

NOVEL MUTATIONS IN PAX9 GENE ASSOCIATED WITH DENTAL ANOMALIES- A REVIEW

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Abstract

Dental anomalies such as tooth agenesis are caused by disturbances and gene mutations that occur during odontogenesis. Human genetic variations have long been of researcher's interest as it is partly responsible for the inter-individual response to drugs, infections and several other phenotypes related to development and progression of the disease. Tooth development is an intricate process which involves complex interplay of genes acting in symphony to exhibit the trait. Mutations or genetic errors in the DNA sequences encoding proteins involved in the process of odontogenesis have been identified in recent years. Some of the genes of prime importance are the homeobox genes which are known to play an important role in tooth development. Several genes such as PAX, MSX, AXIN, DLX have been implicated in the process of odontogenesis. Animal models used in earlier studies reported that PAX9-deficient knockout mice exhibit missing molars due to an arrest of tooth development at the bud stage. The aim of this review was to prepare an exhaustive collection of PAX9 mutation panel reported to be associated with dental anomalies. The literature review will also provide a comprehensive understanding of variations observed in PAX9 gene in association with several common dental anomalies.

Keywords: Homeobox genes, Mutation, Orofacial cleft, PAX9, Polymorphism, Tooth agenesis

Introduction

Fixed prosthodontic treatment deals with the replacement of teeth by artificial substitutes that are not readily removable from the mouth. Its focus is to restore function, esthetics and comfort[1]The fixed partial denture (FPD) is one of the most commonly preferred treatment options for a single missing tooth.[2] FPDs were considered to be the best treatment choice for replacing a single missing tooth[3] Fixed prosthodontics treatment can range from the restoration of a single tooth to rehabilitation [4]

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Homeobox genes are transcription factors that are key regulators of developmental processes such as regional specification, patterning and differentiation. The first homeobox genes were identified in *Drosophila* and homologous genes were remarkably found in all animal species, fungi and plants. The dentition is derived from the first branchial arch, where complex interactions between the stomodeal epithelium (derived from the ectoderm) and the underlying mesenchyme derived from cranial neural crest (CNC, migrating from the neuroectoderm during early neurulation) drive the development of the appendages. Odontogenesis begins from the thickening and invagination of the stomodeal epithelium forming the dental placode. The dental placode gets invaginated into the dental mesenchyme and forms a tooth bud. The mesenchyme, then proliferates and condensates around the tooth bud. At the cap stage, the invaginated epithelium expands laterally and covers the condensed mesenchyme that will become the dental papilla. Different structures can be distinguished in the dental epithelium, including the enamel knot, which is known as an organizing center of tooth morphogenesis. At the late bell stage, cytodifferentiation starts: the cells of the internal enamel epithelium differentiate into ameloblasts (enamel-producing cells) while adjacent cells in the dental papilla differentiate into odontoblasts (dentine-producing cells).

Even though the origin of mandibular (lower jaw) and maxillary (upper jaw) teeth are histologically and morphologically identical, they involve different developmental pathways. The patterning of murine dentition was determined by the complex and specific distribution of homeobox genes in the first arch mesenchyme before the initiation of tooth formation [1] In dentition, these genes play an important role in the specific determination of incisors versus molars while they can also discriminate between maxillary and mandibular teeth. [2] During embryogenesis, *PAX* genes encode a family of transcription factors that play key roles. *PAX9* gene expression marks the prospective sites of tooth development and is maintained in the developing tooth mesenchyme thereafter. Preliminary analyses conducted [3] show that *Pax9* is vital for the tooth development to proceed beyond the bud stage.

The basic steps in genetic mapping of a disease gene include identification of the mode of inheritance; genetic mapping of the disease gene; identification and screening of candidate genes; and evaluation of the functional consequences of the mutation(s) identified. The categories of mutations that can occur in human DNA are single base-pair change, deletion, insertion, inversion and chromosomal abnormalities. A disease mutation in a gene can ultimately affect the function of the encoded protein in many ways like transcription, mRNA stability, translation, or protein stability, localization, or function [4].

