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Genetic basis of non-syndromic anomalies of human tooth number

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ABSTRACT

Teeth organogenesis develops through a well-ordered series of inductive events involving genes and BMP, FGF, SHH and WNT represent the main signalling pathways that regulate epithelial–mesenchymal interactions. Moreover, progress in genetics and molecular biology indicates that more than 300 genes are involved in different phases of teeth development. Mutations in genes involved in odontogenesis are responsible for many dental anomalies, including a number of dental anomalies that can be associated with other systemic skeletal or organic manifestations (syndromic dental anomalies) or not (non-syndromic dental anomalies). The knowledge of the genetic development mechanisms of the latter is of major interest. Understanding the mechanisms of pathogenesis of non-syndromic teeth anomalies would also clarify the role of teeth in craniofacial development, and this would represent an important contribution to the diagnosis, treatment and prognosis of congenital malformations, and the eventual association to other severe diseases. Future research in this area is likely to lead to the development of tests for doctors to formulate an early diagnosis of these anomalies.

1. Introduction

The tooth is a specialised body of the maxillofacial skeleton, the development of which is made possible by a long and complex series of steps. Tooth development is under genetic control and is regulated by inductive interactions between epithelial and mesenchymal cells.[1,2] During 7–11 embryonic weeks in humans the oral epithelium that lines the inside of the oral cavity shows a local thickening, the dental placode. Cells of the dental placode proliferate and further invaginate in the mesenchyme that condenses around the epithelium forming the bud stage. Later, the epithelium expands deeper and becomes surrounded by the condensing mesenchyme forming a tooth cap and later a bell. During these stages the mutual interaction between the epithelium and mesenchyme lead to the formation of the different anatomical and functional parts of the tooth. Mesenchymal cells differentiate into dentine-producing odontoblasts and the adjacent epithelial cells differentiate into enamel-secreting ameloblasts. Fig. 1 shows the principal stages of tooth development.[2]

Most odontogenic studies using mice dentition as a development model showed that the position, number, dimension, and shape of several teeth are controlled by a complex system of genes whose modifications can cause dental anomalies. Depending on the development stage in which the alteration takes place, different anomalies could occur: number anomalies (hyperdontism, anodontism, and hypodontism), structural abnormalities (amelogenesis imperfecta, dentinogenesis imperfecta, and dentinal dysplasia) and/or shape abnormalities (macrodontia, microdontia, and taurodontism).

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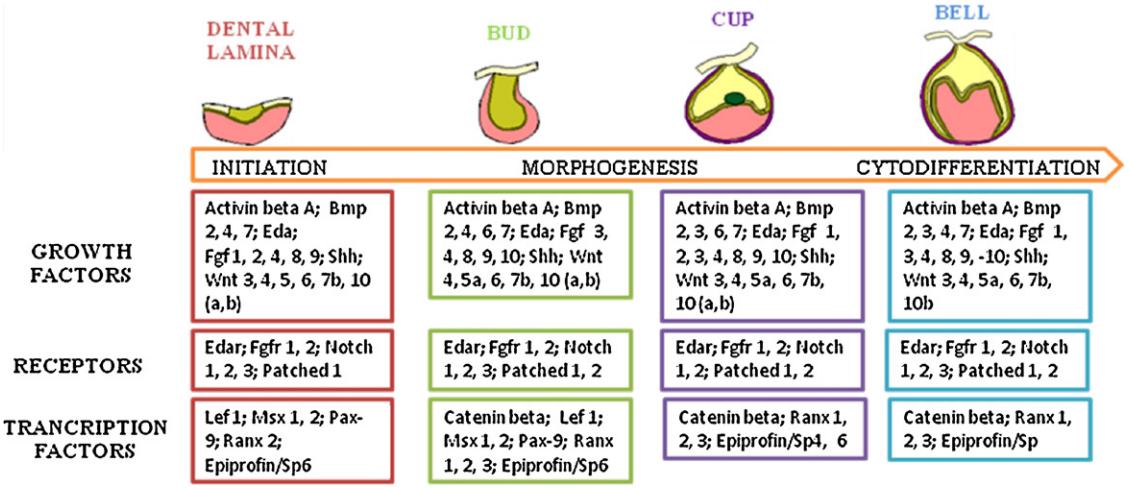

Fig. 1 – Stages and basic players involved in tooth development (growth factors, receptors, transcription factors) in human and mouse.
Adapted from Brook[95] and *Gene expression in tooth*: http://bite-it.helsink.fi.

Dental anomalies can either be associated with other systemic disorders (syndromic disease), i.e. hypohidrotic ectodermal dysplasia, cleidocranial dysplasia, Gardener syndrome, or be isolated (non-syndromic disease).

This article reviews the most recent acquisitions amongst non-syndromic anomalies of human tooth number. Although non-syndromic number anomalies are rare conditions, the analysis of the gene functions in this type of disease would be helpful to understand the most common ones.

2. Genetic basis of dental development

The human dentition develops through a process regulated by genetic networks and tissue interactions. These interactive mechanisms are strategic for the serial development of all the teeth within a particular class. Recent findings about the roles of signalling molecules and the expression of homeobox genes in dental development indicate a complementary interaction between the "field" and "clone" theories.[3–7]

The "field" theory was first proposed by Butler and then adapted by Dahlberg; the theory[4,5] postulated that each tooth within a class, e.g. molars, develops number, shape, size and order of development because it belongs to a common field. Nevertheless a field gradient would exist, depending on the position of the tooth in the field.

Osborne[3] in his clone theory, proposed that a single preprogrammed cells clone is responsible for the development of a specific class of tooth.

Morphogenesis is regulated by inductive interactions between cells in the epithelium and the mesenchyme tissue. The molecular interactions involve a complex series of signals made of molecular signals, receptors, and transcription control systems.[8–11]

Major signalling molecules involved in regulation of tooth embryogenesis belong to the BMP (bone morphogenetic protein), the FGF (fibroblast growth factor), the SHH (sonic hedgehog) and the WNT (Wingless) families. Recently, Nakamura has also pointed out the importance of epiprofin/Sp6 (Epfn) as an essential transcription factor in tooth morphogenesis and differentiation.

Fig. 1 summarise the basic players involved in tooth development (genes, growth factors, receptors, and transcription factors) in humans and mouse.

