Original Article

Mutations in the *MSX1* gene in Turkish children with non-syndromic tooth agenesis and other dental anomalies

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Abstract

Aim: To search for mutations on the *MSX1* gene and to present a genetic basis for non-syndromic tooth agenesis in conjunction with dental anomalies in a Turkish population. **Materials and Methods:** The patients included in this study were otherwise healthy, with ages ranging from seven to eighteen years. Eighty-two of them had one to six teeth missing (Group I) and 26 had more than six teeth missing (Group II), except for the third molars,. The missing teeth and dental anomalies were examined clinically and radiographically. The *MSX1* gene was sequenced from the blood samples of patients who consented to the study. **Results:** Mutations or polymorphisms on the *MSX1* gene were identified in six patients. Taurodontism was seen in patients from both groups I and II. The nucleotide changes were identified by mutation screening. **Conclusions:** Performing family studies, screening other candidate genes, and investigation of interactions between genes will provide a basis for better analysis of tooth agenesis models and their association with other dental anomalies.

Key words: Dental anomalies, *MSX1*, tooth agenesis

INTRODUCTION

Odontogenesis begins during the second month of embryogenesis and ends with the eruption of the third molar teeth at adolescence. The developmental process is arranged in a manner similar to that of the ectodermal organs, which are managed by tissue interactions and genetic systems.[1,2] During odontogenesis, abnormalities with regard to the number, size, shape, structure, and color of the teeth, may occur due to various factors.[3] Tooth agenesis or absence of one or more tooth germs, is the most common developmental abnormality that leads to great challenges.[4-6]

Hypodontia is described as having up to six teeth missing, excluding the third molars,[4,7] whereas, oligodontia is defined as having more than six teeth missing, excluding the third molars,[8-10] and anodontia is explained as the absence of all teeth.[11]

In many studies, the most commonly missing teeth, excluding the third molars, have been found to be the mandibular second premolar, maxillary lateral incisor, and maxillary second premolar teeth. Even as the maxillary central incisor, maxillary and mandibular first molar, and canine teeth are known to be rarely missing,[12-20] they are generally observed to be absent in cases of oligodontia.[4] In oligodontia, two of every three missing teeth have been reported to be second premolar or maxillary lateral incisor teeth.[21]

Missing teeth have been reported to cause abnormal occlusion and have negative effects over the growth and development of the alveolar bone and craniofacial structures.[4,22-27] In addition, it has been reported that many dental abnormalities may be encountered along with the missing teeth. These dental abnormalities are, delayed dental development and eruption,[8,19,28-30]

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differences in tooth size and form,[12,19,31-39] abnormally positioned teeth,[34,37,40-48] infraocclusion of the deciduous molars,[19,34,44] teeth with short roots,[49,50] taurodontism,[19,38,51-54] dens invaginatus,[54,55] tooth rotation,[56] and hypocalcification and hypoplasia of the dental tissues.[34,57]

Furthermore, in the databases, it has been reported that missing teeth may be accompanied by 150 different syndromes.[54]

In recent times, besides epidemiological studies, there have also been genetic studies focusing on missing teeth. Reports suggest that both enviromental and genetic factors play a part in the etiology of missing teeth, while indicating involvement of ethnic differences, as well.[54,58-60]

To date, more than 300 genes have been associated with odontogenesis.[61] The most commonly studied genes with regard to odontogenesis are the *HOX* genes. The *Muscle Segment Homeobox (MSX)* gene is an important member of the *HOX* gene family. Among humans, two *MSX* genes have been isolated: *MSX1* and *MSX2. MSX1* is reported to encode some of the transcription factors required during various phases of dental development, such as, patterning, morphogenesis, and histogenesis.[62-66] Furthermore, *MSX1* mutation in humans is noted to cause severe generalized tooth agenesis.[67]

In many countries, studies investigating the genetic factors involved in cases of missing teeth have revealed genetic defects responsible for various types of missing teeth. To our knowledge, in Turkey, there has been no study investigating the mutation in the *MSX1* gene among people with missing teeth, with presenting results and dental characteristics. The aim of this study is to identify the mutations on the *MSX1* gene, which play an important role in dental development and have been associated with tooth agenesis in children and adolescents with missing teeth, and to present the genetic basis of non-syndromic tooth agenesis in conjunction with dental anomalies, using a population from Turkey. As our study is not an epidemiological one, we only aim to present both dental and genetic characteristics of individuals with missing teeth.

