

## GENES AND DENTAL DISORDERS

MIRCEA GHERGIE<sup>1</sup>, ELVIRA COCÎRLA<sup>1</sup>, IULIA LUPAN<sup>2</sup>,  
BEATRICE S. KELEMEN<sup>2</sup>, OCTAVIAN POPESCU<sup>2,3</sup>

<sup>1</sup>Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine  
and Pharmacy, Cluj-Napoca, Romania

<sup>2</sup>Molecular Biology Center, Institute for Interdisciplinary Research  
on Bio-Nano-Sciences, Babeș-Bolyai University, Cluj-Napoca, Romania

<sup>3</sup>Institute of Biology, Romanian Academy, Bucharest, Romania

### Abstract

*In recent decades with the advancement of molecular research, information regarding specific molecular mechanisms has exploded. In the present review we present the molecular basis of dental pathologies that are of particular interest to clinicians.*

**Keywords:** dentogenesis, craniofacial disease, homeobox genes, transcription factors.

---

### Introduction

The publication in 2001 of an article regarding the human genome [1,2] is considered the most important scientific achievement of all time [3]. The explosion of scientific information, coupled with technological advances in bioinformatics, genetics, molecular biology, physics and chemistry and the interdisciplinary approach have opened new avenues for multiple research topics from the medical field, including dental medicine. Integrating information from all these areas will change the approach to dental health issues, providing effective strategies, dentist diagnosis, prevention, intervention and treatment of craniofacial diseases.

Pursuing oral health starting with the patient's childhood, the dentist is thus able to observe the various abnormalities and to intervene early to remedy the situation, and in more complex cases, recommend patients to specialists in medical genetics and/or genetic counseling [4,5]. For this purpose, the dentist himself must have thorough knowledge of genetic research [3,6,7,8,9].

According to the National Institute of Dental and Craniofacial Research Genetics (2008) in the U.S., from the approximately 5500 known genetic disorders in humans, more than 700 are craniofacial disorders. Only in 20% of all known diseases is their genetic determinism also known [10].

### Molecular mechanism of dentogenesis

Recently many efforts have been made for the

understanding of molecular and cellular mechanisms that control the development and dental pathology. Additional information regarding tooth organogenesis was obtained using mouse embryos [11] and birds [12] as experimental material. These studies revealed a strict genetic control of odontogenesis, which determines the position, number, size and shape of the teeth.

During the bell stage the cyto-differentiation of dental epithelial occurs; cells near the mesenchyme which are differentiated into ameloblastes will produce enamel. The adjacent mesenchymal cells will differentiate into odontoblasts and will be involved in dentin formation. Mesenchyme surrounding the tooth bud will develop forming the supporting structure of the tooth, for example the periodontal ligament that anchors the tooth to the alveolar bone [13,14,15,16].

Like other processes during the embryonic development, morphogenesis and differentiation of teeth is the result of complex interactions at molecular level between the ectoderm and the mesenchyma [13,15,17,18,19,20]. Until now more than 200 genes involved in these processes have been identified [14,21]. A crucial role was attributed to those transcription factors that have a homeodomain. The homeodomain consists of 60 amino acids with a helix-turn-helix DNA binding motif and is encoded by a homeobox sequence: short chains of 180 bp, located in the vicinity of the gene's 3' end. In addition to the homeodomain that facilitates the binding to DNA, the transcription factors also contain a transactivation domain that interacts with a RNA polymerase. The homeodomain transcription factors in turn are involved in the regulation of homeobox gene expression sites, thus having a role in

the activation of gene expression in multicellular organisms during embryonic development [20,22].

The first genes containing homeobox sites were identified in the Hox cluster (cluster-related group of genes located on the same chromosome, each coding for a particular protein which are often regulated by the same cellular mechanisms). This cluster is highly conserved during evolution; sequences have remained relatively unchanged (75-90%) for hundreds of millions of years [23,24]. In embryogenesis, Hox genes cluster controls the development plan of the embryo during development. During tooth morphogenesis, expression of the homeobox genes is under the control of signaling cascades initiated by the interaction of certain proteins (either growth factors or other proteins secreted or available on the surface of neighboring cells) with receptors on the surface of target cells.

