ORIGINAL ARTICLE

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Investigating the etiology of multiple tooth agenesis in three sisters with severe oligodontia

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Structured Abstract

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Objectives – To describe the dentofacial phenotypes of three sisters with severe non-syndromic oligodontia, to report on the mutation analysis in three genes, previously shown to cause various phenotypes of non-syndromic oligodontia and in two other suspected genes. Based on the phenotypes in the pedigree of this family, the different possible patterns of transmission are discussed.

Methods – Anamnestic data and a panoramic radiograph were taken to study the phenotype of the three sisters and their first-degree relatives. Blood samples were also taken to obtain their karyotypes and DNA samples. Mutational screening was performed for the *MSX1*, *PAX9*, *AXIN2*, *DLX1* and *DLX2* genes.

Results – The probands' pedigree showed evidence for a recessive or multifactorial inheritance pattern. Normal chromosomal karyotypes were found and – despite the severe oligodontia present in all three sisters – no mutation appeared to be present in the five genes studied so far in these patients.

Conclusions – In the three sisters reported, their common oligodontia phenotype is not caused by mutations in the coding regions of *MSX1*, *PAX9*, *AXIN2*, *DLX1* or *DLX2* genes, but genetic factors most probably play a role as all three sisters were affected. Environmental and epigenetic factors as well as genes regulating odontogenesis need further *in vivo* and *in vitro* investigation to explain the phenotypic heterogeneity and to increase our understanding of the odontogenic processes.

Key words: *AXIN2*; *DLX1*; *DLX2*; etiology; *MSX1*; mutation screening; oligodontia; *PAX9*

Introduction

The absence of one or more teeth is a common developmental anomaly in man. The incidence of missing permanent teeth has been reported to vary from 1.6% to 9.6% in various populations, excluding the third molars, which are absent in around 20% (1). The incidence of missing teeth in the primary dentition is considerably lower (0.08–1.55%) (2). After the third molars, the second premolars (3.4%) and the maxillary lateral incisors (2.2%) are most commonly affected (3). Agenesis involving first and second molars is extremely rare. Oligodontia is the term conventionally used in cases where six or more teeth are missing.

The etiology of tooth agenesis is still largely unknown (4); however, a definite familial trend has been reported (5, 6). Brook (6) suggests that most cases of hypodontia have a polygenic inheritance pattern and that the risk of relatives having hypodontia will depend on a combination of numerous genetic and environmental factors, each with a small effect. Hypodontia may occur in isolation or in association with such syndromes as ectodermal dysplasia, Down's syndrome, Ellis-van Creveld syndrome and such conditions as cleft lip and palate (7–10). Familial tooth agenesis is transmitted as an autosomal dominant, a recessive or an X-linked condition (11).

Over 200 candidate genes have been demonstrated to be active in tooth development (12). Theoretically, a mutation in any of these developmental genes could result in a failure of tooth development. Studies of the molecular mechanisms involved in murine tooth development have led to the identification of many candidate genes that might be involved in human hypodontia. Vastardis (13) recommended the use of human molecular genetics combined with 'family studies' (pedigree analysis) to identify the genes involved in hypodontia. Also much of the understanding regarding the importance of genes during tooth development has been obtained from studies of knockout and transgenic mice. In *MSX1* and *PAX9* knockout mice tooth development arrests at the bud stage while teeth in *MSX1/MSX2* double mutants arrest at the lamina stage. To date, mutations in three different genes and two loci have been identified to cause non-syndromic oligodontia in humans (14, 15). Mutations of the *MSX1* (16, 17) or the *PAX9* (18–26) genes create an impairment of one or more molecular processes that regulate tooth formation (27). More recently, *AXIN2* mutations have been reported to cause colon cancer in combination with oligodontia (28). In a large Chinese kindred oligodontia, called He-Zhao deficiency, was transmitted in an autosomal dominant fashion with incomplete penetrance (29). The affected members had normal primary dentition, followed by the absence of most permanent teeth, excluding first and/or second permanent molar and central maxillary incisors. The gene locus was mapped to chromosome 10q11.2 (14). *KROX-26/ZNF22* expressed in human tooth development is a potential candidate gene (30). Another locus associated with autosomal recessive hypodontia has been reported in a Pakistan kindred on chromosome 16q12.1 (15).

The objective of the present study was to screen likely candidate genes causing the severe oligodontia in three sisters and to identify the genotype/phenotype correlation.