Paired-box gene 9 (*PAX9*) mutation is potentially associated with impaction among patients. The relationship between *PAX 9* polymorphism and the occurrence of maxillary canine impaction was analysed in a study [5]. The results of this study are presented in Table 1. The transcription factors involved in the development of dentition in humans is Paired box 9 (*PAX9*). Mutations in *PAX9* gene influence the number, position and morphology of the teeth in an affected person. Numerous mutations have been reported by several researchers globally to discuss the association of *PAX9* mutations or polymorphisms with syndromic or nonsyndromic dental anomalies. The most common consequence of *PAX9* gene mutation is the autosomal-dominant isolated (non-syndromic) oligodontia or hypodontia [6]. A comprehensive meta-analysis by Zhang et al, [7] evaluated the association between paired box 9 (*PAX9*) gene polymorphisms and tooth agenesis among isolated humans. The results of this study are summarized in Table 1.

A study was conducted to perform screening for mutations and/or polymorphisms in the critical regions of *PAX9* and *MSX1* genes. The screening results are shown in Table 1. The authors hypothesize that the lower expression of *PAX9* protein could have contributed to the development of tooth agenesis among affected subjects. [8] Mutations in *AXIN2*, *PAX9* and *MSX1* have been determined in families with dental agenesis. The absence of one or more primary or permanent teeth is considered dental agenesis in children. Data for congenital tooth agenesis prevalence vary between 0.3 and 11.3% for both males and females. The prevalence of congenital tooth agenesis was found to be higher in females than in males. [9]

Table 1: Studies demonstrating the association of Paired box 9 (*PAX9*) gene polymorphisms or mutations with different dental abnormalities.

Author Name / Year	Study population	Mutation/variation identified	Recessive/dominant- Phenotype	Clinical presentation/Results
Vitria et. al, 2019	Patients with and without maxillary canine impaction	Single nucleotide polymorphism (SNP) genotypes	Maxillary canine impaction	<p>The presence of SNPs 3 and 4 is associated with increased likelihood of suffering from maxillary canine impaction.</p> <p>Patients with a CC genotype at SNP 3 and a CC genotype at SNP 4 were more likely to have maxillary canine impaction. All SNPs were located in exon 3 of <i>PAX 9</i> and in the region sequenced by the primer pair</p>
Zhang et.al, 2014	Case-control population with a total of 855 hypodontia cases and 1201 healthy controls.	G allele and G carrier (AG + GG) of A1031G	Hypodontia	<p>4 genetic sites of the <i>PAX9</i> gene involved in hypodontia cases, of which 3 sites may be risk factors and 1 may have a protective role.</p> <p>-C allele and C carrier showed no significant association with oligodontia.</p> <p>-The G allele and G carrier in the <i>PAX9</i> gene were not related factors.</p> <p>-The genotype (AG + GG) of IVS2-54 in the <i>PAX9</i> gene may be a protective factor for oligodontia</p> <p>-No significant differences were found in the allele frequency of IVS2-54 in the <i>PAX9</i> polymorphism between controls and subjects with sporadic tooth agenesis.</p>

Sery et. al ,2015	270 individuals with tooth agenesis and in 30 healthy subjects of Czech origin	<i>PAX9</i> and <i>MSX1</i> genes	Oligodontia	Screening results revealed a previously unknown heterozygous g.9527G>T mutation in the <i>PAX9</i> gene in monozygotic twins with oligodontia and three additional affected family members. The same variant was not found in healthy relatives.
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Table 2: Studies demonstrating the association of *PAX9* and tooth agenesis

