

Review Article

The role of Msx1 and Pax9 in pathogenetic mechanisms of tooth agenesis

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ABSTRACT

Background: Tooth agenesis is one of the most common developmental anomalies in human, which one or a few teeth are absent because they have never formed, may cause cosmetic or occlusal harm, while severe agenesis which are relatively rare require clinical attention to support and maintain the dental function. Molecular studies have demonstrated that tooth development is under strict genetic control. **Purpose:** This article want to review the genetic regulating that are responsible for tooth agenesis especially the role of Msx1 and Pax9 in pathogenetic mechanisms of tooth agenesis. **Review:** Tooth agenesis is a consequence of a qualitatively or quantitatively impaired function of genetic networks, which regulate tooth development. Mutations in Msx1 and Pax9 genes are dominant for tooth agenesis in humans. The Pax9 gene, which codes for a paired domain-containing transcription factor that plays an essential role in the development of mammal dentition, has been associated with selective tooth agenesis in humans and mice. **Conclusion:** Reduced amount of functional Msx1 or Pax9 protein in the tooth forming cells is able to cause severe and selective tooth agenesis. There are differences in the frequency of agenesis of specific teeth associated with the defects in Msx1 and defects in Pax9.

Key words: tooth agenesis, genetic regulation, pathogenetic mechanisms, Msx1, Pax9

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INTRODUCTION

During the last decades after the advent of molecular biology and genetics, the new technologies have been extensively used to elucidate developmental mechanisms and the genetic regulation of tooth development. The positional cloning of several genes that cause different developmental dental anomalies, have contributed to understanding of the genetic regulation of developmental and patterning of the human dentition.¹

Agenesis of one or more teeth is one of the most common of human developmental anomalies. The term oligodontia refers to congenital absence of many but not all teeth whereas the term hypodontia implies the absence of only a few teeth. In the permanent dentition, hypodontia has a prevalence of 1.6% to 9.6%, excluding agenesis of the third molars. Oligodontia has a population prevalence of 0.3% in the permanent dentition. It occurs more frequently in girls at a ratio of 3:2. Agenesis of only the third molars

has prevalence between 9% and 37%. In the deciduous dentition, hypodontia occurs less often (0.1%-0.9%) and has no significant sex distribution.²

Both environmental and genetic factors can cause failure of tooth development. Numerous different genes have been implicated in tooth development by genes expression and experimental studies in the mouse, and any of these genes may cause tooth agenesis. Variability in expression includes the number and region of missing teeth, and various other dental features associated with the trait.^{3,4}

The present study confirmed the results of Garn and Lewis⁵ showing other physical dental traits associated with the occurrence of tooth agenesis. It contributes to mount the evidence that agenesis and its associated abnormalities are under genetic control. The possible explanation is that a single genetic defect may give rise to different anomalies, so that two or more dental anomalies in the same patient may present a common genetic origin. Studies of families, as well as investigations of the association of agenesis and

other types of dental anomalies, previously highlighted the role played by genetic mechanisms in the etiology of various dental anomalies.^{5,6}

Dental anomalies are ideal conditions for the geneticist to study the hereditary factors involved in their pathogenesis. This article reviewed the genetic regulating that are responsible for tooth agenesis especially the role of *Msx1* and *Pax9* in pathogenetic mechanisms of tooth agenesis.

Tooth morphogenesis

In the late bud stage, a group of cells at the tip of the epithelial bud, the primary enamel knot stop to proliferate and then removed by apoptosis. The enamel knot deviates significantly from the surrounding epithelium because of its gene expression. It expresses several transcription factors and numerous signalling molecules as well as signalling inhibitors with a specific schedule of appearance, thus having potential to act as a signalling centre that orchestrates the development of the surrounding tissues. The primary enamel knot is apparently induced and maintained by signals emanating from the underlying mesenchyme. On the other hand, formation of the primary knot seems to be a prerequisite for the advancing of the tooth development to the cap stage.⁷

The dental lamina in 6th week, and later the enamel organs, represent the epithelial portion of the oral cavity with potential capacity to generate the ectodermal components of the teeth. In subsequent development, the adjacent mesenchymal tissue will proliferates and condenses to form other components and portions of the future teeth. The permanent-teeth germs are developed later. They originate from the accessory dental lamina, in the case of molars, or from growth of the free edge of the dental lamina on its lingual side for the remaining permanent teeth. The emergence or eruption of permanent dentition takes place over an extended period ranging from 7 to 12 years, apart from the third molars, which erupt between 13 and 25 years, although sometimes they fail to appear at all. Most dental anomalies are more frequent in permanent than in deciduous dentition. With regard to permanent dentition, the lack of one or more teeth is evident in about 1–2 % of the population.⁸