Materials and methods

Three sisters, V.H.J., V.H.A. and V.H.L., with congenitally missing deciduous and permanent teeth presented at the orthodontic clinic (Department of Orthodontics, Catholic University Leuven) for diagnostic evaluation and treatment planning of their oligodontia. At the time of their first consultation, these Caucasian girls were aged, respectively, 9 years, 8 years and 5 years.

Pedigree construction was made by interviews and clinical examination. An intra-oral examination was done to assess the presence of the teeth, the tooth size, tooth morphology and eventual enamel abnormalities. A panorex radiograph from available members of the family was taken to assess the dental development (Fig. 1). Extra- and intra-oral pictures of the three sisters were made (Figs 2 and 3). Anamnestic data of medical history were recorded and a familial anamnesis for the occurrence of agenesis was also performed. A thorough clinical examination of other tissues of ectodermal origin – the skin, hair, nails, sweat glands, ears and eyes – was also done.

A blood sample was taken from the three sisters and their parents for obtaining the karyotype and DNA samples. Karyotypes were studied by G-banding. Genomic DNA was isolated from peripheral lymphocytes. Mutational screening was done in Institute of Dentistry, Biomedicum, University of Helsinki, Finland. The coding region and flanking intronic sequences of *AXIN2*, *MSX1*, *PAX9*, *DLX1* and *DLX2* were subjected for 32 cycles of amplification with Dynazyme Ext DNA polymerase (Finnzymes, Espoo, Finland) in a buffer with 1.5 mM $MgCl_2$. The amplification conditions and primers for *AXIN2* were as described in Lammi et al. (28) and for *PAX9* as in Nieminen et al. (19). The conditions and primers for *MSX1*, *DLX1* and *DLX2* are described in Table 1. The PCR products were purified enzymatically with ExoSAP-IT (USB, Cleveland, OH, USA) according to the manufacturer's instructions. PCR products were sequenced with dye terminator chemistry (ABI Prism® BigDyeTM Terminator Cycle sequencing kit, version 3.1; Applied Biosystems, Foster

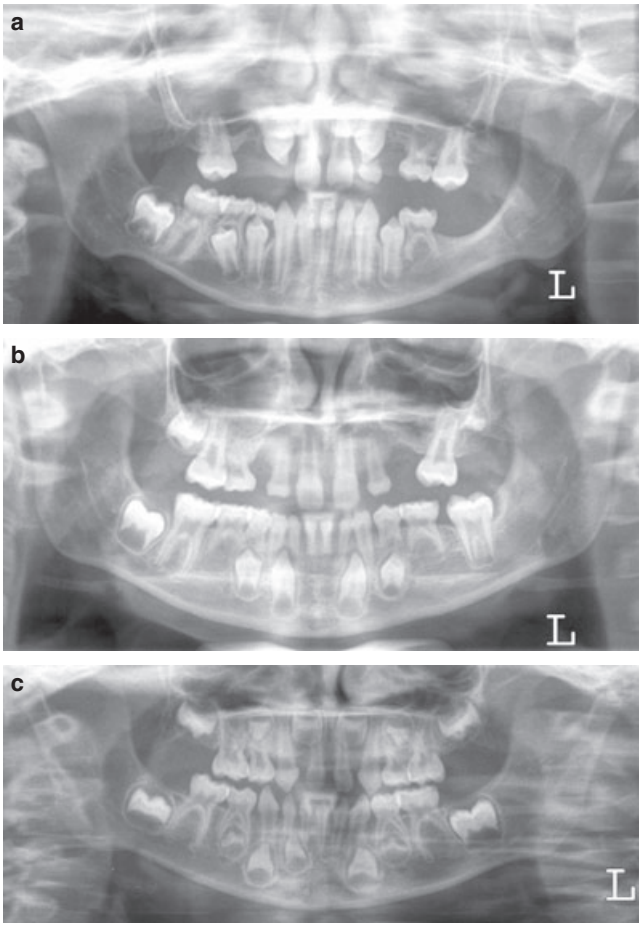

Fig. 1. Panorex radiographs. (a) V.H.J. (b) V.H.A. (c) V.H.L.

City, CA, USA) and subjected to capillary electrophoresis in an ABI3730 Automatic DNA sequencer in the Molecular medicine sequencing laboratory, Biomedicum, Helsinki, Finland. Sequencing results were compared with the wild-type reference sequences with bl2seq.exe software (NCBI, Bethesda, MD, USA).