MATERIALS AND METHODS

Patient selection and study groups
In this study, files of 452 patients, who presented to the Department of Pedodontics, Faculty of Dentistry, Süleyman Demirel University (SDU), and who were eventually diagnosed as cases of missing teeth, based on radiographs, were reviewed. Otherwise healthy patients (no systemic disease or syndrome) with at least one congenital permanent missing tooth, excluding the third molars, and a history of completed or ongoing treatment were selected. The approval of the Ethics Committee of the Faculty of Medicine at SDU was obtained for the study (dated April 16, 2008, and numbered decision 03/09). The selected patients were invited to our clinic. The ones who were available and willing to participate in the study were included in the study after obtaining a written informed consent. The patients who were healthy based on their first medical evaluation record, but developed medical problems later on, had a suspicious medical history, had missing teeth due to unknown reasons, or those that dropped out of the study after giving consent were excluded from the study. Furthermore, individuals who had fluorosis on their teeth or those who lived in regions known to have a tap water with high fluoride levels, were also excluded from the study.

The study population, aged between seven and eighteen years, was split into two groups: Group I was comprised of individuals with one to six missing teeth excluding the third molars (hypodontia) [Figure 1] and Group II was comprised of individuals with more than six missing teeth, excluding the third molars (oligodontia) [Figure 2].

Group I had 82 individuals (54 females and 28 males) and Group II had 26 individuals (13 females and 13 males) that met the criteria of the study.

Definition of the characteristics of missing teeth
In addition to the medical and dental history taking, clinical and radiological examinations of the missing teeth were also performed and recorded on new forms. The radiological examinations were carried out by evaluating the panoramic views obtained by an X-ray device (Panoramic X-ray Unit, Planmeca Oy, Helsinki, Finland) on a negatoscope (Illuminator 5000, RP Beard Ltd, London, UK).

Evaluation of dental anomalies
In total, 108 cases (82 with hypodontia and 26 with oligodontia) were clinically and/or radiologically reviewed for dental anomalies, such as, differences in tooth size or form (conical or wedge-shaped teeth, changed number of tubercles), short root, taurodontism, dens invaginatus, hypocalcification and hypoplasia of the dental tissues, abnormal position or rotation of the teeth, and infraocclusion of the deciduous molars.
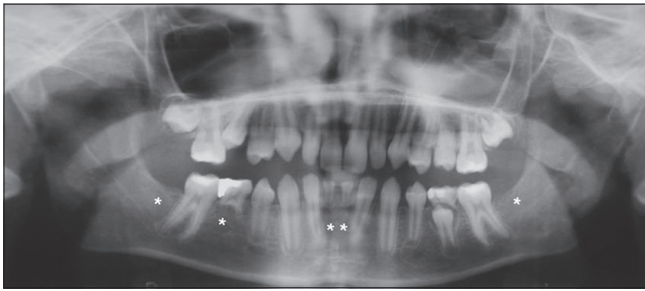
Figure 1: In Group I, the panoramic film of a 11-year-old female individual with five missing teeth excluding the third molars (hypodontia)
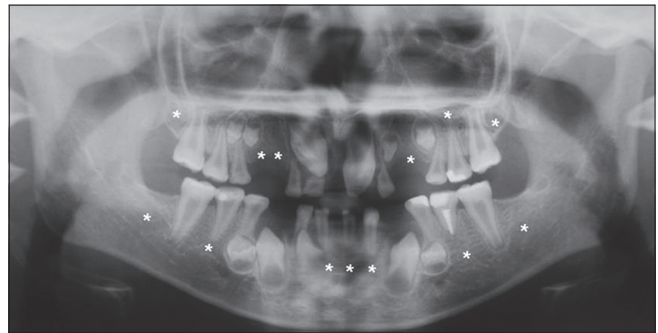

