Eur J Oral Sci 2003; 111: 365–370
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European Journal of
Oral Sciences
ISSN 0909-8836

Focus

Molecular basis of non-syndromic tooth agenesis: mutations of *MSX1* and *PAX9* reflect their role in patterning human dentition

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Mostowska A, Kobielak A, Trzeciak WH. Molecular basis of non-syndromic tooth agenesis: mutations of MSX1 and PAX9 reflect their role in patterning human dentition. Eur J Oral Sci 2003; 111: 365–370. © Eur J Oral Sci, 2003

Tooth agenesis constitutes the most common anomalies of dental development in man. Despite this, little is known about the genetic defects responsible for this complex condition. To date, the only genes associated with the non-syndromic form of tooth agenesis are *MSX1* and *PAX9*, which encode transcription factors that play a critical role during tooth development. This paper aims to review current literature about the molecular mechanisms responsible for selective tooth agenesis in humans.

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Key words: hypodontia; oligodontia; *MSX1*; *PAX9*; mutations

Accepted for publication July 2003

Molecular studies of odontogenesis, using the mouse tooth as a model system, have shown that tooth development is under strict genetic control, which determines tooth position, number, size, and shape (1–3). More than 200 genes are involved in odontogenesis in mice (4), and mutations in several of these genes can cause an arrest in tooth development (Table 1).

Congenital agenesis of one or more permanent teeth is the most common abnormality of the human dentition (5). It occurs in association with other genetic diseases or as an independent oral trait (6). Non-syndromic hypodontia shows a wide phenotypic heterogeneity and is classified as a sporadic or familial form, inherited in an autosomal-dominant, autosomal-recessive or X-linked mode (7). Population studies have revealed that the most commonly missing teeth are third molars (10–25% affected) followed by a deficiency of lower second premolars or upper lateral incisors. Agenesis of the first and second molars is very rare (3, 8, 9). When more than six teeth, excluding third molars, are missing, the condition is referred to as severe hypodontia or oligodontia; it occurs in the general population with an estimated prevalence of 0.25% (10, 11). An extreme case of hypodontia is anodontia, denoting absence of all teeth (3).

Although tooth agenesis is occasionally caused by environmental factors, such as various kinds of trauma of the dental region (12) or by multi-reagent chemotherapy or radiotherapy (13), the majority of cases of hypodontia and oligodontia are due to genetic factors. Mutations of several genes associated with syndromic tooth agenesis, including ectodermal dysplasias (14), Rieger (15) and Witkope (16) syndromes as well as those causing oligodontia as an isolated trait, have been identified.

To date, a non-syndromic form of familial and sporadic tooth agenesis has been associated with mutations in *MSX1* and *PAX9* (Table 2). Protein products of these genes serve as transcription factors that are responsible for the crosstalk between dental tissues and are essential for the establishment of the odontogenic potential of the mesenchyme (2, 17–19). In mice embryos homozygous for the *Msx1* or *Pax9* deletion, odontogenesis is arrested at the bud stage, suggesting that the expression of these genes is critical for the development of the dentition (20, 21).

MSX1

MSX1 (Fig. 1A) contains a highly conserved homeobox sequence encoding a 60 amino acid-long DNA-binding homeodomain (22). The protein product of *MSX1* functions as a repressor of gene transcription and interacts with TATA box-binding protein (TBP) and other components of the core transcriptional complex, as well as with other homeoproteins, including members of the DLX family (23–25). Mice homozygous for *Msx1* deletion exhibit secondary cleft palate, deficiency of alveolar process of the mandible and maxilla, failure of incisor development, and an arrest of molar development at the bud stage. Abnormalities of nasal, frontal and parietal bones as well as of the malleus in the middle ear are also observed in these animals (20).