Author Name / Year	Study population	Mutation/variation identified	Recessive/dominant-Phenotype	Clinical presentation/Results
Wong et. al, 2018	Chinese families with non-syndromic (NS) tooth agenesis	c.140G>C, c.167T>A, c.332G>C, c.194C>A, c.271A>T, c.146delC, c.185_189dup, c.256_262dup, c.592delG, 2 known heterozygous mutations in the <i>PAX9</i> gene among 120 probands	Tooth agenesis	Families segregating a <i>PAX9</i> mutation reveal that all affected individuals were missing the mandibular second molar and maxillary central incisors are most susceptible to microdontia. A significant reduction of bitter taste perception was seen among 3 individuals harbouring <i>PAX9</i> mutations
Murakami et. al. 2017	nine patients with non-syndromic tooth agenesis	<i>P20L</i> , of <i>PAX9</i> in a single familial case involving three patients in two generations	Tooth agenesis	Defects were shown primarily in the first and second molars, which is typical for cases attributable to <i>PAX9</i> mutation.
Salvi et.al, 2016	16 individuals affected by tooth agenesis	None	Tooth agenesis	Two individuals were siblings and also carried a heterozygous functional variant in <i>EDAR</i> -associated death domain (<i>EDARADD</i>) (rs114632254), another disease-causing gene

Abu-Siniyeh et. al, 2018	72 unrelated subjects with hypodontia and 72 normal healthy unrelated control individuals in Jordan	<i>PAX9</i> c.-912T>C (rs2073247) and c.-1031G>A (rs2073244) promoter polymorphisms	Hypodontia	Hypodontia group had a significantly higher -1031GG genotype (P<0.01) and a significantly lower -912TC genotype (P<0.01) compared with the control group. The transcriptional activity of <i>PAX9</i> gene is affected by polymorphisms in the promoter region of this gene and is associated with hypodontia phenotype.
Yang et .al,2020	Two unrelated individuals with non-syndromic tooth agenesis and their families	A missense mutation c.572 T>C and a frameshift mutation c.590_594 dup <i>TGTCC</i>	Tooth agenesis	There is a correlation between the observed phenotypes and alterations in hydrogen bond formation, thereby potentially affecting protein binding
Martha et al, 2019	97 NSH subjects (70 females and 27 males) from patients referred to orthodontic treatment, and matched to each NSH subject a control by age and sex.	None	Non-syndromic hypodontia	The variant genotype and variant T allele of the <i>MSX1</i> rs8670 SNP increased the risk of hypodontia in the studied population. The presence of the variant A allele of <i>AXIN2</i> rs2240308 is associated with frontal agenesis but not with lateral agenesis.
Rodrigues et. al ,2020	594 orthodontic Brazilians		Tooth agenesis	Genotypes and allele distributions for the <i>FGF3</i> rs1893047 were significantly different according to the skeletal malocclusion. Carrying at least one G allele increased in more than two times the chance of presenting skeletal class III malocclusion. No association between another skeletal craniofacial pattern and some polymorphism assessed was found in the study.

Koskinen et al, 2019	Family members of a Regional odontodysplasia patient and with family history of oligodontia	<i>MSXI</i> , <i>PAX9</i> , <i>AXIN2</i> , and <i>WNT10A</i>	Regional odontodysplasia	An initiation codon mutation of the <i>PAX9</i> gene was found in the proband and segregating with oligodontia in the family. The etiology of regional odontodysplasia (RO) may be genetic and the same genes can be involved both in RO and tooth agenesis. The results gave new insights into the aetiology of regional odontodysplasia
Mitsui et al, (2014)	Two unrelated Japanese patients with non-syndromic tooth agenesis	2 novel mutations in the paired domain of <i>PAX9</i> , a three-nucleotide deletion (73-75 delATC) and a missense mutation (<i>C146T</i>)	non-syndromic tooth agenesis	The individual with the 73-75del ATC mutation was missing all maxillary molars and mandibular second and third molars. The individual with the <i>C146T</i> mutation was missing the mandibular central incisors, maxillary second premolars, and first molars, along with all second and third molars. Both mutations affected amino acids that are highly conserved among different species and are critical for DNA binding. When both mutants were transfected to COS7 cells, nuclear localization of <i>PAX9</i> proteins was not affected.
Kirac et al, 2016	31 patients and 30 controls	<i>PAX9</i> and/or <i>MSXI</i>	Hypodontia	22 variations were detected in <i>PAX9</i> in which 18 of them are novel. In addition, 7 variations were found in <i>MSXI</i> in which 5 of them are novel and one of them lead to amino acid change. Statistically significant relations were found between detected variations and tooth sizes.
Hlousková et al, 2015	Patients with tooth agenesis and controls among Czech population	<i>PAX9</i>	Tooth agenesis	Several novel variants in the <i>PAX9</i> gene were found. In subjects with full dentition, polymorphisms were observed.