Figure 2: In Group II, the panoramic film of an eight-year-old male individual with 13 missing teeth excluding the third molars (oligodontia)

Screening for *MSX1* mutation
Study group selection
In 44 patients (33 females and 11 males) in Group I and 26 patients in Group II (13 females and 13 males), who gave consent for blood collection, a blood specimen of 2 ml, required for Deoxyribonucleic acid (DNA) isolation, was obtained, and the specimens were put into ethylenediamine tetra acetic acid (EDTA) containing tubes.

In children with normal dental development, who showed no tooth agenesis phenotype clinically or radiologically, blood collection was not applied, as the likelihood of a mutation presence in the *MSX1* gene was very low,[68] and moreover, the parents of the children did not give consent for the blood collection required for DNA isolation.

Deoxyribonucleic acid isolation
Deoxyribonucleic acid isolation was performed on the collected blood specimens by using a DNA isolation kit (Gentra PureGene Blood Kit, Minneapolis, MN).

Single nucleotide polymorphism genotyping and automated Deoxyribonucleic acid sequencing analysis
The acquired DNA samples were processed to amplify the nucleotide sequences of the two coding exons of the *MSX1* gene by applying the polymerase chain reaction (PCR) method, using appropriate primers [Table 1]. The patients with nucleotide changes were determined by the SNP genotyping method, using the real-time PCR device (Bioneer, ExiCycler™-96, Korea). Those patients were subjected to a double-stranded DNA sequence analysis by the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems) in order to detect a mutation. The chromatograms obtained from these analyses were evaluated by comparing them with the ones showing normal nucleotide sequences.

RESULTS

The mean ages in Group I (*n* = 82, 54 females and 28 males) for females, males, and in total, were

Table 1: *MSX1* gene and primer sets for amplification

MSX1 gene		
Exon	2	
Transcript length	1,926 base pairs	
Translation length	303 aminoacids	
Localization on chromosome 4	4 912 307-4 916 564	
Primer sets for the *MSX1* gene		
Exon 1F:	5'-CCCGGAGCCCATGC CCGGCGGCTG-3'	Binding temperature
Exon 1R:	5'-CTCCCTCTGCGCCT GGGTTCTGGCT-3' [(+%5 Dimethylsulfoxide (DMS0)]	64°C
Exon 2F:	5'-TGATCATGCT CCAATGCTTC-3'	Binding temperature
Exon 2R:	5'-CACCAGGGC TGGAGGAAT-3'	58°C

MSX1: Muscle segment homeobox 1

11.85 ± 0.38, 11,57 ± 0.57, and 11.76 ± 0.32, respectively. The mean ages in Group II (*n* = 26, 13 females and 13 males) for females, males, and in total were, 13.31 ± 1.00, 14.46 ± 0.67, and 13.89 ± 0.60, respectively.

Definition of the characteristics of missing teeth
The most common missing teeth in Group I were the mandibular second premolars, followed by maxillary lateral incisors, maxillary second premolars, and mandibular central incisor teeth, respectively. The least common missing teeth were the mandibular first molars.

The most common missing teeth in Group II were the mandibular second premolars, followed by the maxillary second premolars, maxillary lateral incisors, and the mandibular central incisior teeth, respectively. The least common missing teeth were the maxillary central incisors.

Dental anomalies
In Group I (*n* = 82), which included individuals with hypodontia, 29 (35.4%) had conical/wedge-shaped

teeth or teeth with changes in the tubercle number, eight (9.8%) had short roots in various teeth, 40 (48.8%) had taurodontism in the molars, 32 (39%) had dens invaginatus in the maxillary incisors, and 24 (29.3%) had primary teeth with infraocclusion.