2.1. *BMP*

One of the first signals identified in inductive interactions between the epithelium and the mesenchyme are growth factors belonging to the family of the BMP (bone morphogenetic proteins).[3] These proteins are very common throughout the animal kingdom and seem to be used several times during tooth morphogenesis (BMP2, BMP4 and BMP7 are expressed early in the dental epithelium, BMP2 and BMP7 during the bud stage, and BMP4 during the thickening of dental lamina) and they are apparently able to act like bidirectional signalling factors between the epithelium and the mesenchyme. In fact, the expression of BMP4 that starts initially in the epithelium, switches to the mesenchyme when inductive possibilities are acquired from the latter, suggesting how this molecule is able to induce even its own expression in mesenchymal cells.

The BMP proteins stimulate the expression, at the mesenchymal layer, of the transcription factors MSX1, MSX2,[12] the EGR1 (early growth-response), and the HMG domain of the LEF-1 transcription factor.

MSX1 is largely expressed in the mesenchyme during every step of morphogenesis; MSX2 is initially expressed only in the mesenchyme beneath the future area of the dental lamina, then, in the mesenchyme of the dental papillae and in the enamel knot epithelial cells.[13]

The intense mesenchymal expression of BMP4 during the bud phase could be linked to the subsequent transfer of the inductive ability to the epithelium, leading to the enamel knot formation.

2.2. FGF

In mammals, the FGF family is composed of 19 growth factors (FGF 1–19) which regulate gene expression in the mesenchyme and stimulate the epithelial cellular division and proliferation during the early phases of morphogenesis, in the early epithelial invagination that will later generate the bud, and during the assessment of the epithelial folds that will generate the dental cuspids.[10]

The biological effects of FGF are regulated by their high affinity for the TRK receptor (located on both cellular populations that will generate the bud), and by the proteoglycan heparan-sulphate on the cellular surface used as co-receptors. The FGF, are also able to activate the mesenchymal cells, stimulating the production of the heparan-sulphate Syndecan-1 that further modulates the signals sent by the growth factor.

The first fibroblast growth factor discovered was FGF3, which is expressed together with FGF10 in the dental papillae.[14] Their TRK receptors (FGFR1b and FGFR2b) are located in the dental epithelium. Their inhibition is associated with the progression of morphogenesis, particularly with odontoblasts maturation, and the termination of the proliferation of epithelial cells and their subsequent differentiation into ameloblasts (Fig. 2).

FGF4, FGF8 and FGF9, instead, are exclusively expressed in the dental epithelium: FGF8 is involved during early morphogenesis as an early epithelial signal; FGF4 is expressed from the cells of the primary and secondary enamel knot, regulating cuspid development; FGF9 is expressed from the epithelium during the bell stage and is associated with the differentiation of odontoblasts and ameloblasts. During the first steps of morphogenesis, the epithelial FGFs stimulate the mesenchymal expression of the MSX1, PAX9, Activin βA e RUNX2 (Cbfa1) genes. Differently from the BMPs that stimulate the expression of both MSX genes, the FGFs are not able to induce any mesenchymal activation of the MSX2 gene.

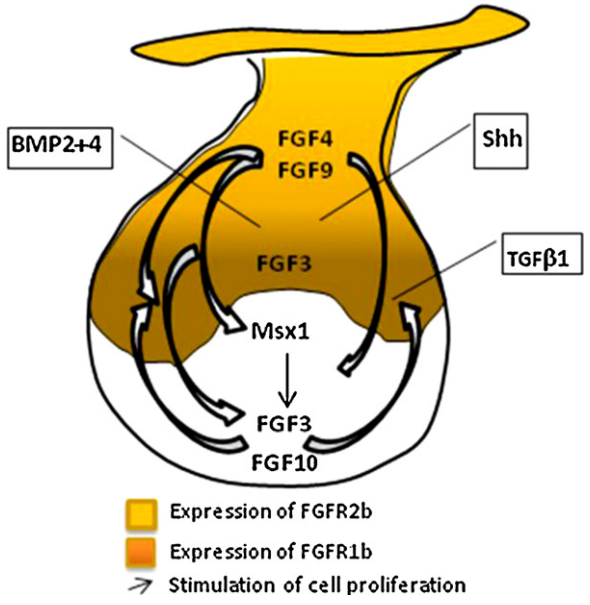

Fig. 2 – Scheme of the possible functions of FGF during the cup stage of dental development.[14]

The FGFs are able to act even as autocrine signalling systems on epithelial cells, and FGF 4–8 are able to induce FGF 3 production in the mesenchyme.

2.3. SHH (Sonic Hedgehog)

SHH are additional signals produced by the dental epithelial cells during the first phases of the thickening of the dental lamina in the enamel knot, and by the ameloblast progenitor cells.[15,16] Their receptor (Patch) is a multipass protein largely expressed in the dental mesenchyme, totally absent in the epithelium. This highly localised presence suggests that they are involved in the regulation of the dental bud assessment. Moreover, its expression in the epithelium during the first phases of morphogenesis seems to follow the expression of FGF8 and FGF9, suggesting the possibility of their role in the regulation of the SHH signal. Dassule[16] showed that SHH signalling is essential for growth and morphogenesis, but not for the differentiation of the mammalian tooth.

2.4. WNT (Wingless)

Another group of molecules involved in the regulation of tooth development is WNT.[17] WNT10 protein binds to the ZF2 (frizzled) on the cell surface, starting an intracellular signalling cascade that involves the β-catenin nuclear proteins and the LEF1 transcription factor.