Table 3: Studies demonstrating the association of *PAX9* and Cleft Lip/Palate & other dental anomalies

Author Name / Year	Study population	Mutation/variation identified	Recessive/dominant-Phenotype	Clinical presentation/Results
Howe et. al, 2015	3,811 individuals; 660 cases with clefts, 1,922 unaffected relatives, and 1,229 controls.	None	Orofacial clefting, Agenesis, tooth displacement	Dental anomalies were more found in cleft lip with cleft palate than other cleft types. Agenesis and tooth displacements were the most common dental anomalies in case probands for primary and permanent dentitions. Compared with controls, unaffected siblings and parents showed an increase in anomalies of the maxillary permanent dentition.
Jia et. al, 2017	well-characterized Pax9 ^{-/-} mouse model	None	Cleft palate	The functional interactions between Pax9 and Dkk1 were shown by the genetic rescue of secondary palate clefts in Pax9 ^{-/-} Dkk1f ⁺ ;Wnt1Cre embryos. The controlled intravenous delivery of small-molecule Wnt agonists (Dkk inhibitors) into pregnant Pax9 ^{+/-} mice restored Wnt signaling. This has led to the growth and fusion of palatal shelves, as shown by an increase in cell proliferation and osteogenesis in utero, while other organ defects remain uncorrected
Devi et .al, 2019	Random population sample of fifty individuals with palatally impacted maxillary canines and 50 gender and age-matched controls	<i>MSX1</i> and <i>PAX9</i> genes	Palatal impaction of maxillary canines	Single nucleotide polymorphisms [<i>MSX1</i>] and [<i>PAX9</i>] showed a statistically significant association with palatal impaction of maxillary canines. The study results suggested that polymorphisms of genes <i>MSX1</i> and <i>PAX9</i> are positively associated with palatal impaction of maxillary canines

Mutations in nine genes (*MSX1*, *PAX9*, *AXIN2*, *WNT10A*, *EDA*, *EDAR*, *EDARADD*, *NEMO* and *KRT17*) have been associated with non-syndromic oligodontia till today. The first and second genes to be identified in non-syndromic oligodontia were *MSX1* and *PAX9*. These two gene proteins are vital for the formation of the odontogenic potential of the mesenchyme. A study group [10] investigated six genes (*MSX1*, *PAX9*, *AXIN2*, *WNT10A*, *EDA* and *EDARADD*) in a patient with sporadic non-syndromic oligodontia. An extensive analysis of publicly accessible databases revealed 15 causative genes responsible for non-syndromic TA (tooth agenesis). Among 198 different mutations, about 15 genes are responsible for non-syndromic TA. The findings have shown new lights on the discovery of novel molecular mechanisms associated with tooth agenesis. [11]