Molecular regulation of tooth development

The technologies of molecular biology and genetics have been extensively used to elucidate developmental mechanisms and the genetic regulation of tooth development. The most usual model has been the mandibular molar teeth of the mouse, the most practical laboratory animal that develops teeth. Immunohistology and in situ hybridization have been used to study gene expression during mouse tooth development and differentiation. Natural and transgenic mutant mice have been utilized to reveal gene function. Tissue culture of whole tooth or jaw explants as well as culture of recombined tissues has been used to study effects of proteins and mutations. This knowledge is applicable to humans and other mammals because of the conservation of the basic genetic and developmental mechanisms.^{1,9}

Molecular studies have revealed that the instructive and permissive tissue interactions during mouse tooth development described above are mainly mediated by growth factor signalling. Development from initiation to eruption is governed by a sequential and reciprocal signalling process rather than simple one-way messages. The signalling involves all major signalling pathways, including transforming growth factor β (TGF β), fibroblast growth factor (FGF), sonic hedgehog (Shh), anhidrotic ectodermal dysplasia (*Eda*), and epidermal growth factor (EGF) signalling, and studies with mouse mutants have shown that they are needed simultaneously during critical stages of development.⁹

Msx1 and *Pax9* are transcription factors intimately involved in the genetic networks regulating tooth development. *Msx1* contains a homeobox which binds to specific target sequences in the DNA but is also capable to proteins interaction. *Msx1* has often been considered rather as a repressor than activator of gene expression. *Pax9* belongs to the paired-box containing transcription factor family, and is one of the earliest mesenchymal markers of the future tooth forming positions in mouse. *Pax9* is regulated by epithelial signals, especially FGF8, and it apparently regulates reciprocal signalling from the mesenchyme. In mice with hypomorphic *Pax9* mutations, a partial failure of tooth development was observed, affecting in a dose-dependent manner the third molars and incisors and to a smaller extent the other molars. The ameloblast differentiation and dentinogenesis were also affected.¹⁰

It has been suggested that the key role of *Msx1* and *Pax9* is to facilitate the bud to cap stage transition. There is signals emanating from the epithelium and mesenchymal during tooth development and molecular regulation (Figure 1). Mesenchymal *Msx1* expression is initially activated by the epithelial bone

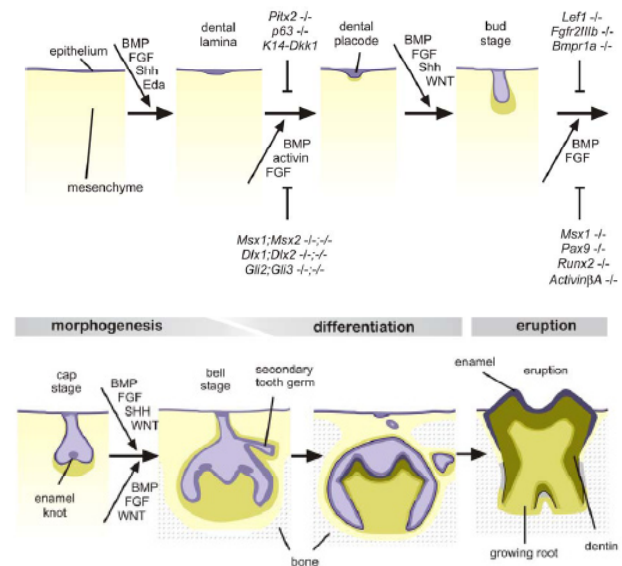


Figure 1. Tooth development and molecular regulation. Signals emanating from the epithelium are shown above and signals from the mesenchyme below the scheme.¹

morphogenetic protein 4 (BMP4) signal, and needed for a reciprocal BMP4 signal from the mesenchyme. BMP4 and Msx1 thus form an autoregulatory loop. BMP4 signal to the epithelium is crucial for the formation of the epithelial signalling centre, the enamel knot, and the arrest of the development in Msx1 null mutant teeth can be rescued by external BMP4 or transgenically activated BMP4 expression. The expression of Pax9 is apparently needed to maintain and, by the synergism with Msx1, to enhance this loop and also needed later in tooth development.¹¹