When the cell receives the WNT signal, β-catenin is stabilised and bonded to the FGF transcription factors that further regulate the expression of the WNT target genes. If the WNT signal is missing, the β-catenin is phosphorylated and then degraded by a protein complex made up of APC (produced from the adenomatous polyposis coli gene), and AXIN1 or its homologous. AXIN2 expression induced by WNT signalling produces a protein that works with a negative feedback mechanism on the signalling mechanism itself.[18,19]

β-Catenin plays two roles in the cells, interacting directly with the LEF1 transcription factor in the cytosol, managing its transport process into the nucleus, and interacting with the E-cadherin protein in the cell adhesion process. Chen have shown that β-catenin function is also required in the developing tooth mesenchyme for the induction of the primary enamel knot formation.[19]

2.5. Epiprofin/Sp6

Epiprofin/Sp6 (Epfn) is a zinc-finger transcription factor (belonging to the specificity protein – Sp – subfamily) of the Kruppel-like factors (KLF) family, expressed in dental tissues but also in other ectodermal appendages.[20] Epfn appears to be strongly involved in the reciprocal inductive BMP and WNT signalling pathway.[21] Epfn shows high homology with the Sp6 gene, and it has been reported that Epfn and Sp6 are different transcripts, perhaps alternatively generated by different promoters from the same gene. In any case, they code for the same protein.[22] At the moment, nine members (Sp1–Sp9) of the Sp subgroup have been described in mammals, and their function is either to induce or to repress the expression of the target genes.[23] The Sp3 protein is expressed in odontoblasts and ameloblasts in the highest amount during the production

phase of the enamel-matrix proteins (amelogenin and ameloblastin).[24] Therefore ameloblasts from Sp3-knockout mice do not express these enamel-matrix proteins, resulting in the disruption of the enamel-dentine layer.[25] Dental mesenchymal cells express Sp4, but in Sp4 mice deficient, tooth development appears to be normal.[26]

3. Teeth number anomalies

The teeth number anomalies are pathologies characterised by a different number of teeth, arising from alterations either of the tooth lamina or the dental germ. These anomalies can exist in deciduous and/or permanent dentition, and can be either in excess or in deficiency.

The numerical decrees can be found in either deciduous or permanent dentition, and is referred to as anodontia when the teeth are totally absent in the arches, agenodontia and ablastodontia when the missing teeth are, respectively, in deciduous dentition or permanent dentition. Anodontia is rare; relatively more frequent is hypodontia when one or more teeth are missing, or oligodontia when it involves more than a half of the total dentition.

The prevalence of agenesis (congenital absence of a tooth) of one or more teeth of the same dentition (permanent or deciduous) is between 1.6 and 9.6%. The prevalence is between 0.4 and 0.9% in the European population, and 2.3 and 10% in the world population. Agenesis in the deciduous dentition is definitely rarer (0.08–1.55%).[27–33]

A meta-analysis of the literature shows the prevalence of agenesis by country and by gender (males/females). The prevalence seems to be higher in Europe (males 4.6%, females 6.3%) and in Australia (males 5.5%, females 7.6%) than in the North American Caucasian population (males 3.2%, females 4.6%). Moreover, the prevalence in females is about 1.37 times that in males.[27]

The most agenetic teeth are the third molars (9–30% of the population), followed by the second lower premolars (3–4%), the lateral upper incisors (2.2%) and the second upper premolars.

The agenesis of the first and second upper and lower molars, central lower incisors and canines is quite rare. Unilateral agenesis is more frequent than bilateral agenesis, except for the lateral upper incisors, which are more frequently agenetic in bilateral form. The left and right seem to have an equal distribution, except for the lateral upper incisors amongst which the most common agenetic ones seem to be the right incisors.[27–33]

The excess of teeth number (hyperdontia) is rare in deciduous dentures (0.3–0.8%), with an equal distribution between genders and arches. However, it is more frequent in the permanent dentition (0.1–3.8%), and, most frequently, these cases are represented by a conoid tooth localised between the upper incisors (mesiodens). In permanent dentition, the supernumerary teeth are more frequently found in the upper maxillae in the anterior area (60%) and the tuber, than the lower jaw where the most common area is the premolars. They are more common in males, with a rate equal to double of that in females.[27,34,35]

Most of the patients with these anomalies have just one supernumerary tooth (76–86%), rarely two teeth (12–23%), and multiples of supernumerary teeth are just 1% of the total, and are generally associated with other morphogenetic disorders.[35]

3.1. *Etiopathogenesis of tooth agenesis*

In recent years many theories have been developed to explain agenesis. These can be divided into two groups: evolutional and anatomical theories.

Evolutional theories explain tooth agenesis by the antero-posterior shrinking of the maxillamandibular complex and the following adaptive reduction of the number of teeth, because of the smaller arches, but also by the reduced functional chewing by eating mainly soft and processed foods.[30,31,33,36,37]

Clayton,[38] observing that the most commonly missing teeth were the last of each ''class'' (incisors, premolars, and molars), hypothesised that those elements were just vestigial bodies that became obsolete during the evolution process, because it does not give any advantage to the species.

The most supported theories are those of Svinhufvud and di Kajaer.

Svinhufvud et al.[39] hypothesised the existence of dental lamina areas more sensitive to epigenetic influences (''Fragile lamina sites'') during the maturation of teeth and than even to anomalies such as missing teeth. Some examples related to this hypothesis are: (1) the agenesis of the upper lateral incisors, which develops in the area of the fusion between the lateral maxillae process and the medial nasal bone process; (2) the second lower premolar that originates in another fragile area of the dental laminae; and (3) the central lower incisors that arises from the initial fusion area of the jaw.

Kjaer[40] instead affirmed that agenesis could be explained by referring to the development areas of the neural maxillae (incisors area, canine/premolar area, and molar area), and states that the most sensitive area is the one where the innervation develops the last.

Several studies have demonstrated that dental development is under strict genetic control of position, number dimension and the shape of the tooth.

Grahen[41] in 1956 was the first to consider agenesis as a hereditary anomaly whose transmission is determined by a dominant autosome, with incomplete penetrance and variable expressivity; this is currently the most agreed upon definition.

Studies by Grahen have shown that the penetrance (defined as the percentage of individuals with a particular gene combination showing the respective characteristic at a particular degree) is 86%, whereas the variable expressivity (the degree of phenotypic expression in an individual) means that the inheritor teeth, when they are not agenetic, can be shown to be modified in shape and or size.

Recently, advances in genetic research have made possible the identification and sequencing of the genes involved in tooth morphogenesis, and the investigation of the molecular networks that control the different steps of this process and also the molecular mechanisms leading to agenesis.

3.1.1. *Genetics of non-syndromic tooth agenesis*

Non syndromic congenital absence of teeth can be sporadic or familial. Genetic transmission can be X-linked, recessive or dominant.

There are several genes implicated in tooth agenesis,[42,43] but mutations occurring in MSX1, PAX9, AXIN2, and EDA are shown to be involved in non-syndromic human tooth agenesis.