Tooth agenesis affects the function and esthetics. In the patterning and morphogenesis of tooth and taste buds, *PAX9* plays a critical role. Mutations of *PAX9* occur in conditions like tooth agenesis. Familial analysis of nonsyndromic tooth agenesis of multiple Chinese populations were carried out using DNA sequencing. The genotype and phenotype investigations revealed 9 novel (as mentioned in Table 2) and 2 known heterozygous mutations in the *PAX9* gene among 120 probands. The clinical characteristics and the results of this study are presented in Table 2. The tooth agenesis was attributed to *PAX9* haploinsufficiency or loss of function of the genes, which was confirmed by functional analysis.[12] The discovery of genetic mutations that unlock the causes of non-syndromic tooth agenesis are being carried out. Interaction of several genes are involved in tooth development in relation to tooth epithelium and mesenchyme odontogenesis. Mutation of candidate genes *PAX9* and *MSX1* are identified as the main causes of hypodontia and oligodontia. A knockout mice model exhibiting *PAX9* deficiency was reported to present with missing molars which resulted due to the arrest of bud phase during tooth development. [13] Murakami et al, stated that *PAX9* and *MSX1* play crucial roles in the development of permanent teeth at the bud stage, and their loss-of-function variants have been associated with congenital tooth agenesis [14]. Sequencing the coding regions of the *PAX9* and *MSX1* genes from nine patients with non-syndromic tooth agenesis was done. The clinical presentation resulted in this study is shown in Table 2.

***PAX9* and tooth agenesis**

Tooth agenesis may occur either in association with genetic syndromes, based on the presence of inherited abnormalities, or as a non-syndromic trait, with both familiar and sporadic cases. A study was conducted by direct Sanger sequencing of *PAX9* and *MSX1* genes. The results of the study are shown in Table 2. It is confirmed that the *WNT10A* played a major role in tooth agenesis and genetic heterogeneity of this disease. *WES* analysis may be an effective approach to search for genetic variants in familial or sporadic tooth agenesis. [15]

A study was conducted to find any association between *PAX9* promoter polymorphisms and the development of hypodontia. The findings of the study are shown in Table 2. The researchers concluded that the promoter polymorphisms viz., *rs2073247* and *rs2073244* of *PAX9* might play a role in the development of hypodontia among the Jordanian population.[16] An association between mutations in *MSX1*, *PAX9*, *EDA*, *AXIN2*, *WNT10A*, *WNT10B* and *LRP6* and human tooth agenesis have been identified by researchers using Sanger sequencing of the candidate genes (as shown in Table 2). The 2 novel mutations identified were further analyzed using structure modeling using computational tools. The mutations were found to result in conformational changes in the *MSX1* homeodomain.[17]. The results reported are shown in Table 2. A study [18] was performed with an objective to elucidate the genetic background of non-syndromic hypodontia (NSH). The NHS group was individually and in groups with frontal and lateral agenesis were assessed for single nucleotide variations. The results of the study are summarised in Table 2. Rodrigues et al, in their research article stated that tooth agenesis may occur in the form of an isolated familial or sporadic anomaly or in association with other genetic diseases like cleft lip/palate. A cross-sectional, multi-centre, genetic study was conducted among orthodontic Brazilians patients to assess if genetic polymorphisms in tooth agenesis (TA)-related genes are associated with craniofacial morphological patterns. The results of the study are summarised in Table 2. The researchers concluded that the genetic polymorphism *rs1893047* in *FGF3* might contribute to variations in the craniofacial sagittal pattern. [19].

In non-syndromic agenesis, gene mutations are said to be the cause [20]. The mutations of *PAX9*, *MSX1*, and *AXIN2* genes are responsible for tooth development. In a case-control study of 306 unrelated Portuguese individuals, single nucleotide polymorphisms in the *PAX9* gene were associated with a high risk of maxillary lateral incisor agenesis. Non-syndromic tooth agenesis is most likely caused by mutations of *MSX1*, *PAX9*, *AXIN2*, and *WNT10A* genes. As the phenotypes of both oligodontia and Regional Odontodysplasia (RO) co-occur in one Finnish family, the study was conducted [21] to investigate the genetic aetiology of the two

conditions. A mutation screening of the genes was performed for the family members of a RO patient and family history of oligodontia. The study results are shown in Table 2. In humans, heterozygous mutations in *PAX9* have been associated with non-syndromic tooth agenesis, predominantly in the molars [22]. Novel mutations identified by the group was a triplet deletion and a missense mutation in *PAX9* gene identified in two Japanese patients who presented with non-syndromic tooth agenesis. The results are discussed in Table 2. The decline in the gene expression was suggestive of haploinsufficiency of *PAX9* gene.

