Tooth agenesis describes the situation when the patients are missing their teeth. Tooth agenesis affects more than 20% of human population. Literature shows that tooth agenesis is one of the most common congenital disorder. The prevalence of missing primary dentition is found in 0.2–0.9%, and the frequency of missing primary dentition in females is found to be 1.37 times higher than in males. The most common missing teeth are the wisdom teeth (25–35%). Reported studies have indicated that many factors, such as genetic, hormonal, environmental and infections are closely associated with tooth agenesis. However, a large proportion of missing teeth remains unclear, but increasing genetic conditions act as a risk factor for the failure of tooth development. Genetic anomalies or mutations in MSX1, PAX9, AXIN2 and EDA genes, appear to be most critical during the development of tooth, leading to various forms of tooth agenesis and systemic features. Reported studies show that haploinsufficiency for MSX1 and PAX9 genes are associated with a severe form of tooth agenesis. Mutations in several other genes have also been identified in rare forms of tooth agenesis. The present paper aims to review the scientific literature related to genetic influence of familial and non-syndromic forms of tooth disorder at the molecular level.

**ABSTRACT**

Tooth agenesis is one of the most prevalent craniofacial congenital anomalies found in some people. Some genes, such as homeobox gene (MSX1), paired domain transcription factor (PAX9), axis inhibition protein 2 (AXIN2), and Ectodysplasin-A (EDA) are involved in tooth development and encodes the transcription factor, which plays an important role during tooth development. Gene anomalies or mutations in MSX1, PAX9, AXIN2 and EDA genes, appear to be most critical during the development of tooth, leading to various forms of tooth agenesis and systemic features. Reported studies show that haploinsufficiency for MSX1 and PAX9 genes are associated with a severe form of tooth agenesis. Mutations in several other genes have also been identified in rare forms of tooth agenesis. The present paper aims to review the scientific literature related to genetic influence of familial and non-syndromic forms of tooth disorder at the molecular level.

**Key words:** Genetics, Hypodontia, Oligodontia, Anodontia, and Review.

By genetic anomalies in homeobox gene (MSX1), paired domain transcription factor (PAX9) gene, axis inhibition protein 2 (AXIN2) and Ectodysplasin-A (EDA) genes are closely associated with tooth agenesis and systemic feature, like colorectal cancer. Tooth agenesis is characterized by the developmental absence of 1–6 teeth. The severe form of tooth agenesis is known as oligodontia or severe hypodontia, in which the number of missing teeth is more than six (excluding third molars). Another severe condition is referred to as anodontia, in which there is a complete absence of all teeth. Tooth agenesis can be classified as familial or sporadic, and be associated with either syndromic or non-syndromic, respectively. Literature shows that genetic factors interact with environmental factors. Genetic factor, such as mutations in some related genes, disturbs the regulatory process of tooth formation. It has been observed that defects in PAX9, MSX1, EDA, AXIN2 and other genes may be associated with familial and non-syndromic oligodontia or partial and complete failure of tooth development.

**INTRODUCTION**

Tooth agenesis describes the situation when the patients are missing their teeth. Tooth agenesis affects more than 20% of human population. Literature shows that tooth agenesis is one of the most common congenital disorder. The prevalence of missing primary dentition is found in 0.2–0.9%, and the frequency of missing primary dentition in females is found to be 1.37 times higher than in males. The most common missing teeth are the wisdom teeth (25–35%). Reported studies have indicated that many factors, such as genetic, hormonal, environmental and infections are closely associated with tooth agenesis. However, a large proportion of missing teeth remains unclear, but increasing genetic conditions act as a risk factor for the failure of tooth development. Genetic anomalies in homeobox gene (MSX1), paired domain transcription factor (PAX9) gene, axis inhibition protein 2 (AXIN2) and Ectodysplasin-A (EDA) genes are closely associated with tooth agenesis and systemic feature, like colorectal cancer.