3.1.1.1. *MSX1*. The MSX1 gene is co-expressed in the mesenchyme with the PAX9; both genes code for transcription factors that play an important role in maintaining the expression of the BMP4 in the mesenchyme. The correct interaction between these three factors is essential to obtain the progression of morphogenesis from the bud stage to the cap stage[11] (Fig. 3).

PAX9 is able to regulate directly the transcription of the MSX1 gene; moreover, it interacts with MSX1 at a protein level enhancing the skill of the PAX9 to transactivate the expression of MSX1 and BMP4 during tooth development.[43–45]

MSX1 and the related gene MSX2 are homeobox genes, i.e. they are genes encoding transcription factors that can control the expression of other genes. These genes code for a sixty amino acid protein (homeodomain) able to bind specific DNA sequences. The Msx1 protein inhibits transcription of the target genes through its interaction with other transcription factors, such as the DLX (DLX2 and DLX5), LHX2, PAX3 and PAX9, and also with other complex transcription components in the nucleus, including the ''TATA box'' binding proteins (TBP, the DNA sequence located at the 5′ end of the gene about 25–30 bp to the starting transcription site that is located more precisely just by this sequence), the Sp1 site or other cAMP binding proteins (CBP/p300).[46–48]

Homozygous MSX1-deficient mice develop a secondary cleft palate, either mandibular and maxillary alveolar processes deficit, lack of development of incisors and interruption of molar development at the bud stage.[47,49]

In most hypodontia cases, a dominant autosomal[50–58] transmission seems to prevail. Moreover, Chishti[59,60] found a recessive autosomal transmitted oligondontia in two distantly related Pakistani families.

The first MSX1 gene mutation associated with human agenesis was described by Vastardis et al. in 1996.[50] Analyzing a large family with a severe form of oligodontia autosomal dominating transmission involving II premolar and III molars, the authors revealed the presence of a mutation located on the 4p chromosome on the MSX1 locus. This missense mutation (substitution of a single DNA base that changes the coded amino acid) located on the homeobox, determines the substitution of an arginine with a proline on amino acid 196 of the homeodomain (Arg196Pro); the mutant protein has a lower thermal stability than the natural one.

In 1998, Hu et al.[53] made a biochemical and functional analysis of the mutant MSX1 protein (R31P) to determine if the agenetic phenotype as a result of this mutation was the direct consequence of a negative dominance mechanism or a haploinsufficiency. The experiments showed that the mutant protein has less or no ability to interact with the DNA or with other protein factors, and it loses its inhibitory function over transcription because of the introduction of a proline into the second alpha-helix of the homeodomain, which is normally responsible for protein stability. Because of this mutation, the protein is not active ''in vivo'' and does not antagonise the normal activity of MSX1, the researchers deduced that the phenotype of the subjects having this specific agenesis is due to the gene haploinsufficiency, which needs the expression of both alleles to work correctly.

Kim et al.[55] in 2006 identified a frameshift mutation with duplication of the G nucleotide in position 62 (g.62dupG) of the MSX gene exon 1. This modification has been identified in a household of five members with oligodontia. Every patient missed several permanent teeth, including all the second premolars and lower incisors. Even in this case, the mutated protein is probably inactive, and interferes with the normal allele function.[53]

Mostowska,[56] studying a family of a patient with 14 agenetic permanent teeth (II and III molars, premolars and central lower incisors), found a new mutation in c.581C>T, causing a substitution at the 194 homeodomain position of an alanine with a valine. Moreover, DNA sequencing revealed a heterozygotic mutation in the proband and in his parents. The existence of this mutation even in the parents means that, in this case, the agenesis is the result of an incomplete penetrance mutation.

Recently, Pawlowska et al.[58] suggested that two polymorphisms in two untranslated regions (intron) of MSX1 (rs8670 e rs12532), could be involved in humans in sporadic and familial tooth agenesis. This evidence could support the hypothesis that regions other than the DNA binding domain, could also be

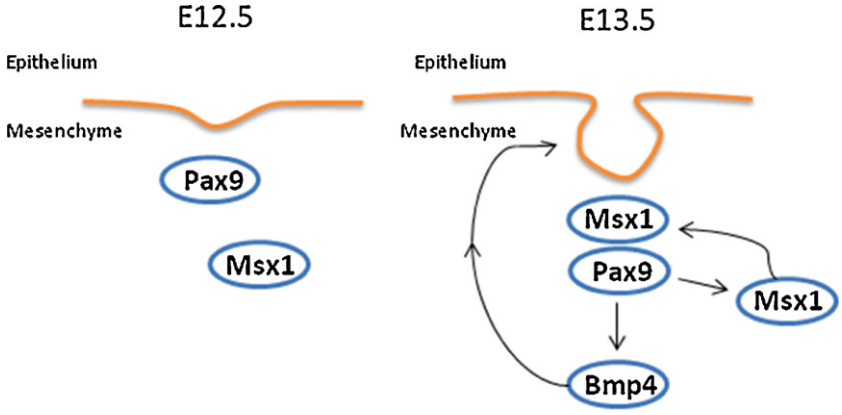

Fig. 3 – Scheme of the interaction of PAX9, MSX1 and BMP4 during the initial phase of dental morphogenesis.[3]

Table 1 – MSX1 gene mutations.

Mutation	Type	Phenotype[a]	Ref.
R196P	Nonsense	FTA	Vastardis et al.[50]
S105X	Nonsense	FTA + cleft lip and palate	van den Boogaard et al.[51]
S202X	Nonsense	Oligodontia; nail dysplasia Witkop Syndrome	Jumlongras et al.[46]
M61K	Nonsense	FTA	Lidral and Reising[54]
G187X	Nonsense	FTA	De Muynck et al.[52]
G22RfsX168	Frameshift	FTA	Kim et al.[55]
A194V	Nonsense	FTA	Mostowska et al.[56]
A219T	Nonsense	FTA	Chishti et al.[59]

[a] FTA, familial tooth agenesis.

related to tooth development. Table 1 summarises MSX1 gene known mutations (Table 1).

3.1.1.2. *PAX9*. In addition to MSX1, the PAX9 gene is responsible for the appearance of non-syndromic agenesis.