Table 1
Mouse models relevant to human tooth agenesis

Gene	Chromosomal localization	Type of molecule	Phenotype of the homozygous knockout mutant	References
Homeobox genes				
Msx1	5	Transcription factor	Mutants lack all teeth	20
Msx2	13	Transcription factor	In double *Msx1*/*Msx2* mutants tooth development is arrested shortly after initiation	47
Dlx1	2	Transcription factor	Double mutants lack maxillary molars; single mutants have normal teeth	48,49
Dlx2	2			
Pax genes				
Pax9	12	Transcription factor	Mutants lack all teeth	21
Pax6	2	Transcription factor	Most mutants develop additional upper incisors	50
HMG-box genes				
Lef1	9	Transcription factor	Mutants lack all teeth	51
Zinc-finger genes				
Gli2	1	Transcription factor	Mutants lack all teeth	52
Gli3	13			
TGFα superfamily				
Activin βα	13	Extracellular signalling protein	Mutants lack all teeth except upper molars	53
Follistatin	13	Activin-binding protein	Abnormal development of lower incisors	53,54
Activin IIA-R		Cell surface activin receptor	Some mutants lack lower incisors	53,55

Table 2
Mutations of human MSX1 and PAX9

Gene	Mutation	Localization	Effect Protein	Phenotype*	Reference
MSX1	G587C	Exon 2, homeobox sequence	Arg196Pro	FTA	26
	C314A	Exon 1	Ser105Stop	FTA, isolated CP or CLP	28
	C605A	Exon 2, homeobox sequence	Ser202Stop	Witkope syndrome	16
	T182A	Exon 1	Met61Lys	FTA	29
PAX9	219insG	Exon 2, paired box sequence	Frameshift at amino acid 73, premature termination of translation at aa 316	FTA	36
	A340T	Exon 2, paired box sequence	Lys114Stop	FTA	37
	793insC	Exon 4	Frameshift at amino acid 264, premature termination of translation at aa 315	FTA	38
		Deletion of the entire *PAX9* locus	Lack of the protein product	FTA	41
	A271G	Exon 2, paired box sequence	Lys91Glu	FTA	39
	T62C	Exon 2, paired box sequence	Leu21Pro	FTA	
	175ins 288pb	Exon 2, paired box sequence	Frameshift at amino acid 58, premature termination of translation at aa 177.	FTA	
	G151A	Exon 2, paired box sequence	Gly51Ser	NFTA	40

The nucleotide numbers are relative to the translation initiation codon within the coding region.
*FTA, familial tooth agenesis; CP, cleft palate; CLP, cleft lip and palate; NFTA, non-familial tooth agenesis.

The first mutation associated with human tooth agenesis discovered in *MSX1* was described in a large family with a severe form of autosomal-dominant oligodontia (26). This missense mutation (G587C), localized in the homeobox sequence, resulted in an Arg196Pro substitution in the homeodomain of the MSX1 protein. The mutated protein has an abnormal structure and reduced thermostability compared with the normal protein. Functional analysis showed that the DNA binding capacity of the mutant protein, as well as the interactions with other transcription factors, were severely impaired (27).

In another large family with orofacial clefting and a similar pattern of tooth agenesis (lack of third molars and second premolars), a C314A transversion in *MSX1* was reported (28). The presence of this mutation (Ser105Stop) indicates that *MSX1* is one of the candidate genes, whose mutations are responsible not only for tooth agenesis but also for non-syndromic cleft lip and cleft palate.

More recently, a T182A transversion in *MSX1*, resulting in a Met61Lys substitution in the protein product of the gene, was described in a family with oligodontia (29). This mutation was localized in a highly conserved region that interacts with other transcription factors and was responsible for transcriptional repression of target genes (30). In this family, a deficiency of second premolars and third molars was found, supporting the view that mutations in *MSX1* are responsible for a specific pattern of

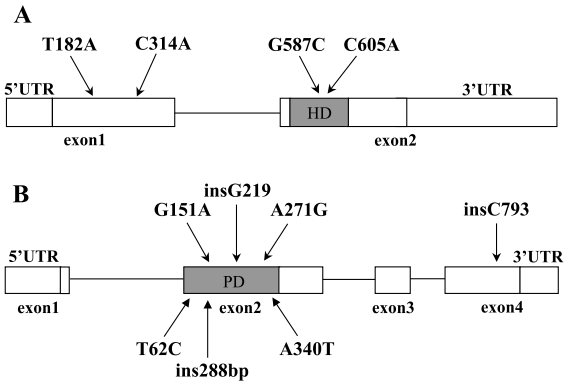

Fig. 1. Schematic view of human *MSX1* (A) and *PAX9* (B) genes. The regions encoding the homeodomain (HD) or paired domain (PD) are shaded. Arrows indicate location of known mutations.

tooth agenesis and corroborating the hypothesis on the odontogenic homeobox code proposed by SHARPE (31).

