from the pedigrees, with 3 males and 4 females representing nonpenetrance of familial hypodontia. Three of these showed no dental anomalies. The dental status of 2 carriers was unavailable due to extraction of most of their teeth.

Four carriers had anomalies: 1 male had a narrow upper lateral incisor, and 2 males had rotated premolars. One female also had a small upper lateral incisor and had undergone orthodontic treatment for labially displaced canines.

2. OLIGODONTIA IN FAMILIES (IV)

Clinical evaluation of family members in the oligodontia study revealed no significant medical problems except for the absence of several teeth in 3 individuals in family 1 and 2 in family 2 (Table 8 and Fig 5). The rest of the family members had normal dentition.

The affected individuals in family 1 lacked all second and third permanent molars as well as both maxillary lateral incisors. The proband and his 2 brothers were too young for definitive diagnosis of the absence of their third molars. This proband and his mother also lacked all first permanent molars and several second premolars. All second molars were missing in the proband‘s primary dentition. The affected brother had all primary teeth, but the second primary molars were submerged. The mother and the affected brother had a malposition of the upper permanent canine. In addition, some permanent teeth appeared smaller than normal in affected patients.

The oligodontia phenotype was even more severe in family 2. The proband lacked 8 permanent molars, and 16 other permanent teeth. One of her 4 molars

Table 8 Oligodontia phenotypes

	right molars 8	7	6	prem. 5	4	c. 3	incisors 2	1	1	2	c. 3	prem. 4	5	molars 6	7	8	left
Family 1	*	*	*	*			*			*			*	*	*	*	up
I:2																	ud
born 1969																	ld
	*	*	*											*	*	*	lp
Family 1	*	*					*			*					*	*	up
II:1																	ud
born 1985																	ld
	*	*							*						*	*	lp
Family 1	?	*	*	*			*			*			*	*	*	?	up
II:4				*									*				ud
born 1994				*									*				ld
	?	*	*										*	*	*	?	lp
Family 2	*	*	*	*	*	*	*			*	*	*	*	*	*	*	up
I:1																	ud
born 1944																	ld
	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	lp
Family 2	*	*		*	*	*	*			*	*	*	*	*	*	*	up
II:1																	ud
born 1977																	ld
	*	*		*		*	*	*	*	*	*		*			*	lp

*Table 8. Oligodontia phenotypes. *, tooth missing; ?, diagnosis impossible (too young); prem., premolars; c, canines; up, upper permanent teeth; ud, upper primary teeth, ld, lower primary teeth; lp, lower permanent teeth. Pedigree numbering in left column.*

showed taurodontism, another was single-rooted, and she had invaginations in her upper central incisors. The affected father had only 3 permanent teeth: upper central incisors and one lower lateral incisor. Both affected individuals in family 2 had had all their primary teeth. The small size of the families does not allow complete segregation analyses to be made, but an autosomal dominant mode of inheritance seems most probable.