Results

The radiographic examination showed the absence of, respectively, 12 (V.H.J.) and 15 (V.H.A.) permanent teeth in the two oldest sisters, excluding the wisdom teeth (Fig. 4a, b and Table 2). Absence of primary teeth could not be confirmed in the two oldest sisters because no early dental radiographs were available. The radiographic examination of the youngest sister (V.H.L.) showed the absence of at least seven permanent teeth (Fig. 4c and Table 2). Owing to the early stage of development, the presence of second premolars, second and third molars could not be confirmed at this time. Only for the youngest sister (V.H.A.), two missing primary teeth could be ascertained, i.e. the upper deciduous lateral incisors.

The intra-oral examination showed reduced crown sizes, especially the incisors presenting a peg-shaped

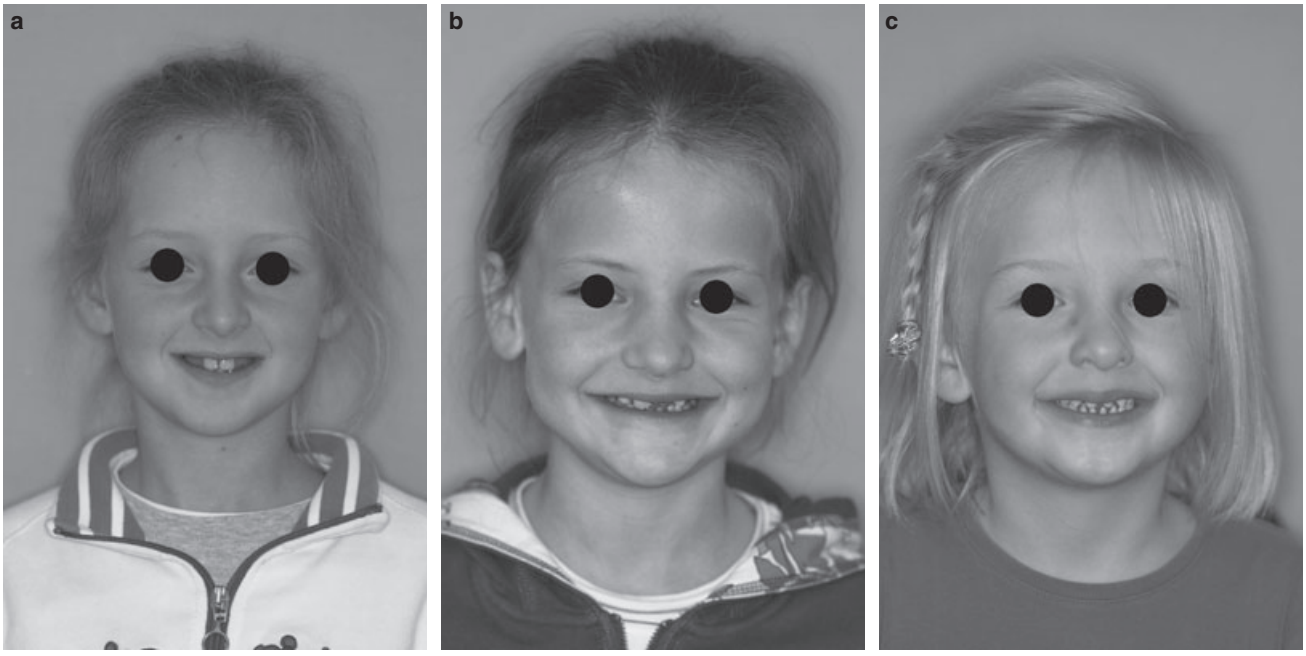

Fig. 2. Extra-oral pictures of the three sisters. (a) V.H.J. (b) V.H.A. (c) V.H.L.