Missing permanent molars and second premolars are found to be caused by defects in mutations of *PAX9* and/or *MSX1* genes. It was also found in few studies that *PAX9* and *MSX1* gene mutations may change tooth size. All of these factors were investigated in the study conducted by [23]. The study results are summarised in Table 2. *PAX9*, *MSX1*, *AXIN2*, *WNT10a*, and *EDA* genes mutation have been associated with tooth agenesis during tooth morphogenesis. Hlouskova et. al, also conducted a similar study to investigate the relationship between the *PAX9* gene variants and tooth agenesis in the Czech population. The results of the study are summarised in Table 2. The authors conclude that tooth agenesis among these patients is caused by mutations in regions different from *PAX9* exons analysed in this study. [24]

***Pax9* gene and orofacial clefting**

Children with oral clefts show dental anomalies, adding complexity to understanding the phenotypic spectrum of orofacial clefting. A study conducted by Howe et al, [25] showed that cases had higher rates of dental anomalies in the maxillary arch than did controls for primary and permanent dentitions but not in the mandible. The other clinical presentations seen in this study are summarised in Table 3. Cleft palate and/or lip is the most common human craniofacial malformations and caused by multiple genetic and environmental factors. A study was conducted on a well-characterized *Pax9*^{-/-} mouse model with a consistent cleft palate phenotype to test small-molecule Wnt agonist therapies. The functional interactions and results are summarised in Table 3. [26] In the *Pax9*-mediated regulation of development of the secondary palate, Canonical Wnt signaling is vital. Jia et al, demonstrated that reduced expression of *Axin2*, a target of canonical Wnt signaling, was accompanied by *Pax-9* deficient embryos who developed characteristics of cleft palate. The data also identified a crucial role for canonical Wnt signaling in acting downstream of *Pax9* to regulate palate morphogenesis. [27]

***Pax9* gene and tooth impaction**

Maxillary canines are the second-most commonly impacted teeth. Most of the impacted maxillary canines are impacted in the palates. About 40% of cases with palatal impaction of maxillary canines presented with agenesis of third molars. Polymorphisms in the *MSX1* and *PAX9* genes are found to be associated with Sporadic agenesis of third molars. A study conducted by [28], to evaluate the association between polymorphisms of *PAX9*, *MSX1* and palatally impacted canines. The study results are seen in Table 3. [28] The potential role of *PAX9* in squamous cell differentiation and carcinogenesis of the oro-esophageal epithelium have been discussed in previous studies. Not only does *PAX9* have an effect on tooth development, the decreased expression has been found to be associated with oro-esophageal squamous cell carcinoma. The down-regulation of the same has been associated with alcohol drinking.

Taken together, several studies have recently populated the polymorphism and mutation data of *PAX9* gene which could be of importance to the clinicians to trace the pattern of inheritance and deduce the probability of acquiring the disease. The team of researchers of our institution have carried out studies encompassing several topics pertaining to the clinical studies and genetic aspects of tooth agenesis, syndromes associated with orofacial clefting, periodontal and other dental infections [30-44]. The data available from the resources helped us in preparing the structure of the present literature review. More research into this field would open up new avenues for translating the experimental data into clinically relevant applications.

CONCLUSION:

This review has provided a comprehensive source of information related to *PAX9* gene functions and its association with several dental anomalies. The number, position and morphology of the teeth are influenced by mutations in *PAX9* gene in an affected individual. Numerous mutations reported in the gene so far have been associated with different types of dental agenesis and other dental defects. In the present review, the authors have summarized to the best of their knowledge, all known *PAX9* mutations associated with some common dental anomalies. The authors believe that this review would benefit dental students, dental practitioners in the application and dental practice.

AUTHOR'S CONTRIBUTIONS:

All the authors contributed to the design and implementation of the research, and to write the review and approved the final manuscript.

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CONFLICT OF INTEREST:

None

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