Recently, a complex rearrangement of *MSX1* involving a GAG insertion and C deletion at nucleotide 250 was identified (S. A. FRAZIER-BOWERS *et al.*, Abstract No. 20, 32nd Annual Meeting of the AADR, 2003). This mutation was described in four individuals from a family with selective posterior tooth agenesis, transmitted as an autosomal dominant trait.

All mutations in *MSX1* associated with selective tooth agenesis were found in the heterozygous state, suggesting that the mutant phenotype was caused by haploinsufficiency, rather than a dominant negative type of inheritance, as originally proposed (27).

It was found that the C605A transversion in *MSX1* resulting in generation of a stop codon at amino acid 37 of the homeodomain, was responsible for Witkope syndrome, which includes agenesis of tooth and nail dysgenesis (16). The truncated protein, which lacks part of the homeodomain and the entire *C*-terminal region, was presumably improperly folded and unable to bind to DNA.

PAX9

PAX9 (Fig. 1B) is a member of a gene family encoding transcription factors that play a key role during embryogenesis (32, 33). Proteins encoded by *PAX* genes share a unique 128-amino acid long DNA-binding paired domain (34, 35). In mouse embryos, *Pax9* is expressed in the mandibular arch mesenchyme prior to any morphological signs of odontogenesis, and its expression is maintained thereafter in the developing tooth mesenchyme (18). Mice homozygous for *Pax9* deletion die shortly after birth because of breathing problems. These animals lack all teeth and exhibit a wide range of developmental defects, including secondary cleft palate as well as other abnormalities in craniofacial bones and cartilages. In *Pax9*-deficient mouse embryos, tooth development is arrested at the bud stage (21).

The first mutation described in human *PAX9* gene was an insertion of an additional G within the paired box sequence at nucleotide (nt) 219 of exon 2 in a family with oligodontia (36). This insertion resulted in a frameshift and premature termination of translation 243 codons downstream of the insertion, thereby shortening the protein by 25 amino acids. Affected individuals had a normal primary dentition but lacked most of the permanent molars. It was demonstrated that this insertion was responsible for the loss of DNA-binding activity of the mutated PAX9 protein as well as the observed phenotype (J. MENSAH *et al.* Abstract no. 21, 32nd Annual Meeting of the AADR, 2003).

Another mutation in *PAX9* associated with oligodontia was described in a large family with severe tooth agenesis (37). The A340T transversion created a stop codon at Lys114 and resulted in a protein truncated in the *C*-terminal region of the paired domain. The phenotype of the affected individuals harbouring these two different mutations was nearly identical. They exhibited an absence of all second and third permanent molars and partial lack of permanent first molars and second premolars as well as the reduction in size of some of the teeth.

More recently, an insertion in *PAX9* was found in a large family that shared phenotypic similarities with the families reported previously (38). In this family, the trait was also transmitted in an autosomal-dominant way and involved agenesis of most molars. This Cins793 mutation, leading to premature termination of translation at amino acid 315, was identified in exon 4 of *PAX9*, outside the paired box. Although the role of the region encoded by exon 4 remains unknown, it is possible that the addition of 51 nonsense amino acids may affect proper folding of the protein, leading to a loss of its function.

In 2003, DAS *et al.* (39) described three novel mutations in *PAX9* associated with molar hypodontia. Two were missense mutations, resulting in substitutions of highly conserved amino acids within the paired domain. A T62C transition coded for exchange of Leu21 for Pro, and A271G caused a substitution of positively charged Lys91 for negatively charged Glu. These mutations probably resulted in functional haploinsufficiency owing to decreased DNA-binding capacity of the PAX9 protein. A third and unusual mutation was identified in twin boys affected with hypodontia. This 288-bp insertion within exon 2 resulted in a frameshift at amino acid 58 and a premature termination of translation at amino acid 177.