Important factors in tooth morphogenesis are: the family of fibroblast growth factors (FGF) and transforming growth factors (TGF, including BMP4 - bone morphogenetic protein 4), the family of Wnt (Wingless) and morphogenesis molecule Shh (Sonic hedgehog). The general scheme of dentition is determined even before the development of visible teeth. The proximal area of the molars to be developed is characterized by the expression of growth factors FGF8 and FGF9, while BMP4 is expressed in the distal region of the presumed incisors [12,16,20,25,26,27,28].

Mentioned transcription factors define spatially the domains of expression of the homeobox genes in the developing jaw. Basically every combination of homeobox genes expressed is a "code" that specifies the type of the tooth [16,29]. Tooth formation is a complex process, genetically controlled in two ways: on one side, by specifying the type, size and position of each tooth organ; on the other, by the processes of enamel and dentin formation. Different genes involved in the formation of teeth belong to signaling pathways with functions in regulating morphogenesis of other organs. This explains the fact that mutations in these genes have pleiotropic effects in addition to causing non-syndromic dental abnormalities and dental anomalies associated with different genetic syndromes [30,31,32,33].

**Dental agenesis.** Congenital lack of one or more teeth is the most frequent anomaly in humans. In hypodontia 1-6 teeth (excluding molar 3) are missing; in oligodontia - more than 6 (excluding molar 3). Anodontia, *ie* the complete absence of teeth is very rare, it was demonstrated in a large family in China and believed to be recessive and inherited autosomally [14, 34].

Cases of hypodontia/oligodontia may or may not be associated with various syndromes such as hypohydrotic ectodermal dysplasia, cleft of the lip or palate etc. Frequency of cases of non-syndromic hypodontia/oligodontia is 80% when missing a tooth, less than 10% when missing several teeth and less than 1% when missing a large number of

teeth [35].

Although agenesis is occasionally caused by environmental factors (trauma in the dental region such as fractures, surgical procedures, chemotherapy, radiotherapy), in most cases the causes are genetic.

So far, only 4 genes have been identified to be associated with non-syndromic hypodontia/oligodontia, which after Carels [36] represents less than 5% of total cases. The identified genes are:

- *MSX1* - hypodontia NS [37];
- *PAX9* - oligodontia NS [38];
- *AXIN2* - oligodontia associated with colono-rectal cancer [39];
- *EDA1* - oligodontia NS [40].

Located on the short arm of chromosome 4 (4p16.1-p16.3), the *MSX1* gene has a homeobox sequence and two exons that encode a homeodomain - a 297 amino acid protein [41]. The gene plays an important role in craniofacial development, including odontogenesis. So far, three mutations in exon 1 and 4 in exon 2 have been associated with hypodontia affecting predominantly PM2 and M3 [37] or cleft associated hypodontia [42,43]. *MSX1* phenotypes caused by the protein deficiency depend on the location of the mutations and their effect on the structure and function of the protein.

In 1996, Vastardis et al. [37] identified an Arg to Pro substitution in position 31 of the *MSX1* gene homeodomain that caused hypodontia and was transmitted in an autosomal dominant way in the analyzed family for 4 generations. The mutant protein had an abnormal structure, but low thermal stability compared with the normal protein. The ability of the mutant protein to bind to DNA, and interact with other transcription factors were significantly altered [44]. Chishti et al. [45] identified a new point mutation in the *MSX1* gene which resulted in the substitution of alanine to threonine (A219T) in the *MSX1* homeodomain in 2 pakistani families, causing oligodontia. This mutation was the first recessive mutation identified in the *MSX1* gene.

The *PAX9* gene is a highly conserved gene in humans (14q12-q13), encoding a transcription factor that is involved in the development of teeth [46]. Until now 14 mutations in exons 1, 2 and 4, mostly in exon 2 were found [42] and are associated with different degrees of non-syndromic agenesis. In 2000, Stockton et al. [38] showed that a mutation in the *PAX9* gene (G219 insertion in exon 2) modified the open reading frame (frameshift mutation) causing premature termination of translation. The affected individuals were normal, but lacked most permanent molars. The disease was transmitted in autosomal dominant fashion.

Subsequently, other *PAX9* mutations that led to non-syndromic oligodontia were found:

- transversion (A340T) that created a stop codon at lysine 114 in the DNA binding domain [47];
- a cytosine insertion in exon 4 (insC793), frameshift

mutation that led to the appearance of a premature STOP codon at amino acid position 315 [48];

- three different missense mutations leading to substitution of arginine with proline in the homeodomain (Arg26Pro), glutamic acid with lysine (Glu91Lys) and leucine-to-proline (Leu21Pro) affecting M1 [49];

- transitions (C76T) [50] and (C139T) that led to the replacement of arginine with tryptophan in N-terminus of the homeodomain [51].