**MSX1 gene anomalies belong to tooth agenesis**

MSX1 is a homeobox gene located on chromosome 4 and encodes a DNA-binding protein. The main function of MSX1 protein is to
interact with TATA box-binding protein (TBP) and some transcription factors to increase the rate of the transcription process. This protein regulates gene expression, which is essential for initiating tooth development. MSX1 protein is considered to be critical during early tooth development; it was found to hold sequence specific DNA-binding activity and supposed to regulate other genes involved in tooth development pathways. Some studies show that the alteration in MSX1 gene is generally associated with the autosomal dominant inheritance of hypodontia, while the defects in PAX9 gene are associated with hypodontia and reduction in teeth size. Defects in MSX1 and PAX9 genes influence early tooth development, leading to the loss of maxillary first, second premolars, mandibular second premolars and first, second and third molars, respectively. Phenotype caused by altered or lack of homeobox 1 protein may be decided by nature or the location of mutations. Missense mutations can cause familial or non-syndromic tooth agenesis, while nonsense mutations can lead to more severe tooth agenesis, nail anomalies and orofacial cleft due to the lack of C-terminal end of MSX1 protein. Mutations in MSX1 and PAX9 genes have been frequently identified in patients with tooth agenesis. However, a majority of these patients may have mutations in other genes as well. So far, several mutations have been identified in the MSX1 gene, and several laboratories are involved in the identification of different type of mutations in genes and their effects on protein structure, function and phenotypic features. Identified gene defects, such as deletion, nonsense mutation, missense mutation and point mutation mostly exist in DNA-binding domain. To the best of our knowledge, many mutations have been detected in the MSX1 gene, most of which have different effects on their protein structure, function and phenotypic features. All these studies indicated that mutations in MSX1 gene are closely associated with tooth agenesis. It is hypothesized that those regions, where the detected mutation might be necessary to stabilize the protein-binding activities and DNA-binding and bending capacities, are necessary in proper tooth development process. This paper describes few reported mutations of some genes and their effect on protein structure, functions and phenotypic features of patients. Recent studies showed transition (T671C) mutation with substitution of leucine by proline at position 224 in MSX1 gene in patient, and their family members with autosomal dominant hypodontia. Nonsense mutation (c.332C→A) (Ser111 Stop codon) in exon 1 of MSX1 gene was detected in patient and their seven family members with tooth agenesis. Missense mutation in MSX1 gene (Arg196Pro) with G→C substitution in homodomain protein sequence at codon no. 587 was associated with tooth agenesis. Due to gene mutation, the function of protein may get destroyed and show altered DNA-binding capacity and corrupted manner of interaction with other transcription factors. MSX1 gene mutation and their altered protein structure were also associated with multiple congenitally missing teeth, such as a severe form of autosomal-dominant oligodontia. Nonsense mutation in MSX1 gene with transversion mutation (C→A substitution) at codon no. 314 resulted in Ser 105 Stop codon(premature chain termination) observed in patient with orofacial clefting and tooth agenesis. The substitution of a C-nucleotide with A caused a nonsense mutation, resulting in an altered protein with the loss of C-terminus, which led to severe teeth abnormalities, as well as non-syndromic cleft lip and cleft palate. Another patient with oligodontia showed substitution or replacement of T→A at codon no. 182, resulting in Met61Lys transversion mutation within a highly conserved region. Patient showed alteration in second premolars and third molars, which might have been due to the altered interaction of MSX1 protein with other transcription factors. Many other mutations, such as A194V resulting frame-shift mutation of G22RFsX168 were also identified in patients with tooth agenesis. Several studies show that the nonsense alteration in genes is responsible for reducing the size of functional protein (haploinsufficiency) and that the DNA sequence will be targeted in a false manner by the mutated protein. Patients with tooth agenesis and nail dysplasia carried transversion mutation in MSX1 gene at nucleotide 605 (C→A) with premature chain termination at homeodomain region. This mutation resulted in the lack of the required protein (haploinsufficiency) and was precarious for the proper functioning of MSX1 gene, thus resulting in tooth agenesis and nail dysplasia. Missense and transverse (c.662C>A) mutation within highly conserved homeobox sequence of
MSX1 was also identified in one patient with autosomal dominant oligodontia. Another missense mutation was detected in the Pakistani families affected with oligodontia. Missense mutations at nucleotide c.1091 T → C (p.M364T) were identified in the homeobox of MSX1 gene in two Pakistani families with hypodontia.