Incidence of tooth agenesis

The partial absence of dental germs is a congenital defect of hereditary or acquired origins. **Dental agenesis** can be defined as any situation in which one or more teeth are missing because they have never formed. This can also be called oligodontia, dental aplasia, and congenital absence of teeth or hypodontia. The term "oligodontia" is usually limited to those cases in which three or more teeth are missing; anodontia is the type of agenesis in which all the teeth are missing. When agenesis is of one or a few teeth, it tends to be present more distally.^{9,12}

Congenital agenesis of one or more permanent teeth, also known as hypodontia, is among the most well-recognized morphologic anomalies in humans, and yet the etiology is largely unknown (Figure 2). Oligodontia has been defined as agenesis of more than 6 permanent teeth. In Caucasians, tooth agenesis most commonly involves third molars, with from 10 to 25% of the population affected. Reports on the overall incidence of missing permanent teeth, excluding third molars, vary substantially, from 2% to 10%. In Caucasians, approximately 80% of tooth agenesis cases involve only one or two teeth.⁹

Etiology and pathogenesis of tooth agenesis

Evidence supporting a genetic etiology for tooth agenesis is well established reviewed. Tooth agenesis usually presents as an isolated anomaly. However, it is known to occur in association with syndromes or inherited disorders, many of which have known genetic defects.¹³

Tooth agenesis is one of the most common developmental problems in children. The congenital absence of teeth results from disturbances during the initial stages of tooth formation: initiation and proliferation.

Missing teeth may occur in isolation, or as part of a syndrome. Isolated cases of missing teeth can be familiar or sporadic in nature. Familiar tooth agenesis is transmitted as an autosomal dominant, autosomal recessive, or X-linked genetic condition.⁹

While several potential and verified environmental (post genetic) etiological factors at tooth agenesis have been presented, there is definitive proof that genetic factors play a major role in the etiology. The role of the genetic factors was suggested by observed familial occurrence, prevalence differences between populations, and association with heritable syndromes as well as by twin and family studies, but definitive evidence has been acquired during the molecular genetic era: defects in several genes have been shown to cause agenesis and anomalies in size and morphology. Tooth agenesis and tooth size reductions have been related to trauma, maternal systemic disease and various external factors. Among the maternal systemic disease, diabetes and different infections have been suggested. For example, developmental dental anomalies and tooth size reduction have been described in association of maternal rubella infection during pregnancy.¹⁴

The pathogenesis of human tooth agenesis is perhaps best understood in anhidrotic ectodermal dysplasias. In this case the molecular pathogenesis and the phenotypes in human patients and mouse mutants are directly comparable. The gene defects in anhidrotic ectodermal dysplasia (Eda), Eda-receptor (Edar), immunoglobulin K-gamma (IKKy) and their mouse homologs, i.e. the signalling ligand, its receptor and the intracellular mediators of the signalling, cause complete inactivation of this signalling pathway. In the mutant mice, incisors and third molars commonly fail to develop and first molars are hypoplastic, while in the patients with anhidrotic ectodermal dysplasia, both dentitions are severely affected and tooth morphology is simplified. The phenotypes of the mice with impairment or over expression of Eda signalling suggest that early defects of ectodermal placodes and, in teeth, the enamel knots would explain the ectodermal defects in human patients. Thus, failure of signalling at an early stage leads to anomalies that are present also in the deciduous dentition. On the other hand, as the mutant and disease phenotypes are caused by complete inactivation of the



Figure 2. The case of permanent teeth agenesis at 17th year old woman.

signalling pathway, the partial albeit severe tooth agenesis phenotypes suggest redundancy in the function of the signalling pathways, i.e. that different signalling pathways have overlapping functions. This redundancy adds a further element explaining how different gene defects may cause partial agenesis.¹⁵

The congenital absence of teeth is one of the commonest developmental abnormalities seen in human populations. Familial hypodontia or oligodontia represents an absence of varying numbers of primary and/or secondary teeth as an isolated trait. While much progress has been made in understanding the developmental basis of tooth formation, knowledge of the aetiological basis of inherited tooth loss remains poor. The study of mouse genetics has uncovered a large number of candidate genes for this condition, but mutations in only three have been identified in human pedigrees with familial hypodontia or oligodontia: *Msx1*, *Pax9* and *AXIN2*. This suggests that these conditions may represent a more complex multifactorial trait, influenced by a combination of gene function, environmental interaction and developmental timing.¹⁶