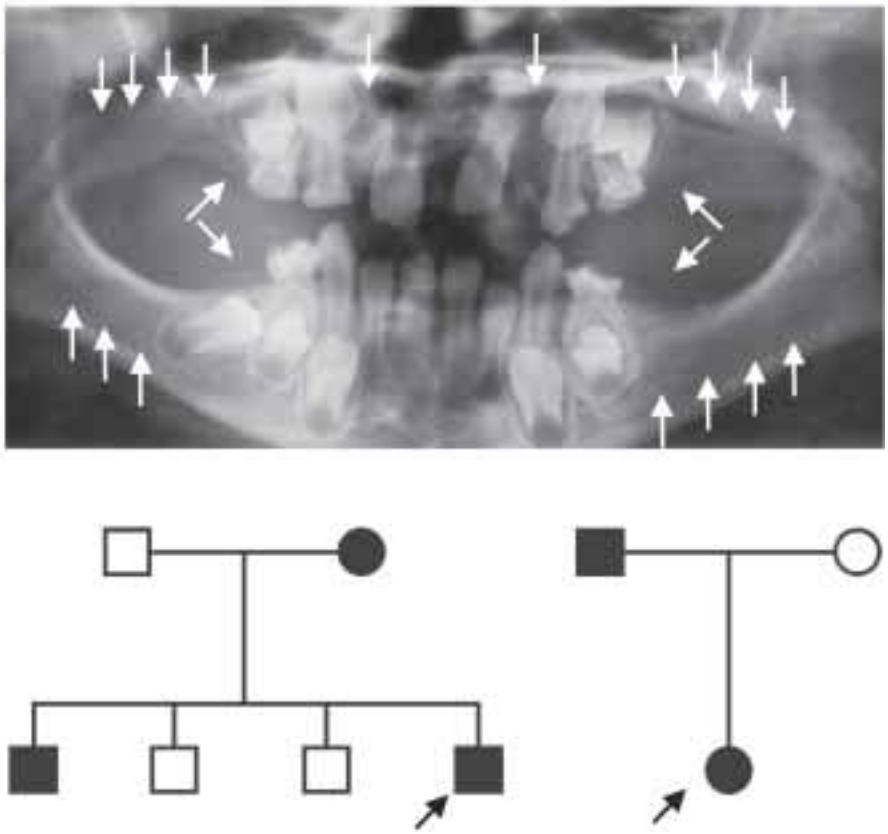

Fig. 5. a. Panoramic radiograph of the proband of Family 1 (II:4) at age 6. Missing teeth depicted with arrows. b. Pedigrees of oligodontia families 1 and 2. Squares, males; circles, females; darkened, affected; arrows, probands.

3. LINKAGE STUDIES (II, III)

Before starting a genome-wide seach for hypodontia locus we studied linkage of hypodontia with some selected candidate genes. The finding that tooth development is inhibited in *Msx1*-negative transgenic mice directly implicates this gene in tooth development (Satokata and Maas, 1994). MSX1 and the closely related MSX2 are homeobox-containing transcription factors, and mutation in the human *MSX2* gene had been identified as the gene causing the Boston-type familial craniosynostosis (Jabs et al., 1993). At the time of this study, *MSX1* had not yet been identified as the cause of oligodontia.

Epidermal growth factor (EGF) has been implicated in early tooth morphogenesis, since tooth development is inhibited in vitro with antisense oligonucleotides to EGF

mRNA (Kronmiller et al., 1991). *EGF* exerts its action on cells via binding to the specific cell-surface receptor, *EGFR*. This receptor is also used by other growth factors in the *EGF* family, including *TGF*α. *EGFR* is expressed in developing teeth from the bud stage onward, and the pattern is developmentally regulated, suggesting involvement in the regulation of tooth development.

Roles for *FGF-3* in tooth development have been suggested, since it is intensely expressed in dental mesenchyme during the cap and bell stages (Wilkinson et al., 1989). Although no defects in dentition are reported in *FGF-3* knockout mice (Mansour et al., 1993), the possibility still exists that a gain-of–function mutation in the *FGF-3* may affect tooth development. It is also possible that this gene defect causes hypodontia in human dentition without having any effect on mouse dentition. *FGF-4* is expressed in structures called enamel knots in the dental epithelium. Experimental analysis has indicated that, in dental tissues, *FGF*s greatly stimulate cell proliferation and it has been speculated that the function of *FGF-4* is to regulate development of tooth form (Jernvall et al., 1995).

We found evidence against linkage of incisor and premolar hypodontia with *MSX1* and *MSX2* (II) as well as with *EGF*, *EGFR*, and *FGF-3* genes (III). The linkage of *MSX1* and *MSX2* was studied in 5 families. 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 the *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 resulted in negative lod scores of less than –2 with recombination fractions 0.0 and 0.01. We used the polymorphic microsatellites in *EGF*, *EGFR*, and *FGF-3*. The tetranucleotide repeat polymorphism within the gene for EGF in chromosome 4 (4q25-27) resides in the intron 10 of the gene (Murray et al., 1992) and the dinucleotide repeat polymorphism within the gene for EGFR (7p12-14) in the first intron (Chi et al., 1992). In the *FGF-3* gene in 11q13.3, there is a dinucleotide repeat polymorphism in the immediate 3´-flanking region (Polymeropoulos et al., 1990). The *FGF-3* and *FGF-4* genes are located as a cluster, and the interval between the genes is less than 35kb (Brookes et al., 1992). The genotypes of these 3 marker loci in 7 Finnish families with hypodontia were determined, and the lod scores with hypodontia calculated (III). The lod scores obtained with recombination fractions 0.00 and 0.01 were less than –2 in the case of the *EGF* and *FGF-3* microsatellites. According to convention, assuming homogeneity, this result would exclude linkage of incisor-premolar hypodontia with these genes. During the genome scan (see below) additional families and also neighboring markers