In all these cases, permanent parts of the molars were missing, which emphasizes the importance of this gene in development. The sequencing of *PAX9* gene in samples from a Chinese family with many cases of oligodontia showed a transition (A → G) in the initiator AUG codon, in exon 1. This is the first mutation found in an initiator codon that supposedly caused a severe inhibition of translation [21].

**AXIN2 gene** (17q23-q24) encodes the Axin2 protein that has an important role in regulating the stability of β-catenin, which is involved in the Wnt signalling pathway (wingless). When cells receive Wnt signals, β-catenin binds to stabilized transcription factors (TCF family), regulating the expression of Wnt target genes. It was found that changes in the functioning of the Wnt signaling pathway leads to cancer predisposition.

In 2004 Lammi et al. [39] identified mutations that caused severe oligodontia in a Finnish family (11 members lacked at least eight permanent teeth, two of whom developed only 3 permanent teeth) with a predisposition for colorectal cancer (8 patients). It was found that oligodontia and predisposition to cancer was caused by a nonsense mutation (Arg656Stop) in the *AXIN2* gene. In another unrelated patient with severe agenesis, an insertion (1994-1995insG) in the *AXIN2* gene was identified that caused a frameshift mutation. Both mutations activate the Wnt signalling pathway. These results prove the importance of this signalling pathway in the normal development of teeth.

Oligodontia as the results of mutations in the *AXIN2* gene was more severe than that described for mutations in *MSX1* and *PAX9* genes; there were more missing molars, premolars, upper lateral incisors and lower incisors, but upper central incisors were present.

**EDA1 gene** (Xq12-q13.1). Mutations in this gene cause X-linked hypohydrotic ectodermal dysplasia (HED), a rare disease characterized by hypoplasia or absence of sweat glands, dry skin, sparse hair and pronounced oligodontia. In 2010 Khabour et al. [52] identified a nonsense mutation (transition 463C> T) in the *EDA* gene in a Jordanian family. The mutation resulted in the replacement of arginine with cysteine that has led to intolerance to heat, the absence of 17 teeth, speech problems and anhidrosis (reduced sweating) in affected individuals. In 2006 Tao et al. [40] found a point mutation (cross c.193C> G, the replacement of arginine with glycine) in the *EDA* gene in a Mongolian family in which affected males (females are carriers) did not present other features of the disease than

hypodontia. Other cases of non-syndromic hypodontia were described by Li et al. [53], in 2008 in two families in China with two nonsense mutations in the same gene (947A> G substitution Glu316Gly and 1013C> T substitution in the protein Thr338Met). Threonine substitution with methionine in position 338 (Thr338Met) is accompanied by the lack of central and lateral incisors, and canine teeth of the maxilla and mandible [54].

In 2009 Song et al. [55] identified three new mutations of the *EDA* gene (Ala259Glu, Arg289Cys, Arg334His) in four male individuals (27%) from 15 analyzed individuals with non-syndromic oligodontia.

In addition to genes *MSX1*, *PAX9*, *AXIN2* and *EDA1*, Kantaputra and Sripathomsawat (2011) reported that non-syndromic hypodontia can be caused by mutations in *WNT10A* gene. This gene is part of the *WNT* gene family encoding the expression of signalling proteins on the cell surface and is associated with several syndromes (ectodermal dysplasia), but also non-syndromic hypodontia.

In conclusion, the genetic causes of dental pathologies are multiple. Phenotype and severity is dependent on the affected gene, the type and location of the mutations. We still do not know all the causes of the dental diseases, but their genetic basis is not a neglected factor.