In Group II (*n* = 26), which included individuals with oligodontia, 18 (69.2%) had conical/wedge-shaped teeth or teeth with changes in the tubercle number, three (11.5%) had short roots in the maxillary central incisors and premolars, 12 (46.2%) had taurodontism in the molars, four (15.4%) had dens invaginatus in the maxillary incisors, and 11 (42.3%) had primary teeth with infraocclusion.

None of the groups demonstrated hypoplasia or hypocalcification of the dental tissues. Although data concerning the abnormal position and rotation of the teeth were recorded, as there were many factors involved in these anomalies, we did not want to present the results, because they could be misleading.

MSX1 mutation
Single nucleotide polymorphism genotyping method results
The evaluation of the data obtained from the SNP genotyping method revealed that among the 70 individuals that we included in our study, six (8.6%) had nucleotide changes in *MSX1* gene, whereas, the others were found to show no nucleotide change in this gene. Among the six individuals who had nucleotide sequence change (five heterozygotic and one homozygotic genotypes), four (9.1%) were in Group I and two (7.7%) were in Group II [Table 2].

Table 2: SNP genotyping data obtained using real-time PCR device, and characteristics of individuals with nucleotide sequence change

Group	Sample number	Genotype	Missing teeth	Dental anomalies
I	DHD-012	Heterozygote	45	Taurodontism
I	DHD-021	Heterozygote	15, 12, 22, 25	Taurodontism
I	DHD-022	Heterozygote	15, 12, 22, 25, 35, 45	Taurodontism Dens invaginatus
I	DHD-040	Heterozygote	15, 25, 35	Taurodontism Dens invaginatus
II	DHD-003	Heterozygote	17, 16, 15, 14, 12, 22, 24, 25, 26, 27, 37, 36, 35, 45, 46	Taurodontism Differences in tooth form Infraocclusion
II	DHD-004	Homozygote	17, 15, 14, 12, 22, 24, 25, 26, 27, 37, 35, 45, 46, 47	Taurodontism Differences in tooth form Infraocclusion

SNP: Single nucleotide polymorphism, PCR: Polymerase chain reaction

Data acquired by automated Deoxyribonucleic acid sequence analysis
Deoxyribonucleic acid (DNA) sequence analysis performed on the six individuals with nucleotide sequence change showed that there was a base change in four different areas in the coding region of the first exon and one base change in the uncoded 3' untranslated region (UTR) region of the second exon.

In Group I, the individual with mandibular right second premolar tooth missing and taurodontism, who was represented by the sample number DHD-012 showed a cytosine-to-guanine change (c. 119C > G) at nucleotide 119, in the coding region of the first exon, and an alanine-to-glycine change at codon 40 (Ala40Gly) [Figure 3]. To our knowledge, this nucleotide change, which we define as a missense mutation, has not been reported in literature.

There was a cytosine-to-thymine change at nucleotide 347 in the coding region of the first exon (c. 347C > T) and the coding codon of glycine at position 116 was observed to change into another codon still coding glycine (Gly116Gly), in Group I, for sample DHD-021, who had maxillary lateral incisor and second premolar teeth missing and taurodontism [Figure 4]. This silent mutation has not yet been reported in literature.

There was a cytosine-to-adenine change at nucleotide 463 in the coding region of the first exon (c. 463C > A) and a proline-to-glutamine change at position 155 (Pro155Gln) in Group I for sample DHD-022, with the maxillary lateral incisor and all the second premolar teeth missing, taurodontism, and dens invaginatus, and for sample DHD-040, with maxillary second premolar teeth and mandibular left second premolar tooth missing, taurodontism, and dens invaginatus [Figure 5]. As there was no such missense mutation reported in the available literature or in the genetic databases (www.hgmd.cf.ac.uk and www.ensembl.org), we defined this as a novel mutation.