TABLE 9 **Exclusion of candidate genes for incisor-premolar hypodontia**

intragenic marker	location	lod score max (thetas ≤ 0.02)[a]	recombinations in families[b] 1	2	3	4	5	6	7	9	91[c]	92[c]	93[c]	94[c]	11
MSX1	4p16	-2.739	1	*3*	0	1	1	*2*		3	*3*	1	2	2	1
MSX2	5q34-q35	-1.649	1	2	0	1	*1*	1		2	1	0	*1*	1	0
EGF	4q25-27	-13.385	1	2	*1*	1	1	1	1	5	1	1	2	1	4
FGF3, FGF4	11q13.3	-6.545	2	*1*	*1*	1	1	0	2	5	1	0	1	1	3
EGFR	7p12-14	-2.919	1	1	0	1	1	*1*	1		3				
INHBA	7p15-p13	-5.000	1	1	0	1	1			4	3	0	1	1	

haplotype of neighboring markers	location	gap between[d]	recombinations in families[b] 1	2	3	4	5	6	9	91[c]	92[c]	93[c]	94[c]
BMP2	20p12	1.5 cM	1	1	0	1	0		4	2	0	2	1
BMP4	14q22-q23	4 cM		1	0	*1*	0	*1*	2	2	0	2	1
DLX1, DLX2	2q32	8.5 cM		0		1			2	1	1	2	1
GLI2	2q14	2 cM	*2*	2	0	1	1	0	2	2	*1*	2	1
GLI3	7p13	10 cM	1	1	0	0	1	*1*	4	3	1	1	
LEF1	4q23-q25	14 cM	1	2	0	1	1		1	*4*	1	*1*	*2*
PAX9	14q12-q14	5 cM	*1*	2	*1*	*1*	0	*1*	2	1	1	2	1

[a]thetas (recombination fraction) ≤ 0.02 corresponds to a genetic distance ≤ 2 cM, and covers on average 2 million basepairs around the gene.

[b]bold numbers= recombinations between supposed disease allele or haplotype and IPH in affected persons, italic numbers= healthy persons having supposed disease allele or haplotype, 0= no recombinations detected, blank= family either not genotyped or not informative.

[c]smaller families obtained from family 9 when great-grandparents were omitted.

[d]genetic distance between the two nearest markers to the gene.

were genotyped. Combined results demonstrated that all the genes described above showed recombinations in most families studied, and the total lod scores, except for *MSX2*, were far below –2 (Table 9).

In the second stage, a genome-wide scan was performed with 327 markers in family 9 and with 270 markers in families 1, 2, 6, and 9. During the course of the study it emerged that a family (family 9) may segregate more than one locus causing hypodontia. This family was therefore divided into 4 different families, and the results were calculated also for these. The lod scores were calculated by pairwise and affecteds-only methods. The affecteds-only method excludes any possible mistake in the calculated penetrance of the gene. When results were tested also for genetic heterogeneity with the Homog program, several regions with positive lod scores were detected (Fig 6). These regions were studied further by genotyping more families, but no statistically significant levels were reached. In all these regions, however, recombinations were detected with incisor-premolar hypodontia in several of the families. Thus, the heterogeneity of this trait is obvious.