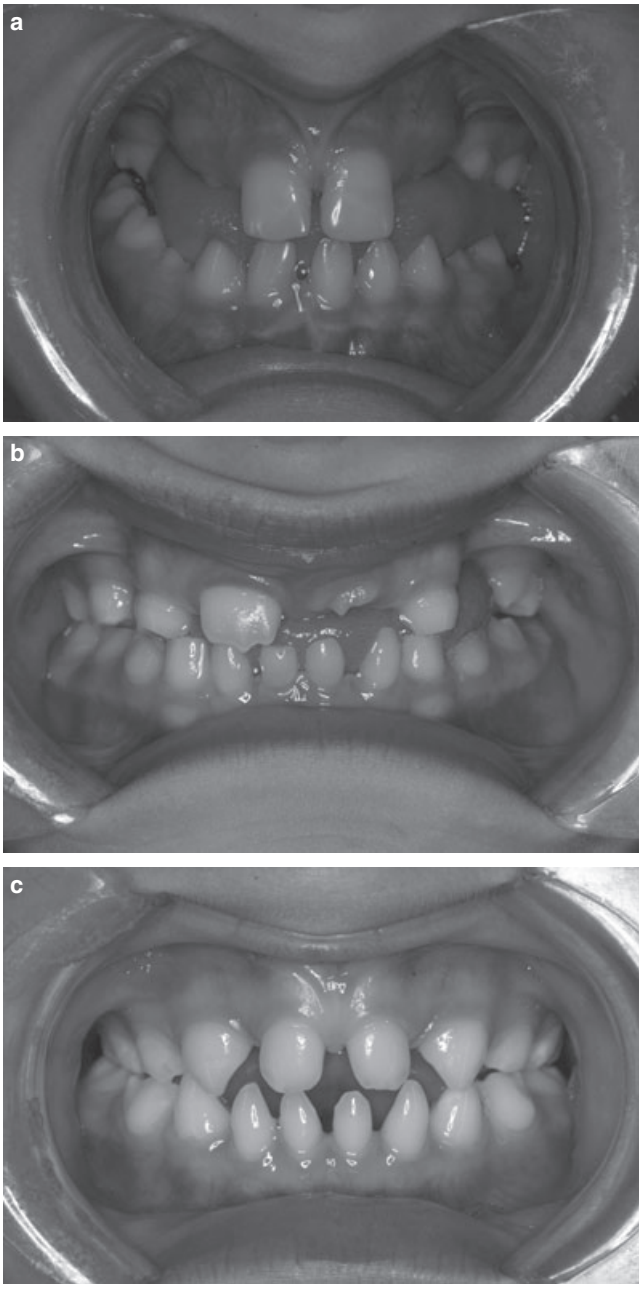

Fig. 3. Intra-oralpictures. (a) V.H.J. (b) V.H.A. (c) V.H.L.

form (Fig. 3). No enamel hypoplasia was found. The clinical examination showed no abnormalities in other ectodermal tissues pointing to isolated non-syndromic oligodontia in all three sisters. The clinical examination of the other available members of the family revealed that:

1) the mother of the girls has a full complement of teeth, while the father has agenesis of the two lower second premolars; none of them has clinical ectodermal abnormalities;
2) one sister of the mother has peg-shaped upper laterals; and
3) two female cousins of the father lack several permanent teeth.

Karyotype was normal, 46,XX after G-banding in the three sisters. The mutation screening did not reveal any mutations in the *MSX1*, *PAX9*, *AXIN2*, *DLX1* or *DLX2* coding regions or exon–intron junctions.

Discussion

To date, mutations in three different genes have been identified to cause non-syndromic oligodontia in humans: *MSX1*, *PAX9* and *AXIN2* (31).

PAX9 belongs to a gene family encoding for transcription factors during global embryogenesis. It contains a paired box, a sequence encoding a specific DNA binding domain. During odontogenesis, *PAX9* plays an important role in the sequential and reciprocal signaling cascades between epithelial and mesenchymal cell layers. Up to now, 15 heterozygous mutations of the *PAX9* gene have been reported (18–26). Most of these were associated with familial, non-syndromic form of tooth agenesis. Although there is considerable phenotypic heterogeneity, molars are the most affected teeth. However, in this family the upper lateral incisors are the most frequently missing teeth, followed by the upper canines and premolars, the lower lateral incisors and the lower second premolars.

The *MSX1* gene is expressed in the dental mesenchyme during odontogenesis. As a member of the homeobox family, this gene encodes for a DNA binding sequence. The MSX1 protein represses transcription and, besides PAX9, it also interacts with other components during the signaling pathways of odontogenesis like the DLX-family or TATA-binding protein. So far, seven *MSX1* mutations as well as some whole gene deletions have been discovered in oligodontia patients, all heterozygous (16, 17). Third molars, second premolars and incisors seem to be the most frequently missing teeth.