It is noteworthy that two members of the family in which Leu21Pro missense mutation was described also displayed cleft lip and palate (39). However, only one of the affected individuals carried this mutation. It is possible that the presence of the T62C transition may have decreased the threshold for orofacial clefting in this individual.

Interestingly, a mutation in *PAX9* was recently identified in a person with non-familial tooth agenesis (40). This individual lacked third molars, second premolars and some incisors, resembling phenotypes associated with mutations in *MSX1*, as well as other mutations in *PAX9*. This novel G151A transition resulted in a

Gly51Ser substitution in the PAX9 protein, and was localized the in helix–turn–helix motif of the *N*-terminal half of the paired domain which determines DNA binding capacity.

All heterozygous mutations of *PAX9* associated with tooth agenesis were likely due to a loss of function of one copy of the protein, leading to haploinsufficiency (36–40). Das *et al*. (41) recently described a small nuclear family harbouring a heterozygous deletion of the entire *PAX9* locus. The female proband and her father were had severe hypodontia involving agenesis of all primary and permanent molars. This confirmed that *PAX9* is a dosage-sensitive gene.

It is noteworthy that in knockout mice heterozygous for *Pax9* deletion, no abnormalities in tooth development were found. Thus, *Pax9* does not appear to be dosage-sensitive in mice. This might reflect the usage of different expression factors and a markedly different dental pattern between mice and men (41).

Concluding remarks

The studies reviewed strongly emphasize the importance of *MSX1* and *PAX9* in tooth development, and suggest that mutations in these genes are responsible for a specific pattern of inherited tooth agenesis. Haploinsufficiency of MSX1 protein affects the development of all teeth, preferentially third molars and second premolars, while reduced amount of PAX9 protein specifically affects molar development. The effect of haploinsufficiency of PAX9 on the development of incisors and premolars is probably caused by a deficiency of MSX1 protein (37, 40), since expression of *Msx1* in knockout mice homozygous for a *Pax9* deletion is attenuated (21). The observed variations in the pattern of tooth agenesis suggest that other mutated gene products, acting in the same signalling pathways as MSX1 and PAX9, may influence the patterning of dentition.

Specific patterns of hypodontia in families harbouring mutations in *MSX1* might reflect the odontogenic homeobox code proposed by SHARPE *et al*. (31). Prior to initiation of tooth development, they identified, in mouse facial mesenchyme, overlapping spatially restricted centres of homeobox gene expression, designated as 'domains', which might determine the identity of each individual tooth. Homeobox genes *Msx1* and *Msx2* are expressed in the presumptive incisor region, while at the same developmental stage *Barx1, Dlx1*, and *Dlx2* are coexpressed in the presumptive molar region and probably play a role in the development of molars.

The existence of the odontogenic homeobox code was extended by Peck *et al*. (42), who examined relationship between malpositions of permanent canines and tooth agenesis in humans. They showed that within the anterior orofacial field, the transposition of maxillary canine–first premolar was associated with agenesis of maxillary second incisors, whereas within the posterior orofacial field, palatally displaced canine and mandibular lateral incisor–canine transposition were associated with increased agenesis of molars. According to their findings, both *MSX1* and *PAX9* would be candidate genes that specify the posterior orofacial field, since mutations in *MSX1* were linked mainly to agenesis of third molars and second premolars, while mutations of *PAX9* were linked to agenesis of all molars. *MSX1* would also be a candidate gene for specification of the anterior orofacial field, since haploinsufficiency of MSX1 also affected another tooth families.

It is also noteworthy that phenotypes caused by deficiency of MSX1 protein might depend on the localization of mutations and their effect on the protein structure and function. Two substitution mutations, Arg196Pro (26) and Met61Lys (29) cause only familial non-syndromic tooth agenesis. Frameshift mutations, Ser202Stop mutation (16), resulting in a protein that lacks the *C*-terminal end of the homeodomain, impairs not only teeth but also nail formation, while Ser105Stop mutation (28), causing complete absence of the MSX1 homeodomain, is responsible for the most severe phenotype, which includes orofacial clefts with accompanied tooth agenesis.