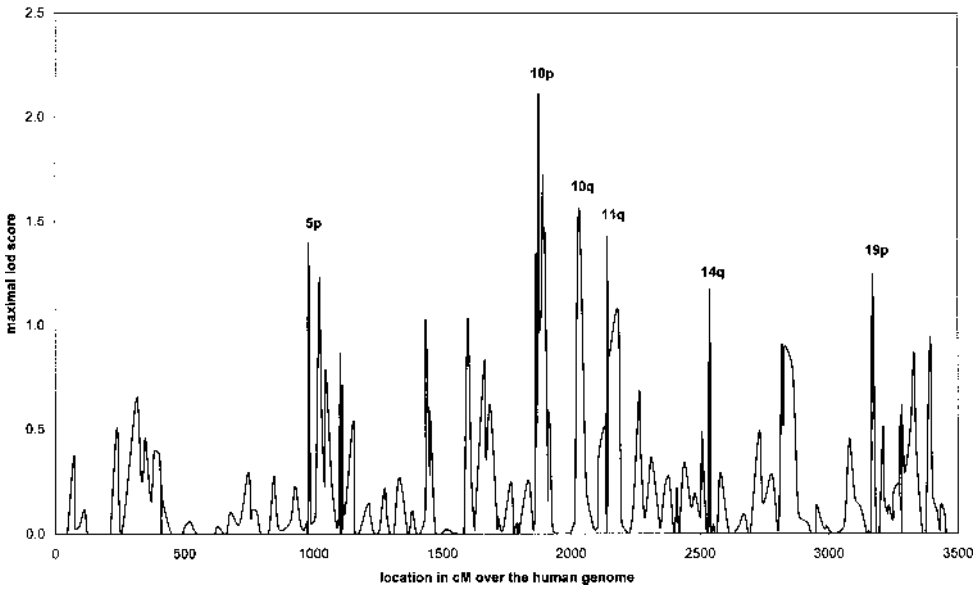

Fig. 6. Results of genome-wide scan. Lod scores were calculated in families 1, 2, 6, and 9, using information from all genotypes or only the affecteds and testing for genetic heterogeneity. Only the highest value of each marker presented. Genetic lengths in cM were summed over the chromosomes from 1 to 22. Chromosomal locations of the highest lod scores depicted. p= short arm of chromosome, q= long arm of chromosome.

We found no evidence in our families for linkage of hypodontia and *BMP-2*, *BMP-4*, *DLX1*, *DLX2*, *LEF1*, *GLI2*, *GLI3*, or *PAX9* (Table 9, Nieminen et al., unpublished results). *BMP-2* and *BMP-4* are members of the TGFβ superfamily and act as morphogens in various animal species; they have been involved in the regulation of early tooth morphogenesis (Vainio et al., 1993). The *DLX*, *DLX2*, *LEF1*, *GLI2*, *GLI3*, and *PAX9* genes are interesting because they, in addition to *MSX1* and *MSX2*, are transcription factors and show clear associations with the initiation and patterning of tooth development (for review, Jernvall and Thesleff, 2000). In mice, a deletion in *Msx1* results in inhibition of the expression of *Bmp4* and *Fgf3*, which act as reciprocal signals to the epithelium (for review, Thesleff, 2000). Recently, it has been shown that adding BMP4 to cultures of *Msx1* mutant tooth germs can restore their development (Bei et al., 2000).

4. IDENTIFICATION OF PAX9 MUTATION IN OLIGODONTIA (IV)

A frameshift mutation in the *PAX9* gene has been identified to cause oligodontia in a family in which affected individuals lacked most molars (Stockton et al., 2000). We

decided to analyze the *PAX9* gene in 2 families with oligodontia because most molars were also affected in our families (IV).