There was a cytosine-to-thymine change at nucleotide 95 in the coding region of the first exon (c. 95C > T) and an alanine-to-valine change at codon 32 (Ala32Val) in Group II for sample DHD-003, which demonstrated 16, 15, 14, 12, 22, 24, 25, 26, 27, 37, 36, 35, 45, and 46 numbered teeth missing, taurodontism, differences in tooth shape, and infraocclusion [Figure 6]. As there was no such missense mutation reported in the available literature or in the genetic databases (www.hgmd.cf.ac.uk and www.ensembl.org), we defined this as a novel mutation.

There was a cytosine-to-thymine change in the uncoded 3' UTR region of the second exon (c. *6C > T) in Group II
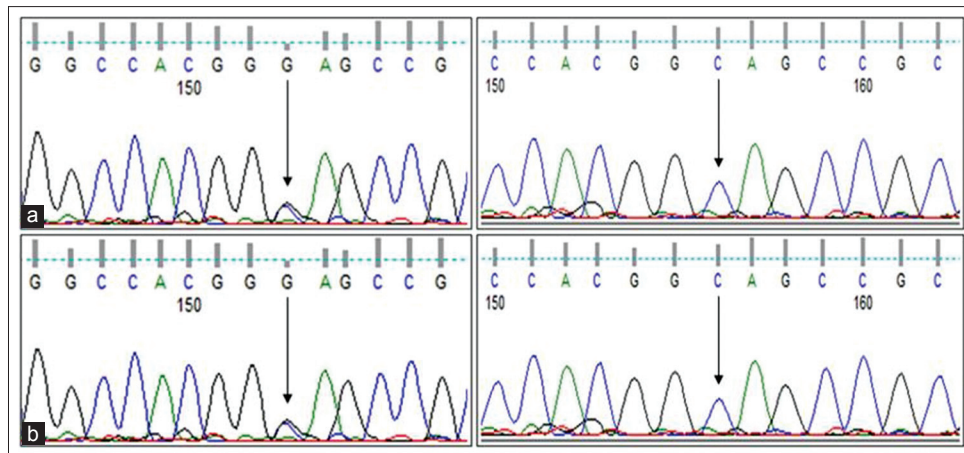
Figure 3: (a) The chromatogram showing heterozygote c. 119C > G (Ala40Gly) nucleotide change belonging to the individual who was represented by sample number DHD-012. (b) The chromatogram shows the normal nucleotide sequence for the same region
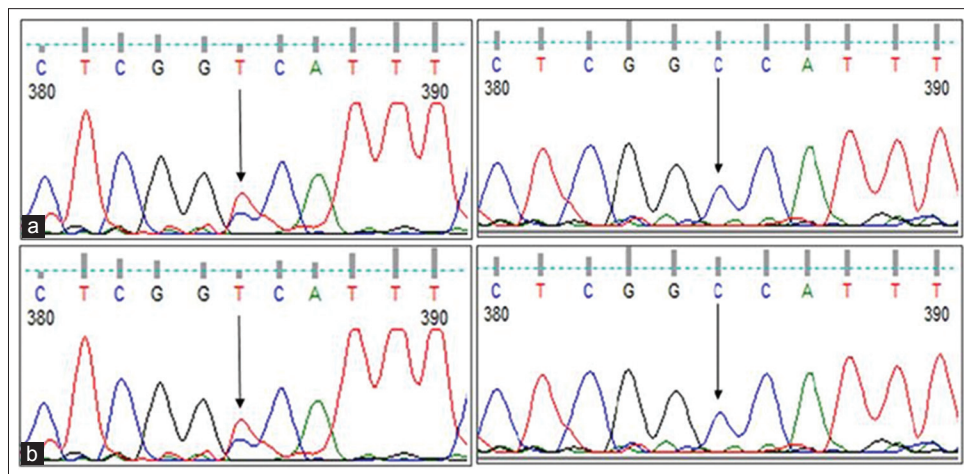