The PAX9 gene is located on chromosome 14 (14q12–q13), and is one of the genes of the transcription factors family genes, which are essential during the first years of development in several multicellular organisms. The proteins produced from the PAX genes (nine in mammals, PAX1–PAX9) constitute a homeodomain (paired domain) made of 128 amino acids able to bind particular DNA sequences and an additional homeodomain, functionally separated and selectively activated by PAX genes that transactivate the first domain.[43–45,60]

In mouse embryos, the PAX9 gene is expressed in the mandibular arch mesenchyme, before any sign of dental morphogenesis; high gene expression levels are maintained until the late bell stage, when PAX9 production gets inhibited. Mice with homozygotic deletion of the PAX9 gene (PAX9−/−), die a few months after birth because of breathing difficulties. They lack all their teeth, with arrest of morphogenesis at the bud stage and several development problems, including secondary cleft palate, skull and cartilage anomalies. Instead, heterozygous mice for the deletion (PAX9+/−) develop normally.[61]

Recently Nakatomi et al.[61] provided evidence for the interaction between PAX9 and MSX1 in craniofacial and lower incisor development. They showed that the same tooth types are affected in mice with concomitant heterozygosis of PAX9 and MSX1, and they also discovered an unknown function of the genes at different stages of odontogenesis.

Today it is well accepted that many different mutations of the PAX gene are able to cause agenesis in affected individuals. (Table 2).

The PAX9 mutations, either missense or stop, must be heterozygous and show an autosomal dominant transmission; the resulting phenotype would result from either the haplo-insufficiency, dominant negative activity or a different action of the new protein.[62–75]

Severe hypodontia is probably the result of gene haplo-insufficiency, as Das has hypothesised.[6] He studied a small family in which a father and his daughter were affected by a severe hypodontia presenting with the agenesis of all molars, both deciduous and permanent, resulting from the deletion of the entire PAX9 gene in one of the two 14th chromosomes.

Nevertheless, haplo-insufficiency is not enough to totally explain the mechanisms working in other mutations responsible for some missing molars.[30,63–70,76] These studies report the hypothesis that the allele involved is hypomorphic, i.e. an allele presenting a reduced but not totally missed function;[76] thus, the combined activity of the wild-type and mutated genes will not reach the threshold to develop the teeth in a normal fashion. Alternatively, less serious phenotypes would be the consequence of the allele mutation that further generates a mutated protein, which acts in a negative dominant way or develops a new function.

The first mutation in PAX9 was reported by Stockton et al. in 2000,[62] in a 43-member family, in which it was possible to reconstruct the family tree since 1645. Twenty-one of the family members presenting missing molars in all the four

Table 2 – Known gene mutations in PAX9.

Mutation	Nucleotide variation	Mutated amino acid	Phenotype	Ref.
Frameshift	218-219insG	G73fsX243	Oligodontia	Stockton et al.[62]
Delation	Heterozygous	Deletion	Hypodontia	Das et al. (2001)
Nonsense	A340T	Lys114stop	Oligodontia	Nieminen et al.[63]
Frameshift	792_793insC	V265fsX315	Oligodontia	Frazier-Bowers et al.[65]
Missense	A217G	Lys91glu	Hypodontia	Das et al.[66]
Missense	G151A	Gly51Ser	Oligodontia	Mostowska et al.[60,68]
Missense	T62C	Leu21Pro	Hypodontia	Das et al.[66]
Frameshift	175_183del/ins288bp	R59fsX177	Oligodontia	Das et al.[66]
Missense	C76T	Arg26Tyr	Oligodontia	Lammi et al.[67]
Missense	G83C	Arg28Pro	Oligodontia	Jumlongras et al. (2004)
Transition	A1G	M1V	Oligodontia	Klein et al. (2005)
Missense	A259T	Lys91Glu	Oligodontia	Kapadia et al.[70]
Nonsense	C175T	Arg59stop	Oligodontia	Tallon-Wolton[71]
Missense	C139T	Arg47Tyr	Oligodontia	Zhao et al.[72]
Missense	G6R	Gly6Arg	Hypodontia	Wang et al.[73]
	S43K	Ser43Lys	Oligodontia	
Frameshift	321_322insG	mRNA instability	Oligodontia	Suda et al.[75]

quadrants and some of those presented even agenesis of the second premolars (mostly upper) and central lower incisors.

Nieminen et al.[63] identified a Finnish family affected by a severe form of autosomal dominant agenesis. This mutation was phenotypically characterised by all the II and III molars being missing, partial missing of the first molar and the second premolar, and size reduction of some teeth.

In 2003, Das et al.[66] found three other mutations on the PAX9 gene associated with molar agenesis. Patients affected by mutation reported by Lammi et al.[67] have a slightly different phenotype than the previous one, resulting in missing premolars, canines and incisors and smaller teeth. Mostowska et al.[68] analysed 25 either familiarly transmitted or non-familiarly transmitted agenesis patients, for the MSX1 and PAX9 genes mutations. The author has found a novel mutation in the paired box sequence of PAX9 gene in a patient with a sporadic form of agenesis concerning III molars, II premolars and lateral upper incisors and one lower incisor.

Jumlongrans et al.[69] reported a missense mutation causing oligodontia in permanent teeth, especially molars. Kapadia et al.[70] described a heterozygous mutation resulting in posterior tooth agenesis. In 2007 Tallon-Wolton[71] described the analysis of a three-generation Spanish family affected by oligodontia and other dental anomalies and systemic disease including hypercholesterolemia, hypothyroidism, diabetes mellitus, scoliosis and congenital cardiovascular anomalies. The patients affected by agenesis were missing molars and premolars associated with dental anomalies such as upper canine inclusion, root anomalies and microdonties.

Recently, Wang et al.[73,74] reported two different missense mutations in a Chinese population with varying degrees of non-syndromic tooth agenesis. Phenotypes resulting from the two mutations were very different in terms of severity: excluding third molars, the individual with the familial S43K mutation, may be missing most molars, maxillary premolars and mandibular canines. The patients affected by sporadic tooth agenesis (G6R mutation), were missing only mandibular central incisors and maxillary second premolars. A novel mutation of PAX9 was identified (2011) by Suda et al.[75] in a Japanese family with non-syndromic oligodontia, particularly in the molars.