Sequencing of the *PAX9* gene of the affected family members (exons 2, 3, and 4 that include the coding region) revealed 3 nucleotide changes as compared to the published sequences (Hetzer-Egger et al., 2000; Peters et al., 1997). All changes could be verified from both strands. The nucleotide transversion A340T present in the heterozygous state of exon 2 in all 3 affected members of family 1 produces a stop codon at lysine 114 in the end of the DNA-binding paired box of PAX9. This premature termination of translation creates a truncated protein that lacks the last alfa-helix (α6) of the paired box and the entire C-terminal region. The insG219 mutation described by Stockton et al. (2000) destroys the paired box 121 nucleotides earlier, between the N- and C-subdomains (Xu et al., 1999). The lack of the C-terminal region that follows the paired box most probably disrupts the normal function of both mutant proteins. The mildness of these phenotypes as compared to the PAX9 mouse null mutant suggests that both mutations are loss-of-function and exert their effect mainly through inactivation of one copy of a protein, leading to haploinsufficiency. It is possible that the mutant proteins retain some of the DNA-binding capacity, and especially the slightly more severe phenotype of Family 1 may be attributed to the dominant negative effect of the more complete paired box of the Lys114Stop mutant protein. The other nucleotide changes found were in exon 3. The silent change C717T in the codon of His239 was present in the heterozygous state in the healthy mother and affected daughter of family 2. A G718C transversion causes the conservative change Ala240Pro. This change was present in the heterozygous state in all members of family 2 and in the homozygous or heterozygous state in some of the members of family 1.

GENERAL DISCUSSION

1. SUBJECTS

We collected a sample of families in which hypodontia was segregating in an autosomal dominant manner in order to define clinical features of hypodontia and to localize the gene locus behind this anomaly. The frequency of hypodontia was high, more than 30% in these families. The frequency did not differ significantly between males and females, with 34% of females and 35% of males having hypodontia. We therefore combined the sexes but calculated the frequencies of the individual anomalous features separately in first- and second-degree relatives, first cousins and more remotely related relatives. The phenotypes confirmed earlier suggestions as to the hypodontia phenotype as an isolated trait. One individual in these families had a cleft of the soft palate. However, one cleft patient in a sample of 214 individuals corresponds with the frequency of cleft patients in Finland. Missing teeth were premolars in 2 families, incisors in 3 families, and premolars and incisors in 6 families. It probably would have been better to have had families with either incisor or premolar hypodontia to provide even more uniform phenotypes in the molecular genetic part of the study. However, affected premolars and upper lateral incisors seem to appear together very commonly in different combinations, and it may be impossible to separate families according to this feature.

Simulation with the Slink program (Ott,1989; Weeks et al., 1990) showed that the families were sufficiently informative to reveal significant evidence for linkage of hypodontia.We combined data of 5 (II) and 7 (III) families in genetic linkage studies. In addition, we used 4 families in the second stage of linkage studies in which the genome-wide search with a selection of microsatellites with a maximum genetic distance of 15 cM was performed. Large families with several affected individuals are usually very informative in mapping human diseases. In this case, however, we had some unexpected results, probably because the hypodontia gene frequency is high in the population; it is possible that many different genes are involved. In large kindreds, the possibility to have more than one gene causing tooth agenesis in a pedigree is high. In statistical analysis, calculations both in whole pedigrees and pedigrees divided into different families were performed in order to find possible linkage with different loci.

2. PHENOTYPIC ANALYSIS OF HYPODONTIA AND OLIGODONTIA

Many family studies have shown that isolated hypodontia and oligodontia are inherited in a dominant manner with reduced penetrance and variable expression. Our study supports this model. The highest frequency of tooth agenesis in our study was in first-degree relatives, 39%, followed by second-degree relatives (36%) and first cousins (12%). The frequency is higher when the peg-shaped upper lateral incisors are included, being 43%, 42%, and 18% respectively. Most affected individuals with hypodontia lack only 1 or 2 permanent teeth. Hypodontia of the primary teeth was not found, confirming the previous studies. Lower second premolars were the most frequently missing teeth, followed by upper second premolars and upper lateral incisors. These findings are in agreement with other studies.

In our family with oligodontia, affected individuals with *PAX9* mutation lacked all second and third permanent molars as well as both maxillary lateral incisors. In addition, 2 affected family members lacked second premolars. While the reason for these particular teeth to be affected is unknown, it is probably related to the developmental sequence of tooth formation. It is suggested that those teeth in each tooth family that develop latest are less stable and do not reach a critical threshold at an early stage of development.

Even if congenitally missing teeth are an isolated phenomenon, studies have shown many associated dental features. The same anomalies were seen both in hypodontia and oligodontia families. The higher frequency of missing teeth and other dental anomalies has been shown among relatives of the probands than in the population, and our findings confirm this (Grahnen, 1956; Brook, 1984).