The most compelling evidence for the genetic etiology of tooth agenesis has been provided by the identification of gene defects associated with different types of tooth agenesis. Dominant defects in *Msx1*, *Pax9* and *AXIN2* have been described in families with isolated severe tooth agenesis. However, in association with defects in *Msx1*, nail dysplasia and some patients with oral clefts have each been described in single families. In addition to causing severe tooth agenesis phenotype, a defect in *AXIN2* also predisposed to colorectal cancer. Recently, two defects that affected only dentition were also described in Eda. All these gene defects cause severe types of agenesis. However, evidence for association of specific intragenic polymorphisms to tooth agenesis, apparently consisting mostly of common types of tooth agenesis, has been presented for *Msx1*, *Pax9*, *AXIN2*, *TGF α* , *IRF6* and *FGFR1*.¹⁷

The role of genes in tooth agenesis

Identifying a hereditary dental pathology and defining its unique characteristics are the first steps toward the dissection of its genetic basis. A thorough interview of the patient and his or her relatives is the next step to defining the trait as familial; if it proves to be so, it is imperative to define the pattern of inheritance of the anomaly.¹⁸

In this respect, several genes that are pivotal in initiating the development of teeth have been subjected to intense study in the past decade. Mutations in a number of genes were found to interrupt tooth development in mice. However, to date there are only three genes associated with the nonsyndromic form of human tooth agenesis: *AXIN2*, *Msx1*, and *Pax9*. Among them, *Msx1* and *Pax9* was more intensively studied. Recently, the general structure of the Pax paired domain was described and the phylogenetics and relation between the several members of the Pax family were established. In addition, both gene

expression and molecular pathogenesis of *Msx1* and *Pax9* have been relatively well characterized, making it a special candidate to explain at least part of primate tooth variation (Figure 3).¹⁹

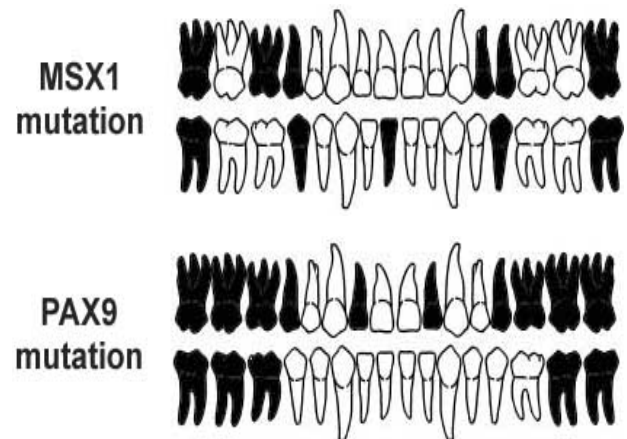


Figure 3. The mutation of two genes tooth development (*Msx1* and *Pax9*) which can lead to tooth agenesis. Darkness of the colour expresses the frequency of agenesis.¹⁹

DISCUSSION

There is considerable evidence suggesting that genes play a fundamental role in the etiology of tooth agenesis. Moreover, there seems to be a genetic relationship in the determination of different dental anomalies, considering the high frequency of patterns of association. A single genetic defect may result in different phenotypic expressions, including such various traits as tooth agenesis, microdontia, ectopic tooth position, and delayed development of different teeth.

As with many other organs, tooth development involves sequential and reciprocal signalling processes between epithelial and mesenchymal cell layers that are orchestrated by a hierarchy of genes encoding secreted growth factors, extra cellular matrix components, and transcriptional regulators. Because the regulatory genes required for tooth formation are common components of signalling cascades involved in development of other embryonic structures. Among the transcriptional regulatory genes required for tooth formation, the *Msx1* homeobox gene is highly expressed in the dental mesenchyme and is essential for tooth development, since targeted gene disruption results in arrested tooth formation at an early stage in *Msx1*. In addition to its expression in the tooth primordia, *Msx1* expression is prominent in regions of epithelial-mesenchymal interactions in several other embryonic structures, including other craniofacial structures and the limb. These findings have led to the hypothesis that *Msx1* is an important component in the signalling events that occur between epithelial and mesenchymal tissues.¹⁹