3.1.1.3. AXIN2. Recently Lammi[30,77] suggested that oligodontia could be determined by AXIN2 gene mutations (axis inhibition protein 2), also detected in colon and liver cancer. The AXIN2 gene is located on chromosome 17 (17q21–q25); it produces a protein that suppresses the WNT pathway suppressive protein promoting the beta-catenin degeneration. The authors observed a four-generation Finnish family affected by autosomal dominant oligodontia associated with colorectal cancer. Most of the family members were missing most or all molars, premolars, lower and upper lateral incisors, and in three cases, even canines. Moreover, six oligodontic members were also affected by colorectal cancer that, instead, was absent in the non-oligodontic members.

The AXIN2 coding region direct sequencing showed a substitution C>T on the 1966 position of exon 7 (1966C>T) resulting the switch of Arg with a stop sequence on the 656 amino acid causing the premature interruption of the protein. This nonsense mutation is detected just in oligodontia-affected family members, but not in the normal ones (checked in over 100 control cases).

The AXIN2 mutation screening in other agenesis-affected patients allowed the identification of the case of a 13-year-old patient with a phenotype similar to that of the family members presenting the insertion of a heterozygote 1-bp (G) after nucleotide 1994 in exon 7. This mutation, which was absent in the parent genes, appeared de novo in the germinal cells of one of the parents.

Because of the limited knowledge regarding the interactions between AXIN2 gene and the appearance of agenesis, Mostowska et al.[78] studied the link between the AXIN2 gene variations and patients presenting selective kind of agenesis, hypodontia and oligodontia resulting from mutations in the PAX9 and MSX1 genes.

The analysis of exons and the borderline regions between exons and intron sequences in patients affected by agenesis revealed six already known polymorphisms (c.148C>T, c.432T>C, c.1365A>G, c.1386C>T, c.1712+19G>T and c.2141+73G>A), and three new genetic variations. Two of these were located in intron regions (c.1060-17C>T) and one was located on exon 7 (c.2062C>T presenting a Leu688Leu substitution). Patients who are carriers of the c.956+16G and c.2062T allele variations are likely to develop agenesis. Moreover, the c.2062C>T transition represents an agenesis-favouring condition due to the alteration of the exonic splicing enhancer (ESE), an indispensable splicing site matching DNA sequence, which affects negatively the cellular concentration of AXIN2.

These results seem to confirm the important role played by WNT in growing tooth.

3.1.1.4. EDA. More recently, other genes were involved in isolated tooth agenesis.

Mutations in EDA gene cause X-linked HED which signs are sparse hair, reduction in the number and size of tooth and cuspid and lack of sweat glands.

The EDA gene[79] occupies a segment of the long arm of the X-chromosome (Xq12-13.1); it encodes a protein belonging to the tumour necrosis factor (TNF) family of ligands. Eight isoforms of EDA transcript are known but only two of them contain the receptor-binding TNF homology domain: EDA1 which binds the EDA ligand EDAR and EDA2 that binds only a receptor called XEDAR. By contrast, from the EDAR receptor and its intracellular transmission signal EDARADD, expressed in enamel knot, ectodysplasin-A (EDA), a type II membrane protein, is expressed from the cells of the external tooth epithelium.

In mouse are found two spontaneous mutations, tabby and downless, leading to typical signs and symptoms of HED. The tabby gene is analogous to ED1 in humans, and it is expressed in the surrounding epithelium; the downless gene is analogous to the human EDAR, being expressed in the enamel knot. The tabby and downless phenotypes appear identical.

Edaradd is mutated in the crinkled mouse mutant, which has an identical phenotype to tabby and downless.[80]

Recently, several studies have reported sporadic hypodontia in families affected by mutations in EDA and EDA receptor (EDAR) gene.[81–87] The probands affected by these mutations, largely male, show only variable degrees of tooth lack, without

the systemic symptoms of HED. The most commonly missing teeth are molar and premolar.

In 2010, Muhammad et al.[88] investigated a Pakistani family demonstrating an X-linked recessive sporadic hypodontia. DNA sequencing in the three affected members detected a novel missense mutation (c.993G>C; p.Q331H) not found in healthy men of the family and not representing a nonpathogenic polymorphism.

In 2011 Bergendal[89] investigated 93 Swedish probands with non-syndromic isolated oligodontia (lack of six or more permanent teeth) for the presence of mutations in MSX1, PAX9, AXIN2, EDA and EDAR genes and the novel candidate gene EDARADD (EDAR-associated death domain), the downstream signalling mediator. One male proband was affected by a novel heterozygous missense mutation in EDARADD (c.308C>T; p.Ser103Phe) resulting in six missed teeth (three molar and three premolar) without any ectodermal symptoms. This report adds a new member in the EDA signalling pathway, EDARADD, which mutation is associated with isolated oligodontia.

3.1.1.5. *Deficit He-Zhao*. Another oligodontia phenotype known as the He-Zhao deficit,[90] was largely found in a Chinese family kinship living in a small village of Shaanxi (Northwestern China). This is an autosomal dominant with incomplete penetrance and high variability anomaly found in 52 out of 328 family members of six different generations.[91] The deciduous dentition was totally conserved but the permanent one presented several different anomalies, ranging from the loss of a few teeth to anodontia. The only conserved teeth were the first and second molars and the upper central incisors.

The genetic locus responsible for the He-Zhao deficit is located on chromosome 10 (10q11.2).[90] Many different genes located in this area are considered capable of generating this anomaly: Dkk-1 is a member of a gene family coding for the WNT signalling system antagonist protein; PRKG1B codes for the cGMP-dependent type 1 kinase; KOX zinc finger genes group, including ZNF11, ZNF22 and the homologous KROX-26[92] and ZNF 25.[29,90]

3.1.1.6. *16q12.1 Chromosome (autosomal recessive hypodontia)*. This is an autosomal recessive type of hypodontia associated with several tooth anomalies e.g. malformations, enamel hypoplasia and missed eruption. It was found in a Pakistani kinship from Sind, having several consanguineous marriages.