The prevalence of malformed lateral incisors in our families was high (I); being 9% in first- and 11% in second-degree relatives, 6% in cousins, and 3% in other relatives and exceeding by 2 to 6 times the frequency in the general population (Grahnen 1956; Alvealo and Portin, 1969). Alvesalo and Portin suggest that missing and peg-shaped upper lateral incisors are different expressions of a dominant autosomal gene, and our results confirm this.

An association has been speculated between missing teeth, delayed tooth development, and tooth size (Grahnen, 1956; Garn and Lewis, 1970; Rune and Sarnäs, 1974; Brook, 1984). In this study, dental age was within normal limits. This is understandable because only one or a few teeth were missing in the affected individuals in our study; they had the mildest form of hypodontia. Based on previous studies, it seems that the more teeth are missing, the greater delay of development the remaining teeth may show (Garn et al., 1961; Rune and Sarnäs 1974). However,

notable individual variation has been observed, even in oligodontia patients some affected persons show a severely delayed tooth formation, whereas others show normal timing. Interestingly, the delay was more obvious in males than females as in one study of oligodontia (Schalk-van der Weide, 1992).

It was not possible to measure the exact dimensions of teeth. In 5 families, however, some single small teeth, especially premolars, were evident.

Ectopic permanent canines have been demonstrated in many studies to belong to the spectrum of dental anomalies related to hypodontia (Peck et al., 1993, 1994; Pirinen et al., 1996), and our study also confirms this finding. A clear difference existed between individuals with and without hypodontia; ectopic upper permanent canines in individuals with hypodontia were observed three times as often than in individuals without hypodontia or in the population.

The association of rotated premolars with hypodontia has been reported by Baccetti (1998); our study lends support also to this finding.

Dens invaginatus, an enamel-lined cavity within a tooth, is a dental malformation mostly affecting maxillary lateral incisors and showing a broad spectrum of morphological variation. Minor forms of tooth invagination seem to be comparatively common. Deep palatal-side clefts were present in nearly 40% of British children (Hallet, 1953), and over 7% had definite invaginations showing some degree of dilation beyond the cervical margin. A frequency of 3% has been reported in the Swedish population (Koch and Thesleff, 2001). The frequency of invaginations in our families was similar to that of British children. However, invagination was noticed less frequently in individuals with hypodontia both in the families studied and in the control group.

The prevalence of taurodontism varies greatly in the literature, mostly because of different criteria for diagnosing the anomaly and the teeth assessed. The frequency of taurodontism of lower molars clearly remains below 10% in European populations (Holt and Brook, 1979; Schalk-van der Weide et al., 1993), with maxillary molars affected more often (Al-Khateeb and Salako, 1997). Taurodontism has been described together with hypodontia in many syndromes (Jorgenson, 1982), sex chromosome anomalies with extra X chromosomes (Varrela and Alvesalo, 1988, 1989), and cleft palate patients (Laatikainen and Ranta, 1996). The association of taurodontism with hypodontia has been shown in two investigations of patients with hypodontia and their siblings (Stenvik et al., 1972; Seow and Lai, 1989) as well as in a study of patients with oligodontia (Schalk-van der Weide, 1993). Seow and Lai (1989) reported a frequency of taurodontism of 35% in persons with hypodontia, and Schalk-van der Weide et al. (1993) 29% those with oligodontia, but only lower molars were assessed. In our study, taurodontism seems to be related to hypodontia,

confirming previous studies. However, that the results were not statistically significant in separate groups of relatives, may relate to the small number of individuals in these groups. Our findings are in agreement with a Finnish study on cleft patients (Laatikainen and Ranta, 1996), showing the frequency of taurodontism to be 41% in 39 pairs of twins with cleft lip and/or palate and hypodontia.

A taurodontic tooth is generally believed to result from a disturbance in Hertwig‘s epithelial root sheath, which is an extension of the dental epithelium after crown morphogenesis has been completed. Based on clinical observations and this theory, the association between taurodontism and hypodontia may result from the same genetic etiology affecting the growth of dental epithelium.