The *AXIN2* gene is located on chromosome 17 and is known as a negative feedback regulator of Wnt-signaling, which regulates early organ differentiation and development and plays a key role in many basic cell functions, like cell homeostasis. Disturbance of

Table 1. **The conditions and primers for *MSX1*, *DLX1* and *DLX2***

Gene	Target	Forward primer	Reverse primer	Ann. T	Added reagent
MSX1	Promoter	AGGAGAGGAGGGCAGAAGAG	ACACCGAGTGGCAAAGAAGT	57	10% DMSO
MSX1	Exon 1	CCCGGAGCCCATGCCCGGCGGCTG	CTCCCTCTGCGCCTGGGTTCTGGCT	64	5% DMSO
MSX1	Exon 2	ACTTGGCGGCACTCAATATC	AGAGGCACCGTAGAGCGAG	59	2% DMSO
DLX1	Exon 1	GACCTTCGCTGAGTCAAAGC	CGCTCCTTCTTTCCCTCTCT	58	
DLX1	Exon 2	TTGAACGTTCTCTCCCTGGT	CAGCGAGCAACAAATGAAGA	58	
DLX1	Exon 3	TTGGGAGGTCCTATCTCTGC	CCTCTGGGTCCTTCTTTTCC	58	
DLX2	Exon 1	GAAAGAGGATGCGACCAGAG	TAGACCGACTCGGCACTCTT	58	2% DMSO
DLX2	Exon 2	GTTCAGTGCACCCACCTCTT	GCAGGCCTGGAAATCCTTA	59	
DLX2	Exon 3	TAGCCAGCGGTTTTTCTCTG	CGGGGTAAGCAATGAGGATA	59	3 mM $MgCl_2$, 5% DMSO

Ann. T, annealing temperature.

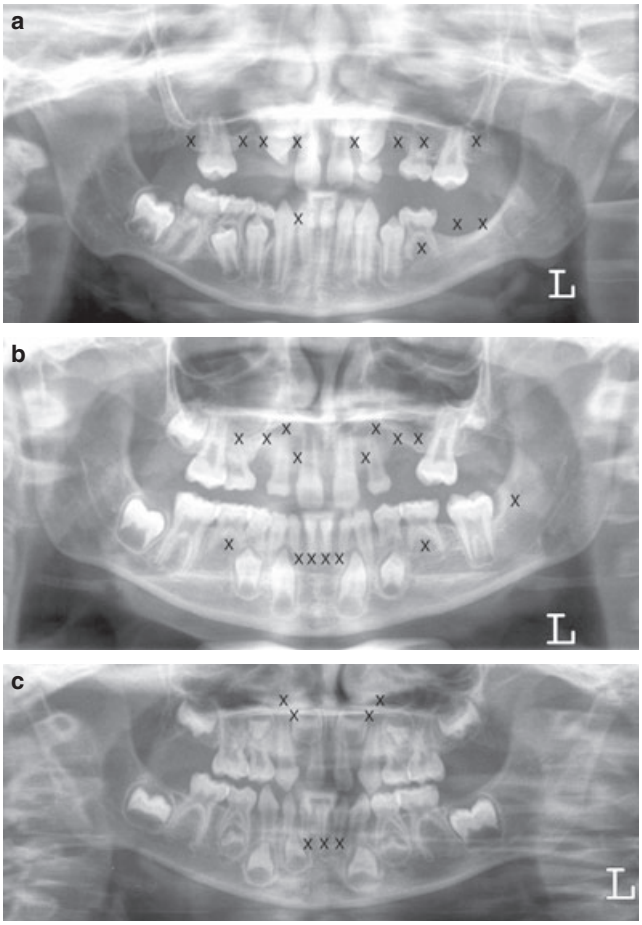

Fig. 4. Panorex radiographs. Each missing permanent tooth is indicated with x. Missing deciduous teeth are not indicated. (a) V.H.J. (b) V.H.A. (c) V.H.L.

Table 2. **Chart of the congenitally missing permanent teeth**

	Congenitally missing permanent teeth														
	Right								Left						
Individuals ID and dental arch	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
V.H.J.																
Maxillary		*		*	*		*			*		*	*		*	
Mandibular							*						*	*	*	
V.H.L.																
Maxillary				*	*	*	*			*	*	*	*			
Mandibular				*			*	*	*	*			*		*	
V.H.A.																
Maxillary						*	*			*	*					
Mandibular								*	*	*						

*Congenitally missing tooth; 1 and 2 = central and lateral incisors, respectively; 3 = canine; 4 and 5 = first and second premolars, respectively; 6, 7 and 8 = first, second and third molars, respectively.

Wnt-signaling may cause cancer. During tooth development, *AXIN2* is expressed in the dental mesenchyme, the odontoblasts and the enamel knot, and it is suggested that it is needed for down-regulation of Wnt-signaling at specific stages. Recently, two *AXIN2* mutations have been reported (28, 32). One was reported in a large family with familial tooth agenesis and colorectal cancer or precancerous lesions of variable types. The oligodontia phenotype here is rather severe as the affected family members lacked most permanent molars, premolars, lower incisors and upper lateral incisors. The other was a *de novo* germline mutation in a 13-year-old patient with an oligodontia phenotype as described above. Because of his young age, the cancer predisposition could not be demonstrated (28).