The responsible gene locus, homozygotically expressed in all the affected relatives, is located on the 16q12.1 chromosome. It is not clear which gene in this site is responsible for the anomaly.[93]

3.2. *The etiopathogenesis of exceeding number anomalies*

The correct aetiology of supernumerary teeth is still not clear, even though many theories have been suggested.[94,95] Smith in 1969[96] suggested the atavism theory, which affirms that supernumerary teeth represent a return to the roots (3 incisors, 1 canine, 4 premolars and 3 molars per arch). By contrast, Anglesio[97] emphasises that supernumerary teeth are rarely found in both dentitions which endorse the casual appearance theory. Primosh[98] considered that the ectopic and isolated appearance cannot match the atavism theory. Taylor[99] indicated that the bud can split itself into two identical or different parts creating two similar teeth ores, one normal and one dysmorphic.

Black and other authors[95] hypothesised a limited and independent proliferation of the dental lamina section triggered genetically, traumatically, or by other means, thereby stimulating the creation of a supernumerary tooth. During the 4–5 years of its activity, the dental lamina divides itself into three sections that are able to work for a limited period before their disintegration: the primitive dental lamina (vestibular lamina) generates all the deciduous teeth; the secondary dental lamina is generated from the oral portion of the primitive one generating the diaphysial teeth; and the accessory dental lamina, a distal extension of the secondary dental lamina which generates the permanent molars. The hyperodontia area is mostly represented by the final portion of each lamina, as the supernumerary distribution appear. The Rose theory[35] affirmed that the dental lamina continued to proliferate because of the failure of the dental lamina to be reabsorbed, producing new, normally shaped buds.

Another important theory is the one that affirms that during the invagination and proliferation of the outer layer of the enamel organ epithelium responsible for the permanent tooth bud adjustment, several more epithelium buttons originate from the distal portions of the lamina to generate all the supernumerary teeth.

The inheritance theory is also considered as another valuable theory. Işil Orhan[100] cites many literature cases reporting supernumerary teeth in relatives. Umweni and Osunbor,[101] analyzing 13 Nigerian patients affected by a non-syndromic hyperdontia, found two pairs of siblings (male–male and male–female) presenting the same hyperdontia, deriving the same autosomal dominant disease. Desai and Shah[102] reported a supernumerary case in two brothers. Marya and Kumar[103] described two mesiodens cases in brothers and a supernumerary case involving the premolar area in a patient already treated for a mesiodens, and having a cousin presenting a mesiodens. Mercuri and O'Neill[104] described a supernumerary case localised in the premolar area in two brothers, the father and the grandfather.

Batra[105] have described recently the case of a 17-year-old girl having eight lower supernumerary teeth (2 in the incisor region and 3 in the right and left premolar regions). The patient's brother had eight lower supernumerary teeth with a different distribution (4 incisors and 4 premolars), and the father presented 4 supernumerary premolars (3 maxillary and 1 mandibular).

Inchingolo[106] described a rare case of non-syndromic hyperdontia. The proband presented an impacted supernumerary tooth (4.9). Supernumerary teeth were also detected in the proband's two sisters. The elder sister presented 1.9, 2.9, 2.10, 3.9, 4.9 supernumerary teeth, and the youngest had 1.9 and 2.9. The proband's mother also had supernumerary teeth in the posterior portion of the superior maxillary bone. The presence of hyperdontia in the mother and her three children led the authors to suggest an autosomal dominant

transmission of the character, with an evident penetrance of the phenotype in the evaluated family.

Many investigators think that it is critical to understand the type of events influence the appearance of supernumerary teeth. Sedano and Gorlin[107] affirmed that the autosomal dominant transmission without penetrance could be an explanation in some of the cases. Bruning et al.[108] suggested the possibility of a gender-related transmission to explain the male prevalence. Cassia et al.[109] suggested the possibility of autosomal recessive transmission in a large Lebanese family having consanguineous marriages, which probably caused the appearance of five supernumerary lower incisors. Cadenat[85] suggested the possibility of an autosomal recessive gene and an inhibitory gene located on the X chromosome. Brook[95] suggested a combined action of genetic and environmental factors. Niswandert[95] provided an autosomal recessive lower penetrance gene in a female, related to supernumerary teeth. To further support the genetic hypothesis, Łangowska-Adamczyk and Karmańska[110] described the case of hyperdontia that affected in an identical manner two monozygous twins and their mother.

Despite all the information coming from the studies that the literature provides us, the genetic and/or molecular aetiology of these anomalies is still not clear, as we cannot identify any gene mutation responsible for the appearance of these non-syndromic supernumerary teeth. Molecular studies in mice mutated to have deficient signalling molecules seem to identify hyperdontic mutations.[94,110,111]

In normal mouse dentition, there are fewer teeth than that in humans: each quadrant has 3 molars and 1 incisor separated by a diastema. The diastema is the result of the bud missing during morphogenesis, because in the mandibular diastema there are two buds; in the maxillary diastema, however, seven buds are generated. The development of these buds stop at the cup step, and their resorption is the result of apoptosis, but it is still not known if the apoptosis arises primarily or secondarily to the cup transition.

Murashima-Suginami et al.,[112,113] studied the growth of molars and incisor supernumerary teeth and fusion between molars and supernumerary teeth in mice with USAG-1 (Uterine Sensitisation Associated Gene-1) gene deficiency. The USAG1 gene and its human analogue Ectodin/wise are BMP protein antagonists. They regulate BMP activity by binding to BMP and neutralising its activity. They also regulate WNT signalling by activating or inhibiting it, depending on the situation.

The gene expression area is the mesenchyme and epithelium of the rudimentary maxillary incisor tooth organ formation, in the region of the incisors and diastema and it regulate apoptosis signal. In fact USAG1 abrogation saves the odontogenic mesenchymal cells from apoptosis.

Epiprofin (Epfn) is also involved in the reciprocal inductive BMP and WNT signalling pathway. Recentely, Nakamura et al.[114] has reported that Epfn-knockout mice show a typical phenotype resulting in a severe hyperdontia, especially concerning incisors and molar teeth. Tooth morphogenesis is strongly affected, resulting in extra teeth lacking enamel (hypoplasia) and lack of ameloblasts because of a failure in ameloblasts differentiation.