## References

1. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature*, 2001; 409:860-921.
2. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science*, 2001; 291:1304-1351.
3. Wright JT, Hart TC. The genome projects: implications for dental practice and education. *J. Dental Educ*, 2002; 66:659-671.
4. Bixler D. Genetic counseling in dentistry. *J. Dental Educ*, 1997; 40:645-649.
5. Pemberton TJ, Mendoza G, Gee J, Patel PI. Inherited dental anomalies: a review and prospects for the future role of clinicians. *J. Calif. Dent. Assoc*, 2007; 35:324-326, 328-333.
6. Behnke AR, Hassell TM. Needs for genetics education in U.S. Dental and dental hygiene programs. *J. Dental Educ*, 2004; 68:819-822.
7. Collins F, Tabask L. A call for increased education in genetics for dental health professionals. *J. Dental Educ*, 2004; 68:807-808.
8. Hart TC, Ferrell RE. Genetic testing considerations for oral medicine. *J. Dental Educ*, 2002; 66:1185-1202.
9. Slavkin HC. The human genome, implications for oral health and diseases, and dental education. *J Dental Educ*, 2001; 65:463-479.
10. Pemberton TJ, Gee J, Patel PI. Gene discovery for dental anomalies: a primer for the dental professional. *J Amer Dent Assoc*, 2006; 137:743-752.
11. Miletich J, Sharpe TP. Normal and abnormal dental development. *Hum Mol Gen*, 2003; 12:69-73.
12. Haworth KE, Healy C, Morgan P, Sharpe PT. Regionalisation of early head ectoderm is regulated by endoderm and prepatterns the orofacial epithelium. *Development*, 2004; 131:4797-4806.
13. Thesleff I. Epithelial-mesenchymal signalling regulating tooth morphogenesis. *J Cell Sci*, 2003; 116:1647-1648
14. Thesleff I, Pirinen S. Dental anomalies: Genetics. *Encyclopedia of Life Sciences*, John Wiley&Sons, Ltd www.els.net, 2005.