DLX1 and *DLX2* are members of a family of genes orthologous to the *Drosophila* distal-less homeobox gene. *DLX1* and *DLX2* are the first genes to be identified

that have a role in odontogenic patterning (33). Mice with targeted null mutations of both *DLX1* and *DLX2* homeobox genes do not develop maxillary molar teeth but incisors and mandibular molars are normal. *DLX1* and *DLX2* thus appear to have a functionally redundant role in development of maxillary molar teeth but are not required for development of incisors or mandibular molars.

So far, mutations in the genes (*MSX1*, *PAX9* and *AXIN2*) studied has been found almost exclusively in families with dominant inheritance (there are a few *de novo* mutations). From literature and experience from dental clinics, it is known that there are plenty of cases of severe agenesis with apparently more complex than simple dominant inheritance and this family seems to belong to this group. The etiology in these cases most probably consists of multiple genetic and environmental factors (6). The genetic changes may be more subtle on the molecular level than dominant mutations. These changes may be present in genes that we have not yet associated with human tooth agenesis, in genes associated with syndromes or in the genes that we studied in this family. In fact there are several common polymorphisms in the latter genes that may be involved but so far one has no good idea of which ones. Although we did not found a mutation in the three sisters, genetic factors are most probably involved in the etiology of the agenesis in this family, because the dental phenotype (severe oligodontia) was present in all three sisters.

The considerable variation in the number and type of missing teeth is typical for familial oligodontia (16, 19, 20, 24). However, the dental phenotypes in this family were more severe than and different from oligodontia in families with mutations reported elsewhere.

The phenotype of these sisters also differed from hypohidrotic ectodermal dysplasia (HED), in which both deciduous and permanent teeth are severely affected. Furthermore, the other ectodermal symptoms in nails, hair or skin typical for HED were not found in the three sisters or the parents. The youngest sister is the only one in which also deciduous teeth were certainly missing.

This is a case of isolated hypodontia in one family with almost no hypodontia in deciduous teeth. In most cases, in which the causal genetic factor was identified – like mutations in the *MSX1*, *PAX9* or *AXIN2* genes, the segregation of the oligodontia phenotype was the result of autosomal dominant inheritance, which is not the case in this family. Therefore, other types of transmission should be considered:

1) *Reduced penetrance*. Recently, a transition mutation was reported in the *MSX1* gene which appeared to be present in one of the healthy parents of the proband (34). As in this family, the sister of the girls' mother has a cone-shaped incisor, the mother could possibly be an asymptomatic carrier of a mutation; moreover as the father shows isolated agenesis of two lower premolars, reduced penetrance should be considered in these cases.
2) *Gonadal mosaicism*. In this segregation pattern, the mutation is only present in part of the gonadal cells, which could explain why the mother is not affected while her sister is.
3) *Autosomal recessive inheritance*. To date, an autosomal recessive pattern of inheritance has been genetically mapped in one family with isolated hypodontia (15). Although it is also unlikely that in case of autosomal recessive inheritance, 100% of the offspring is affected, this remains a theoretical possibility.
4) *Polygenic inheritance*. One parent contributes a predisposing allele of one gene and the other parent an allele of another gene.
5) *De novo* dominant mutation in the germline.
6) *Syndromic condition*. Another possibility is that this oligodontia belong to a syndromic condition without any other clinical manifestations than missing teeth. Variable expressivity is well known for many genetic conditions. For instance, Chranowska et al. (35) described a family with anodontia of permanent teeth as the sole clinical sign of ectrodactyly-ectodermal dysplasia-clefting syndrome. In addition, Tao et al. (36) reported a large kindred with ectodysplasin mutation, hypodontia and no HED characteristics. However, it is unlikely that in the event of ectodermal dysplasia, none of the three siblings or the parents present any additional ectodermal dysplasia signs.

Only a few reports can be found on the negative outcomes of tested patient groups for *MSX1*, *PAX9* or *AXIN2* mutations; Nieminen et al. (37) failed to identify linkage to *MSX1* in five unrelated families with hypodontia. Scarel et al. (38) could not discover any mutations in 20 patients with hypodontia. Frazier-Bowers et al. (39) also did not succeed to identify a *PAX9* nor *MSX1* mutation in

20 Vietnamese families. They also could not find any mutations in a high-risk group of an earlier study (40). Gerits et al. (41) also did not succeed to identify a *MSX1*, *PAX9* or *AXIN2* mutation in eight patients with severe oligodontia phenotype. More consequent reporting about positive as well as negative results of mutation screening (the exact number of probands tested included) can give useful information about the *real* incidence of mutations in hypodontia patients. Therefore, screening for *PAX9*, *MSX1* and *AXIN2* mutations is still interesting and useful. Interest should however also go to other genes and developmental factors known from the mouse model.