Supernumerary teeth in the diastema area are found in mutant mice which (1) lack the genes for SPRY2 and SPRY4 (FGF antagonists), (2) have a gene mutation in the POLARYS gene that modulates the SHH signalling pathway, and (3) overexpress the ectodysplasin (EDA) gene.[115]

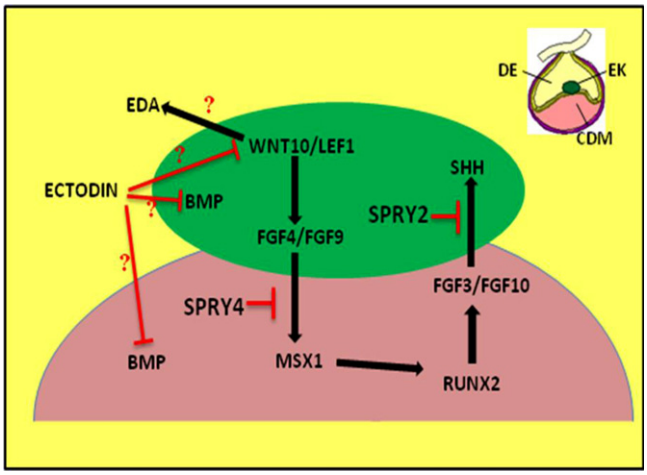

Fig. 4 – Scheme of some critical signalling interactions between enamel knot (EK) in the dental epithelium (DE) and the condensing dental mesenchyme (CDM) in cap stage molar tooth germ. Perturbation of signalling pathways can lead to supernumerary teeth.[111]

Sprouty (SPRY) is an intracellular working gene family that operates a negative feedback on FGF and some receptors such as tyrosine-kinase (TKR) receptor, causing several different biochemical responses. The mice buds begin their first growth in the diastema region when the genetic mechanisms controlling the bud development in the cup phase are not yet operative. One of these genetic mechanisms is the inhibition of the FGF gene, including FGF4 in the enamel knot and FGF3 in the mesenchyme. The Sprouty gene is necessary to avoid dental growth in the diastema. The usual function of SPRY2 is probably to prevent too low levels of FGF10 produced in the bud mesenchyme. Another usual SPRY4 function is to avoid any FGF epithelium signalling, including FGF4 and FGF9 produced in the molar bud and stimulated by the continuous expression of FGF3.

The combined action of SPRY2 and SPRY4 determinates the regression of the bud diastema, avoiding the appearance of diastema teeth in adulthood (Fig. 4).[94,111]

Ohazama[116] pointed out that up-regulation of SHH signalling activity in the mouse diastemal mesenchyme can lead to ectopic tooth formation, as in mice carrying an allele of the IFT88/Polaris ciliary protein. Particularly interesting is the RUNX2 gene, which is one of the mesenchyme targets in the FGF signalling pathway, and is even responsible for cleidocranial dysplasia (CCD).[94,117] In CCD patients with RUNX2 haplo-insufficiency, the supernumerary teeth coming from the permanent teeth can create an actual third dentition; the molecular mechanism proposed to explain this event is the incomplete resorption of the dental lamina.

In RUNX2 (−/−) homozygous mutated mice, molar development is stopped at the cup stage, a morphogenetic phase when RUNX2 is largely expressed in the mesenchyme, whereas in heterozygous mice at this stage a series of events

lead to the formation of supernumerary epithelium buds on the lingual side.

Studies on wild type RUNX2 (+/+), and RUNX2 (+/−) and RUNX2 (−/−) mice[118,119] showed that the lack of RUNX2 expression interferes with the normal morphogenesis of the enamel knot and prevents ectodermal proliferation, thereby interrupting the morphogenesis between the bud stage and the cup stage. An interesting report was the appearance of supernumerary buds on the upper, and in some cases lower, molar lingual side. This event suggests that the normal function of RUNX2 is to prevent supernumerary formation by regulating cellular proliferation or apoptosis. Moreover, it is interesting that the permanent dentitions in humans and mammals are generated by palatal/lingual extensions of the dental lamina.

These observations explain why supernumerary teeth are so frequent in RUNX2 haplo-insufficient humans, and the role of RUNX2 in preventing excessive bud assessment in both humans and mice.

The availability of patients for gene screening in non-syndromic supernumerary anomalies would be very helpful in finding specific mutations that generate these anomalies.

4. Conclusions

Anomalies of defective or excessive teeth number involve alterations in the dentition that can cause dental disharmony, further causing functional and aesthetic alterations. Today, over 300 genes are known to be involved in the development of normal dentition, and a large number of them are responsible, in several different ways, for the appearance of supernumerary teeth and agenesis.[3]

The agenesis of one or a few teeth is responsible for the deficit in the development of alveolar processes, causing diastemas, contiguous teeth migration or inclination, dental retention, antagonist extrusion, and mid-line deviation. These, in turn, can cause chewing loop alterations, muscle contractures and TMJ dysfunctions.[120]

Supernumerary teeth, particularly in the pre-maxilla area, frequently pre-dispose pathologies including tooth retention, late eruption, ectopic eruption, tip and torque alterations, rotation, cysts, and root resorption. These disorders are strictly determined by the space interference of supernumerary teeth on contiguous teeth during the stages of development.[34]

In these cases, an early diagnosis is clearly indispensable to prevent any worse consequences. This is the main reason why it is important to study in depth human anomalies tooth number and their genetic aetiology.

In this work we analysed previous studies to establish the most common genes and mechanisms responsible for anomalies in teeth number. The identification of the genes responsible can give much more information on teeth anomalies and on the biological processes altered by these mutations. The identification of the genetic events responsible for familial transmitted teeth anomalies would even help to improve understanding of the pathogenic events in non-familial, sporadic, transmitted anomalies.

We have to consider that to understand these pathologies totally, we cannot just use the monogenetic model. In fact, the development of anomalies is the result of genetic, epigenetic and environmental interactions.[11]

The identification of mutated genes will help to evaluate how other factors are involved in the phenotypic appearance of these anomalies. Even though mutation-associated non-syndromic teeth number anomalies are rare, gene function analysis would be helpful to understand the most common ones.[29,121,122]

Finally, the identification of the pathogenic mechanisms of non-syndromic teeth anomalies would also clarify the role of teeth in craniofacial development, and would represent an important contribution to the diagnosis, treatment and prognosis of congenital malformations, and the eventual association with other severe diseases. Future research in this area should lead to the development of tests for doctors to make an early diagnosis of these anomalies. The knowledge of genetic mechanisms of these diseases would, therefore, be indispensable for any doctor (paediatricians, dentists and so on) and dental hygienist.

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