To date, the developmental mechanisms by which mutations in *MSX1* and *PAX9* exert their effect on the patient's phenotype have not been elucidated. However, by analogy to the development of dental structures in mice, where expression of *Msx1* and *Pax9* in the mesenchyme is required for transition from the bud to the cap stage (2), one may presume that the human genes might be involved in the same stages of tooth development. In mice, in the bud stage these two transcription factors are required for the maintenance of mesenchymal expression of *Bmp4*, involved in enamel knot formation that directs transition to the cap stage (2, 3). In humans, however, molecular mechanisms underlying enamel knot formation are poorly understood, but one can assume that the processes of transition from the bud to the cap stage might require protein products of the homologous genes.

Although *MSX1* and *PAX9* are the strongest candidate genes for specific forms of tooth agenesis, mutations in these genes were detected only in some affected individuals (43, 44). Genes expressed in the early dental epithelium in mice such as *Bmp4*, *Bmp7*, *Dlx2*, *Dlx5*, *Fgf1*, *Fgf2*, *Fgf4*, *Fgf8*, *Lef1*, *Gli2*, and *Gli3* are also potential candidates (45). Based on existing evidence, it seems possible that both hypodontia and oligodontia are heterogeneous traits, caused by several independent defective genes, which act along or in combination with other genes and lead to specific phenotypes (46).

Suitable genetic models of isolated tooth agenesis are syndromic forms and oral clefts, since tooth development and palate formation share similar developmental mechanisms (46). Studies on the molecular basis of non-syndromic cleft lip and cleft palate, and identification of defective genes responsible for the at least 45 syndromes associated with tooth agenesis may therefore facilitate the search for candidate genes for isolated forms of this developmental anomaly.

Acknowledgements – The editorial assistance of Julia Biernot (MSc) is gratefully acknowledged. This work was supported by Grant no. P04 A 094 22 from the State Committee for

Scientific Research (KBN). A. Mostowska is the recipient of a scholarship from the Postgraduate School of Molecular Medicine in Warsaw.