Conclusion

Considering the discrepancy between the high incidence rate of agenesis and the relatively small number of reported causative mutations, the genetic contribution to oligodontia seems much more heterogeneous than expected so far. In the three sisters reported, their common oligodontia phenotype is not caused by mutations in the coding regions of *MSX1*, *PAX9*, *AXIN2*, *DLX1* or *DLX2* genes, but genetic factors most probably play a role as they were affected all three. Environmental and epigenetic factors as well as other genes regulating odontogenesis need further *in vivo* and *in vitro* investigation to explain the phenotypic heterogeneity and to increase our understanding of the odontogenic processes.

Positive and negative research results as well as the number of probands tested should be reported in this respect. Parents should be informed that tooth agenesis is a familial condition and the children of parents with missing teeth or their families are at risk of agenesis. Different patterns of transmission can apply.

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References

1. Polder BJ, Van't Hof MA, Van der Linden FP, Kuijpers-Jagtman AM. A meta-analysis of the prevalence of dental agenesis of permanent teeth. *Community Dent Oral Epidemiol* 2004;32:217–26.
2. Whittington BR, Durward CS. Survey of anomalies in primary teeth and their correlation with the permanent dentition. *N Z Dent J* 1996;92:4–8.
3. Symons AL, Stritzel F, Stamatiou J. Anomalies associated with hypodontia of the permanent lateral incisor and second premolar. *J Clin Pediatr Dent* 1993;17:109–11.
4. Goodman JR, Jones SP, Hobkirk JA, King PA. Hypodontia: 1. Clinical features and the management of mild to moderate hypodontia. *Dent Update* 1994;21:381–4.
5. 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* 1996;75:1346–52.
6. Brook AH. A unifying aetiological explanation for anomalies of human tooth number and size. *Arch Oral Biol* 1984;29:373–8.
7. Larmour CJ, Mossey PA, Thind BS, Forgie AH, Stirrups DR. Hypodontia – a retrospective review of prevalence and etiology. Part 1. *Quintessence Int* 2005;36:263–70.
8. Kjaer I, Kocsis G, Nodal M, Christensen LR. Aetiological aspects of mandibular tooth agenesis – focusing on the role of nerve, oral mucosa, and supporting tissues. *Eur J Orthod* 1994;16:371–5.
9. Ribeiro LL, Das Neves LT, Costa B, Ribeiro Gomide M. Dental anomalies of the permanent lateral incisors and prevalence of hypodontia outside the cleft area in complete unilateral cleft lip and palate. *Cleft Palate Craniofac J* 2003;40:172–5.
10. Kumasaka S, Miyagi A, Sakai N, Shindo J, Kashima I. Oligodontia: a radiographic comparison of subjects with Down syndrome and normal subjects. *Spec Care Dentist* 1997;17:137–41.
11. Burzynski NJ, Escobar VH. Classification and genetics of numeric anomalies of dentition. *Birth Defects Orig Artic Ser* 1983;19:129–40.
12. Nieminen P, Pekkanen M, Aberg T, Thesleff I. A graphical WWW-database on gene expression in tooth. *Eur J Oral Sci* 1998;106:7–11.
13. Vastardis H. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. *Am J Orthod Dentofacial Orthop* 2000;117:650–6.
14. Liu W, Wang H, Zhao S, Zhao W, Bai S, Zhao Y et al. The novel gene locus for agenesis of permanent teeth (He-Zhao deficiency) maps to chromosome 10q11.2. *J Dent Res* 2001;80:1716–20.
15. Ahmad W, Brancolini V, Haque M, Lam H, Haque S et al. A locus for autosomal recessive hypodontia with associated dental anomalies maps to chromosome 16q12.1. *Am J Hum Genet* 1998;62:987–91.
16. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes evidence tooth agenesis. *Nat Genet* 1996;13:417–21.
17. De Muynck S, Schollen E, Matthijs G, Verdonck A, Devriendt K, Carels C. A novel MSX1 mutation in hypodontia. *Am J Med Genet* 2004;128A:401–3.
18. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. *Nat Genet* 2000;24:18–20.
19. Nieminen P, Arte S, Tanner D, Paulin L, Alaluusua S, Thesleff I et al. Identification of a nonsense mutation in the PAX9 gene in molar oligodontia. *Eur J Hum Genet* 2001;9:743–6.
20. Frazier-Bowers SA, Guo DC, Cavender A, Xue L, Evans B, King T et al. A novel mutation in human PAX9 causes molar oligodontia. *J Dent Res* 2002;81:129–33.
21. Das P, Hai M, Elcock C, Leal SM, Brown DT, Brook AH et al. Novel missense mutations and a 288-bp exonic insertion in PAX9 in families with autosomal dominant hypodontia. *Am J Med Genet* 2003;118A:35–42.
22. Das P, Stockton DW, Bauer C, Shaffer LG, D'Souza RN, Wright JT et al. Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. *Hum Genet* 2002;110:371–6.