3. GENETIC ANALYSIS

Numerous transcription factors, growth factors, and their receptors as well as structural molecules of the cell surface and extracellular matrix have been associated with early tooth morphogenesis (for review, Thesleff and Nieminen, 1996; Jernvall and Thesleff, 2000). Interestingly, several of these genes have been shown to cause arrested tooth development in transgenic mice (Satokata and Maas, 1994; Peters et al., 1998). It is apparent that all these genes are potential candidate genes for hypodontia.

We excluded *EGF*, *EGFR*, and *FGF-3* as genes behind hypodontia (III; Nieminen et al., unpublished results). In addition, we excluded *MSX1* and *MSX2* in our families (II; Nieminen et al., unpublished results). Later, however, a missense mutation in the *MSX1* gene was identified as the first gene defect causing isolated tooth agenesis in man (Vastardis et al.,1996). The phenotypes in our families differed from the phenotype of the family with the *MSX1* mutation, where the prominent feature was bilateral agenesis of second premolars and third molars in both jaws. The number of missing teeth was lower in our families (hypodontia). Further, agenesis of molars (except third molars) was absent in our families with incisor-premolar hypodontia, whereas this was a typical feature of oligodontia both in our family in study IV and in an American family with mutation of *PAX9* (Stockton et al., 2000). The similarity of the phenotypes in our families and the American family motivated our *PAX9*-mutation search. The identification of a new mutation in *PAX9* segregating with oligodontia of molar teeth supports the suggestion that this gene is important especially for the development of the most distal teeth. However, we did not find the same mutation in another oligodontia family with missing molars, nor in the families with incisor-premolar hypodontia. These traits may be caused by mutations in different gene families or perhaps genes acting in the same signaling pathways with *MSX1* and *PAX9*. During our genome-wide scan we detected several regions which may contain mutated genes causing incisor-premolar hypodontia.

CONCLUDING REMARKS

Congenital absence of permanent teeth has interested dentists for a long time, but studies analyzing the molecular pathology with modern DNA techniques have not been performed before the last decade. When this work was begun, several monogenic diseases had been successfully mapped, and this encouraged us to search for the gene behind hypodontia. At that time, genes behind isolated tooth agenesis in man were unknown, and classification of hypodontia was beginning to be renewed. We excluded many important genes which had been implicated in tooth development by performing gene expression and experimental studies in the mouse and causing tooth agenesis in transgenic mice. Mutations in two of these genes, *MSX1* with two different mutations, and *PAX9*, were later identified in two big families where dominantly inherited oligodontia typically affects premolars and molars. We found a new nonsense mutation in *PAX9* in all three affected family members of a family with oligodontia. We must focus on the fact that while mutations in the *MSX1* and *PAX9* genes have been shown to be responsible for tooth agenesis in single families, these genes do not cause this phenotype in other families.

By performing a genome-wide search for the hypodontia gene, we have found some loci that can be linked with variable statistical significance to hypodontia in one or several families. Based on existing evidence, it is obvious that both hypodontia and oligodontia are heterogenous traits, with several mutated genes responsible. In the future, it will be possible to study these loci further and identify more genes behind these traits. Publication of the human genome sequence (Lander et al., 2001; Venter et al., 2001) is valuable for these studies and accelerates the search for mutated genes. Combining clinical and genetic results, classification of the different forms of tooth agenesis will become more exact.

Our results support the findings that ectopic canines, malformed upper lateral insisors, rotation of premolars, and taurodontism are related to hypodontia and oligodontia. Interestingly, the same teeth are most often affected, and the similar associated dental anomalies are seen also in syndromic hypodontia. The last developing teeth in each tooth family seem to be most frequently affected both in isolated and syndromic traits. As genes most obviously play the main role in the etiology of congenitally missing teeth and several dental anomalies, it is important that a family history of hypodontia/oligodontia as well as dental anomalies is observed in clinical paediatric dentistry. Screening children of families with segregating tooth agenesis by clinical and radiographic examinations should be carried out at 6 to 7 years of age to plan the best possible treatment for the developing dentition.

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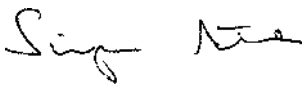

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