References

1. Thesleff I. The genetic basis of normal and abnormal craniofacial development. *Acta Odontol Scand* 1998; **56**: 321–325.
2. Peters H, Balling R. Teeth: where and how to make them. *Trends Genet* 1999; **15**: 59–65.
3. Vastardis H. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. *Am J Orthod Dentofacial Orthop* 2000; **117**: 650–656.
4. *Gene expression in tooth*. http://bite-it.helsinki.fi
5. *Online Mendelian inheritance in man* 2002. http://www3.ncbi.nln.nih.gov/omim/. 106600 hypodontia, #604625 oligodontia.
6. Gorlin R, Cohen M, Levin S. *Syndromes of the head and neck*, 3rd edn. New York: Oxford University Press, 1980.
7. Burzyński NJ, Escobar VH. Classification and genetics of numeric anomalies of dentition. *Birth Defects Orig Artic Ser* 1983; **19**: 95–106.
8. Shapiro SD, Farrington FH. A potpourri of syndromes with anomalies of dentition. *Birth Defects Orig Artic Ser* 1983; **19**: 129–140.
9. Simons AL, Stritzel F, Stamatiou J. Anomalies associated with hypodontia of the permanent lateral incisors and second premolar. *J Clin Pediatr Dent* 1993; **17**: 109–111.
10. Sarnas KV, Rune B. The facial profile in advanced hypodontia. a mixed longitudinal study of 141 children. *Eur J Orthod* 1983; **5**: 133–143.
11. Schalk-van der Weide Y, Beemer FA, Faber JA, Bosman F. Symptomatology of patients with oligodontia. *J Oral Rehabil* 1994; **21**: 247–261.
12. Schalk-van der Weide Y, Steen WH, Bosman F. Distribution of missing teeth and tooth morphology in patients with oligodontia. *ASDC J Dent Child* 1992; **59**: 133–140.
13. Näsman M, Forsberg CM, Dahllöf G. Long-term dental development in children after treatment for malignant disease. *Eur J Orthod* 1997; **19**: 151–159.
14. Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nat Genet* 1996; **13**: 409–416.
15. Semina EV, Reiter R, Leysens NJ, Alward WL, Small KW, Datson NA. Cloning and characterisation of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat Genet* 1996; **14**: 392–399.
16. Jumlongras D, Bei M, Stimson JM, Wang W, Depalma SR, Seidman CE, Felbor U, Maas R, Seidman JG, Olsen BR. A nonsense mutation in *MSX1* causes Witkop syndrome. *Am J Hum Genet* 2001; **69**: 67–74.
17. Vainio S, Karavanova I, Jowett A, Thesleff I. Identification of BMP4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell* 1993; **75**: 45–58.
18. Peters H, Neubuser A, Balling R. Pax genes and organogenesis: *Pax9* meets tooth development. *Eur J Oral Sci* 1998; **106**: 38–43.
19. Tucker AS, Khamis AI, Sharpe PT. Interactions between Bmp4 and Msx1 act to restrict gene expression to odontogenic mesenchyme. *Dev Dyn* 1998; **212**: 533–539.
20. Satokata I, Maas R. *Msx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 1994; **6**: 348–355.
21. Peters H, Neubuser A, Kratochwil K, Balling R. *Pax9*-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Gene Dev* 1998; **12**: 2735–2747.
22. Hewitt JE, Clark LN, Ivens A, Williamson R. Structure and sequence of the human homeobox gene HOX7. *Genomics* 1991; **11**: 670–678.
23. Carton KM, Zhang H, Marshall SC, Instroza JA, Wilson JM, Abate C. Transcriptional repression by Msx1 does not require homeodomain DNA-binding sites. *Mol Cell Biol* 1995; **15**: 861–871.
24. Zhang H, Carton KM, Abate-Shen C. A role for the Msx1 homeodomain in transcriptional regulation: residues in the *N*-terminal arm mediate TATA binding protein interaction and transcriptional repression. *Proc Natl Acad Sci USA* 1996; **93**: 1764–1769.
25. Zhang H, Hu G, Wang H, Sciavolino P, Iler N, Shen M, Abate-Shen C. Heterodimerization of Msx and Dlx homeoproteins results in functional antagonism. *Mol Cell Biol* 1997; **17**: 2920–2932.
26. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missence mutation causes selective tooth agenesis. *Nat Genet* 1996; **13**: 417–421.
27. Hu G, Vastardis H, Bendall AJ, Wang Z, Logan M, Zhang H, Nelson C, Stein S, Greenfield N, Seidman CE, Seidman JG, Abate-Shen C. Haploinsufficiency of *MSX1*: a mechanism for selective tooth agenesis. *Mol Cell Biol* 1998; **18**: 6044–6051.
28. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. *MSX1* mutation is associated with orofacial clefting and tooth agenesis. *Nat Genet* 2000; **24**: 342–343.
29. Lidral AC, Reising BC. The role of MSX1 in human tooth agenesis. *J Dent Res* 2002; **81**: 274–278.
30. Jimenez G, Verrijzer CP, Ish-Horowicz D. A conserved motif in goosecoid mediates groucho-dependent repression in *Drosophila* embryos. *Mol Cell Biol* 1999; **19**: 2080–2087.
31. Sharpe PT. Homeobox genes and orofacial development. *Connect Tissue Res* 1995; **32**: 17–25.
32. Dahl E, Koseki H, Balling R. *Pax* genes and organogenesis. *Bioessays* 1997; **19**: 755–765.
33. Underhill DA. Genetic and biochemical diversity in the Pax gene family. *Biochem Cell Biol* 2000; **78**: 629–638.
34. Walther C, Guenet JL, Simon D, Deutsch U, Jostes B, Goulding MD, Plachov D, Balling R, Gruss P. *Pax*: a murine multigene family of paired box containing genes. *Genomics* 1991; **11**: 424–434.
35. Semenza GL. *PAX proteins*. New York: Oxford University Press, 1998; 169–198.
36. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of *PAX9* is associated with oligodontia. *Nat Genet* 2000; **24**: 18–19.
37. 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* 2001; **9**: 743–746.
38. Frazier-Bowers SA, Guo DC, Cavender A, Xue L, Evans B, King T, Milewicz D, D'Souza RN. A novel mutation in human *PAX9* causes molar oligodontia. *J Dent Res* 2002; **81**: 129–133.
39. Das P, Hai M, Elcock C, Leal SM, Brown DT, Brook AH, Patel PI. Novel missense mutations and a 288-bp exonic insertion in *PAX9* in families with autosomal dominant hypodontia. *Am J Med Genet* 2003; **118**: 35–42.
40. Mostowska A, Kobielak A, Biedziak B, Trzeciak WH. Novel mutation in the paired box sequence of *PAX9* gene in a sporadic case of oligodontia. *Eur J Oral Sci* 2003; **111**: 272–276.
41. Das P, Stockton DW, Bauer C, Shaffer LG, D'Souza R, Wright JT, Patel PI. Haploinsufficiency of *PAX9* is associated with autosomal dominant hypodontia. *Hum Genet* 2002; **110**: 371–376.
42. Peck S, Peck L, Kataja M. Concomitant occurrence of canine malposition and tooth agenesis: evidence of orofacial genetic fields. *Am J Orthodont Dentofac Orthoped* 2002; **122**: 657–660.
43. Scarel RM, Trevilatto PC, di Hipolito O, Camargo LE, Line SR. Absence mutations in the homeodomain of the *MSX1* gene in patients with hypodontia. *Am J Med Genet* 2000; **92**: 46–349.
44. Frazier-Bowers SA, Scott MR, Cavender A, Mensah J, D'Souza RN. Mutational analysis of families affected with molar oligodontia. *Connect Tissue Res* 2002; **43**: 296–300.
45. Tucker AS, Sharpe PT. Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. *J Dent Res* 1999; **78**: 826–834.