Both Msx1 and Pax9 are also needed for the mesenchymal cell condensation around the growing epithelial bud. The reduced condensation which is also seen in the Pax9 hypomorphic mutants, perhaps indicating a decreased amount of committed dental mesenchymal cells, may be related to tooth agenesis. As *Msx1* is known to be important for the commitment of neural crest, an early defect in the migration of neural crest cells could also be responsible for the tooth agenesis, if it caused a reduction in the amount of competent ectomesenchymal cells.²⁰

One of the earliest placodal markers, Eda, is originally expressed throughout the oral epithelium and epidermis, but becomes limited to the placodes at an early stage. When Eda was over expressed in the epithelium, the hair and tooth placodes become larger, probably due to an increased amount of the cells destined to become placode cells. Thus Eda signalling probably acts rather as a modulator of ectodermal placode deformation than as an initiator. Eda signalling may be important as a mediator of effects of Shh and BMPs. Mutation in the Eda and Edar genes in human cause X-linked and autosomal anhidrotic ectodermal dysplasia characterized by failure of sweat development, tooth agenesis and size reduction of teeth.¹⁵

Tooth agenesis is a consequence of a qualitatively and quantitatively impaired function of genetic networks, which regulate tooth development. Impaired function of genetic networks are reflected as reduced signalling or impaired signal regulation, cell proliferation, migration and differentiation. The most critical are the stages of formation of signalling centres that have an organizing role for the future development. The reduction of the "tooth forming potential" may follow from a reduced functional activity of a single gene as in the case of defects in Msx1 and Pax9.²¹

The number and type of teeth are strictly controlled during odontogenesis. Msx1 and Pax9 form a signalling cascade during tooth development. Mutations in Msx1 and Pax9 genes are dominant for tooth agenesis in humans. The gene Pax9 was found to be localized in chromosome 14 (14q12-q13). The disruption of DNA-binding ability of Pax9 that causes hypodontia. Nonsense mutation in exon 1 of Msx1 in chromosome 4 was found to be heterozygous in all affected family members. Nieminen have identified there was gene deletions in Msx1 and Pax9, missense mutation R196P of Msx1 and missense L21P of Pax9.^{21,22}

The key role of Msx1 and Pax9 is to facilitate the bud to cap stage transition. Mesenchymal Msx1 expression is initially activated by the epithelial BMP4 signal. Loss of function defects in Msx1 and Pax9 in humans cause partial failure of tooth development, tooth agenesis. Defects in Msx1 associate especially with agenesis of second premolars and third molars, whereas the defects in Pax9 affect particularly the permanent molars. The size of the permanent teeth may also be reduced. In one of the families with a defect in Msx1, some patients also presented with nail dysplasia and in another family with oral clefts. Several other sequence changes in Msx1 have also been described in

connection with oral clefting. In addition, a micro satellite allele in the intron of Msx1 has been associated with both tooth agenesis and oral clefting, and two promoter region SNP alleles of Pax9 with tooth agenesis.^{17,23}

In the case of Msx1 and Pax9, tooth agenesis has been related to critical function of the mouse homologues of these genes in the formation of the enamel knot and the subsequent transition from bud to cap stages. The Msx1 haploinsufficiency, however, appear to affect only secondary teeth and permanent molars, and it is not obvious how a weakened enamel knot function, which presumably follows from a reduced amount of functional Msx1 protein, is linked to impaired secondary tooth development. It is possible that the late developing teeth are more sensitive to impaired enamel knot function. The development of these teeth normally is a long lasting process and happens surrounded by the alveolar bone. It can also be speculated that enamel knots may regulate the program leading to the secondary tooth formation.²³

It is concluded that based on the molecular and genetic studies of tooth development, tooth agenesis is a consequence of a qualitatively or quantitatively impaired function of genetic networks, which regulate tooth development. Reduced amount of functional Msx1 or Pax9 protein in the tooth forming cells is able to cause severe and selective tooth agenesis. Another conclusion, based on the analysis of the phenotypes associated with the known defects in these genes, is that the phenotypes associated with the defects in Msx1 and those associated with the defects in Pax9 are different. Despite the similarities, there are clearcut differences in the frequency of agenesis of specific teeth.

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