15. Thesleff I, Tummers M. Tooth organogenesis and regeneration. StemBook (internet). Cambridge (MA). Harvard Stem Cell Institute, (<http://www.stembook.org/node/551>), 2008.
16. Tucker AS. Tooth morphogenesis and patterning molecular genetics. Encyclopedia of Life Sciences, John Wiley&Sons LTD, 2009.
17. Dassule HR, McMahon AP. Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. *Dev Biol* 1998; 202:215-227.
18. Kapadia H, Mues G, D'Souza R. Genes affecting tooth morphogenesis. *Orthod Craniofacial Res*, 2007; 10:105-113.
19. Peters H, Balling R. Teeth. Where and how to make them. *Trends Genet*, 1999; 15:59-65.
20. Thesleff I. Homeobox genes and growth factors in regulation of craniofacial and tooth morphogenesis. *Acta Odontol Scand*, 1995; 53:129-134.
21. Klein M, Nieminen P, Lammi L, Niebuhr E, Kreiborg S. Novel mutation of the initiator codon of PAX9 causes oligodontia. *J. Dental Res*, 2005; 84:43-47.
22. Sharpe TP. Homeobox genes in initiation and shape of teeth during development in mammalian embryos. In: *Evolution of Teeth*. Ed. Teaford M.F., Smith, M.M. și Ferguson, M.W.J., Cambridge Univ. Press, New York, 2000.
23. Campbell NA, Reece JB, Mitchell LG. *Biology*. Addison-Wesley Longman, Inc, 1999.
24. Zarnea G, Popescu O. *Dictionar de Microbiologie generală și Biologie Moleculară*. Edit. Acad. Române, București, 2011.
25. Mandler M, Neubüser A. FGF signalling is necessary for the specification of the odontogenic mesenchyme. *Dev Biol*, 2001; 240:548-559.
26. Thesleff I, Nieminen P. Tooth induction. Encyclopedia of Life Sciences. John Wiley&Sons, Ltd [www.els.net](http://www.els.net), 2005.
27. Tucker AS, Matthews KL, Sharpe PT. Transformation of tooth type induced by inhibition of BMP signaling. *Science*, 1998; 282:1136-1138.
28. Tucker AS, Sharpe PT. The cutting-edge of mammalian development; how the embryo makes teeth. *Nature Reviews Genetics*, 2004; 5:499-508.
29. Chen Y, Bei M, Woo I, Maas R. Msx1 controls inductive signaling in mammalian tooth morphogenesis. *Development*, 1996; 122:3035-3044.
30. Bailleul-Forestier I, Molla M, Verloes A, Berdal A. The genetic basis of inherited anomalies of the teeth: Part 1: Clinical and molecular aspects of non-syndromic dental disorders. *Europ. J Med Genet*, 2008; 51:273-291.
31. Coskuni A, Ozdemir O, Gedik R, Ozdemir AK, Gul E, Akyol M. Allelic heterozygous point mutation in homeobox PAX9 gene in a family with hypohidrotic ectodermal dysplasia: clinical and molecular findings. *J Chinese Clinic Med*, 2007; 11:11.
32. Kurisu K, Tabata MJ. Human genes for dental anomalies. *Oral Dis*, 1997; 3:223-228.
33. Kurisu K, Tabata MJ. Hereditary diseases with tooth anomalies and their causal genes. *Kaibogaku Zasshi*, 1998; 73:201-208.
34. Garib DG, Alencar BM, Ferreira FV, Ozawa TO. Associated dental anomalies: the orthodontist decoding the genetics which regulates the dental development disturbances. *Dental Press J. Orthod*, 2010; 138:138-157.
35. De Coster PJ, Marks LA, Mertens LC, Huysseune A. Dental agenesis: genetic and clinical perspectives. *J Oral Pathol & Med*, 2009; 38:1-17.
36. Carels C. Genetic diagnosis of dental anomalies. *Amer. Assoc. Orthod. (AAO), Annual Session*, 2011.
37. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman C.E. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat.Genet*, 1996; 13:417-421.
38. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel P. Mutation of PAX9 is associated with oligodontia. *Nat.Genet*, 2000; 24:18-19.
39. Lammi L, Arte S, Somer M, et al. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Amer J Hum Genet*, 2004; 74:1043-1050.
40. Tao R, Jin B, Guo SZ, et al. A novel missense mutation of the EDA gene in a Mongolian family with congenital hypodontia. *J Hum Genet*, 2006; 51:498-502.
41. Hewitt JE, Clark LN, Ivens A, Williamson R. Structure and sequence of the human homeobox gene HOX7. *Genomics*, 1991; 11:670-678.
42. Matalova E, Fleischmannova J, Sharpe PT, Tucker AS. Tooth agenesis: from molecular genetics to molecular dentistry. *J. Dental Res*, 2008; 87:617-623.
43. Mostowska A, Kobiela A, Trzeciak WH. Molecular basis of non-syndromic tooth agenesis: mutations of MSX1 and PAX9 reflect their role in patterning human dentition. *Europ. J. Oral Sci*, 2003; 11:365-370.
44. Hu G, Vastardis H, Bendall AJ, et al. Haploinsufficiency of MSX1: a mechanism for selective tooth agenesis. *Mol. Cell Biol*, 1998; 18:6044-6051.
45. Chishti M, Muhamad D, Halder M, Ahmad W. A novel missense mutation MSX1 underlies autosomal recessive oligodontia with associated dental anomalies in Pakistani families. *J Hum. Genetics*, 2006; 51:872-878.
46. Kavitha B, Priyadarshini V, Sivapathasundharam B, Saraswathi TR. Role of genes in oro-dental diseases. *Indian J. Dental Res*, 2010; 21:270-274.
47. Nieminen P, Arte S, Tanner D, et al. Identification of a nonsense mutation in the PAX9 gene in molar oligodontia. *Eur J Hum Genet*, 2001; 9:743-746.
48. Frazier-Bowers S, Guo DC, Cavender A, et al. A novel mutation in human PAX9 causes molar oligodontia. *J Dent Res*, 2002; 81:129-133.
49. Das P, Hai M, Elcock C, et al. Novel missense mutations and a 288-bp exonic insertion in PAX9 in families with autosomal dominant hypodontia. *Amer J Med Genet*, 2003; 118A:35-42.
50. 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. *Europ J Hum. Genet* 2003;11:866-871.
51. Zhao J, Hu Q, Chen Y, Luo S, Bao L, Xu Y. A novel missense mutation in the paired domain of human PAX9 causes oligodontia. *Amer J Med Genet*, 2007; 143A:2592-2597.
52. Khabour OF, Mesmar FS, Al-Tamimi F, Al-Batayneh OB, Owais AI. Missense mutation of the EDA gene in a Jordanian family with X-linked hypohidrotic ectodermal dysplasia: phenotypic appearance and speech problems. *Gen Molec Res*, 2010; 9:941-948.
53. Li S, Li J, Cheng J, et al. Non-syndromic tooth genesis in two Chinese families associated with novel missense mutations in the TNF domain of EDA (ectodysplasin A). *PLoS One*, 2008; 3:e2396.
54. Han D, Gong Y, Wu H, Zhang X, Yan M, Wang X. Novel EDA mutation resulting in X-linked non-syndromic hypodontia and the pattern of EDA-associated isolated tooth agenesis. *Europ J Med Genet*, 2008; 51:536-546.
55. Song S, Han D, Qu H, et al. EDA gene mutations underlie non-syndromic oligodontia. *J. Dental Res*, 2009; 88:126-131.