46. Vieira AR. Oral clefts and syndromic forms of tooth agenesis as models for genetics of isolated tooth agenesis. *J Dent Res* 2003; **82**: 162–165.
47. Bei M, Maas R. FGFs and BMP4 induce both Msx1-independent and Msx1-dependent signalling pathways in early tooth development. *Development* 1998; **125**: 4325–4333.
48. Qiu M, Bufone A, Martinez S, Menses JJ, Shimamura K, Pedersen RA, Rubenstein JL. Null mutation of *Dlx2* results in abnormal morphogenesis of proximal first and second brachial arch derivatives and abnormal differentiation in the forebrain. *Genes Dev* 1995; **9**: 2523–2538.
49. Qiu M, Bufone A, Ghattas I, Menses JJ, Sharpe PT, Presley R, Pedersen RA, Rubenstein JL. Role of the *Dlx* homeobox genes in proximodistal patterning of the branchial arches. mutations of *Dlx1, Dlx2, and Dlx1 and Dlx2* alter morphogenesis of proximal skeletal and soft tissue structures derived from the first and second arches. *Dev Biol* 1997; **185**: 165–184.
50. Hanson I, van Heyningen V. Pax6: more than meets the eye. *Trends Genet* 1995; **11**: 268–272.
51. van den Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, Grosschedl R. Development of several organs that require inductive epithelial–mesenchymal interactions is impaired in *LEF1* deficient mice. *Genes Dev* 1994; **8**: 2691–2703.
52. Mo R, Freer AM, Zinyk DL, Crackower MA, Michud J, Heng HH, Chik KW, Shi XM, Tsui LC, Cheng SH, Joyner AL, Hui C. Specific and redundant functions of *Gli 2* and *Gli3* zinc finger genes in skeletal patterning and development. *Development* 1997; **124**: 113–123.
53. Matzuk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. *Nature* 1995; **374**: 356–360.
54. Matzuk MM, Lu N, Vogel H, Selheyer DR, Roop DR, Bradley A. Multiple defects and perinatal death in mice deficient in follistatin. *Nature* 1995; **374**: 360–363.
55. Roberts VJ, Barth SL. Expression of messenger ribonucleic acids encoding the inhibin/activin system during mid- and late-gestation rat embryogenesis. *Endocrinology* 1994; **134**: 914–923.