23. Mostowska A, Kobielak A, Biedziak B, Trzeciak WH. Novel mutation in the paired box sequence of PAX9 gene in a sporadic form of oligodontia. *Eur J Oral Sci* 2003;111:272–6.
24. Lammi L, Halonen K, Pirinen S, Thesleff I, Arte S, Nieminen P. A missense mutation in PAX9 in a family with distinct phenotype of oligodontia. *Eur J Hum Genet* 2003;11:866–71.
25. Klein ML, Nieminen P, Lammi L, Niebuhr E, Kreiborg S. Novel mutation of the initiation codon of PAX9 causes oligodontia. *J Dent Res* 2005;84:43–7.
26. 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;111:365–70.
27. Peters H, Balling R. Teeth. Where and how to make them. *Trends Genet* 1999;15:59–65.
28. Lammi L, Arte S, Somer M, Järvinen H, Lahermo P, Thesleff I et al. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 2004;74:1043–50.
29. Wang H, Zhao S, Zhao W, Feng G, Jiang S, Liu W et al. Congenital absence of permanent teeth in a six-generation Chinese kindred. *Am J Med Genet* 2000;90:193–8.
30. Gao Y, Kobayashi H, Ganss B. The human KROX-26/ZNF22 gene is expressed at sites of tooth formation and maps to the locus for permanent tooth agenesis (He-Zhao Deficiency). *J Dent Res* 2003;82:1002–7.
31. Hu JC, Simmer JP. Developmental biology and genetics of dental malformations. *Orthod Craniofac Res* 2007;10:45–52.
32. Mostowska A, Biedziak B, Jagodzinski PP. Axis inhibition protein 2 (AXIN2) polymorphisms may be a risk factor for selective tooth agenesis. *J Hum Genet* 2006;51:262–6.
33. Thomas BL, Tucker AS, Qui M, Ferguson CA, Hardcastle Z, Rubenstein JLR et al. Role of Dlx-1 and Dlx-2 genes in patterning of the murine dentition. *Development* 1997;124:4811–8.
34. Mostowska A, Biedziak B, Trzeciak WH. A novel c.581C > T transition localized in a highly conserved homeobox sequence of MSX1: is it responsible for oligodontia? *J Appl Genet* 2006;47:159–64.
35. Chranowska KH, Krajewska-Walasek M, Rump Z, Wisniewski L, Fryns JP. Anodontia as the sole clinical sign of the ectrodactyly-ectodermal dysplasia-cleft lip (EEC) syndrome. *Genet Couns* 1990;1:67–73.
36. Tao R, Jin B, Guo SZ, Qing W, Feng GY, Brooks DG et al. A novel missense mutation of the EDA gene in a Mongolian family with congenital hypodontia. *J Hum Genet* 2006;51:498–502.
37. Nieminen P, Arte S, Pirinen S, Peltonen L, Thesleff I. Gene defect in hypodontia: exclusion of MSX1 and MSX2 as candidate genes. *Hum Genet* 1995;96:305–8.
38. Scarel RM, Trevilatto PC, Di Hipolito O, Camargo LEA, Line SRP. Absence of mutations in the homeodomain of the MSX1 gene in patients with hypodontia. *Am J Med Genet* 2000;92:346–9.
39. Frazier-Bowers SA, Pham KY, Le EV, Cavender AC, Kapadia H, King TM et al. A unique form of hypodontia seen in Vietnamese patients: clinical and molecular analysis. *J Med Genet* 2003;40: 79–83.
40. 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.
41. Gerits A, Nieminen P, De Muynck S, Carels C. Exclusion of coding reading mutations in MSX1, PAX9 and AXIN2 in eight patients with severe oligodontia phenotype. *Orthod Craniofac Res* 2006;9:129–36.