PAX9 gene anomalies belong to tooth agenesis

It was observed that PAX9 gene belonged to paired box families and encoded transcription factor that was necessary for positioning, morphogenesis of entire dentition and proper tooth development. Exon 2 of PAX9 genes contain a sequence of specific DNA-binding domain; the defects in paired domain of PAX9 gene lead to tooth agenesis. Studies show that the deletion of PAX9 gene and mutation in initiation codon are closely associated with the most severe defects in the whole post-canine dentition. However, the effects of missense mutations are less severe than those of nonsense and frame-shift mutation. This paper discusses some of the unique reported mutations, like frame-shift, insertion, missense, nonsense and deletions of entire PAX9 gene. These mutations were identified in the DNA-binding paired domain of PAX9 gene, resulting in a disturbed regulatory process occurring for tooth formation. Missense mutations in PAX9 gene at amino acid position Gly6Arg (G6R) and Ser43Lys (S43K) were detected in two Chinese patients with non-syndromic tooth agenesis. Patients and their family members affected with oligodontia and other dental anomalies were carrying transition and nonsense mutation at C175T, resulting in an altered arginine 59 Stop codon, thus leading to premature chain termination (haploinsufficiency) in PAX9 gene. A patient with oligodontia showed missense (C139T transition) mutation with the substitution of an arginine by a tryptophan (R47W) in the paired domain of PAX9 gene and showed dramatically reduced DNA-binding activity. It was assumed that altered gene introduced a non-functional protein, which lacked DNA-binding motif. Nonsense mutation was identified in PAX9 gene in patients with severe forms of hypodontia. Nonsense mutation with premature chain termination (c.433C>T) of PAX9 gene was identified in paired domain region as a result of the Q145X. In this patient, rapid degradation of truncated protein was found, which might inhibit the interchange of PAX9 protein with DNA. A → T transverse at codon no. 340 for pre-mature chain termination at Lys 114 was identified in a patient. Due to nonsense mutation of PAX9 gene, corrupt information is carried, destroying the function of protein; thus, developing the partial lack of permanent first molar and second premolar and lack of all second and third permanent molars, as well as reduced teeth size. They were showing missing C-terminal region, which is considered as essential for proper tooth development. This change may form an electrostatic and hydrophobic interaction with sugar and phosphate, respectively. Due to the loss of C-terminus of PAX9 protein, severe tooth agenesis is developed. Another study shows the insertion of C at nucleotide 793 in codon no. 315. Subsequently, nonsense mutation in exon 4 of PAX9 gene was detected with partial first molars and second premolars and the absence of second and third permanent molars. This result showed nonsense mutation (haploinsufficiency) with the insertion of C at codon no. 315 in PAX9 gene, thus carrying corrupt information resulting in an altered protein at C-terminus. Missense mutations (T62C and A271G) with substitution in highly conserved region within paired domain were identified in two patients with tooth agenesis. These mutations altered the protein sequence affecting the DNA-binding capacity of PAX9, and thus, delivering altered information for phenotypic features. Premature chain termination at amino acid 177 was identified due to the insertion of nucleotide in the patient with hypodontia. Nonsense mutations are critical for the proper functioning of protein; they also convey corrupt information resulting in severe tooth agenesis. Literature shows that nonsense mutations are more treacherous than other mutations. Substitution of nucleotide G to A at codon no. 151 showed Gly to Ser at amino acid 51 in PAX9 protein localized in helix-turn-helix motif of the N-terminal. Another patient showed heterogygotic (718G>C) missense (transversion) mutation resulting Ala240Pro substitution in the patient with tooth agenesis. Frame-shift mutation with the insertion of C at nucleotide 793 in exon 4 of PAX9
gene was observed, resulting in premature chain termination that occurred in patients with non-syndromic hypodontia. So far, many heterozygous mutations have been identified in the PAX9 gene, most of which are associated with either familial or non-syndromic form of tooth agenesis. It was observed that different types of mutations in genes caused variations in DNA-binding bustle, thus leading to tooth agenesis.

**Other genes anomalies associated with tooth agenesis**

Some recent studies show different types of mutations in several genes such as EDA, WNT10A, AXIN2, LTBP3 and TP63 either in patients with oligodontia or in various forms of tooth agenesis. One patient showed mutation with the insertion of four nucleotides in exon 1 (c.119-120ins TGTG) resulting in frame-shift mutation (p.L40fsX100). Missense mutations (c.1141G>C) were found in exon 9 at 1141 position with substitution of Glycine381Arginine. Another study showed an alteration in the functional domain of EDA with five missense mutations (c.200A > T, c.463C > T, c.758T > C, c.926T > G and c.491A > C) in patients with tooth agenesis. The patient with non-syndromic hypodontia showed EDA gene mutation at Thr338Met. Missense (c.993G > C) mutation with substitution of glutamine with histidine (p.Q331H) of EDA gene was also identified in patients with non-syndromic hypodontia. Another patient with hypodontia showed missense (c.1091T→C; p.M364T) mutation in EDA-A1 gene. Recent study showed four base-pairs deletion (c.718-721delAAAG) in EDAR gene family and one missense mutation (c.T1091C; p.M364T) in EDA gene. Another three novel EDA gene (p.Ala259Glu, p.Arg289Cys and Arg334His) mutation was detected in patients with non-syndromic oligodontia. EDA gene encodes the protein ectodysplasia-A (EDA), a member of tumor necrosis factor (TNF) superfamily. TNF homology domain is required for interaction with receptor. It was observed that most of the mutations were located within the TNF domain of EDA; besides, it was assumed that these mutations may affect the interaction of EDA with its receptor, thus resulting in tooth agenesis.

**CONCLUSION**

The studies reviewed strongly emphasize on reported causative mutation in MSX1, PAX9, AXIN2, and EDA genes and their effects on high prevalence rate of various types of tooth agenesis. The high rate of gene anomalies acts as a risk factor, leading to various types of congenital teeth anomalies, such as hypodontia, oligodontia, anodontia, etc. Therefore, molecular genetic analysis of different genes, such as MSX1, PAX9, AXIN2, EDA and some other genes are useful in minimizing the risk of transmitted genetic anomalies. It is strongly suggested that genes and epigenetic screening should be performed in future for better diagnosis, preventive, counseling and treatment approaches.
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