Figure 4: (a) The chromatogram showing heterozygote c. 347C > T (Gly116Gly) nucleotide change belonging to the individual who was represented by sample number DHD-021. (b) The chromatogram shows the normal nucleotide sequence for the same region
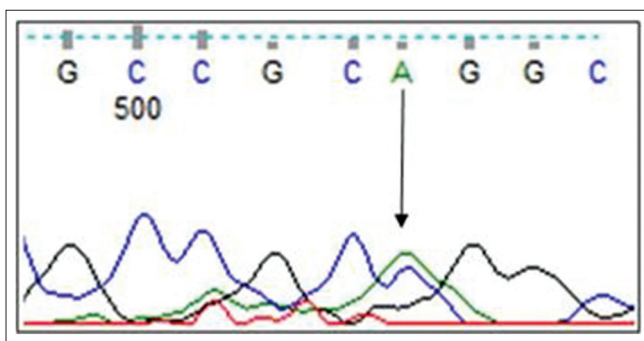

Figure 5: The chromatogram showing heterozygote c. 463C > A (Pro155Glu) nucleotide change belonging to the individuals who were represented by the sample numbers DHD-022 and DHD-040

for sample DHD-004, which had 17, 15, 14, 12, 22, 24, 25, 26, 27, 37, 35, 45, 46, and 47 numbered teeth missing, taurodontism, differences in tooth shape and infraocclusion [Figure 7]. This base change was determined to be a polymorphism recorded with the number rs8670 in the www.ensembl.org database.

The DNA sequence chromatograms of patients with mutation and polymorphism were presented comparatively with the chromatograms of individuals with a normal nucleotide sequence.

DISCUSSION

During odontogenesis, the interaction of genetic, epigenetic, and environmental factors may lead to abnormalities associated with the number, size, form, and/or structure of the teeth. Infections such as rubella, diseases such as syphilis,[69-71] chemotherapy and radiotherapy,[72-74] usage of thalidomide[R] (N-phthaloylglutamimide) during pregnancy,[72] exposure to dioxins,[75-78] different types of traumatic injuries, such as, fractures, surgical procedures, and extraction of deciduous teeth,[8,12] problems associated with the nerve tissue affecting the innervation of the jaws, oral mucosa, and supporting tissues playing a part in the development of teeth,[79,80]
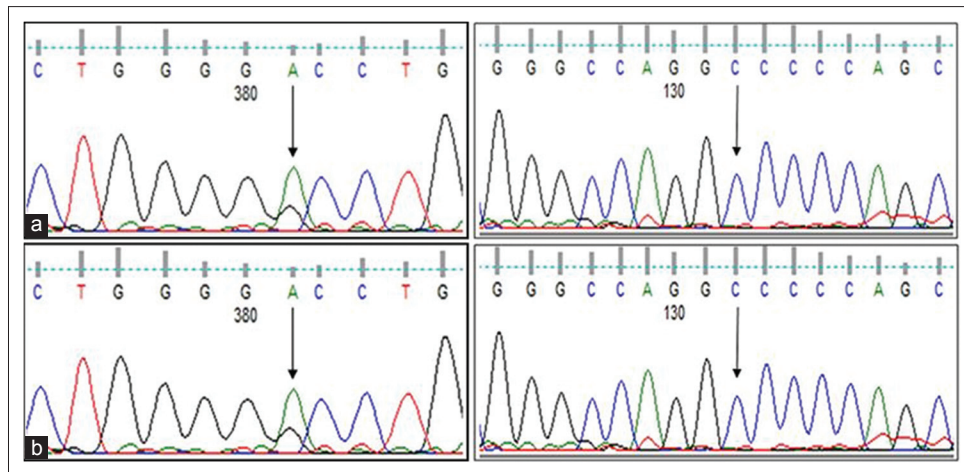
Figure 6: (a) The chromatogram showing heterozygote c. 95C > T (Ala32Val) nucleotide change belonging to the individual who was represented by sample number DHD-003. (b) The forward chromatogram shows the normal nucleotide sequence for the same region
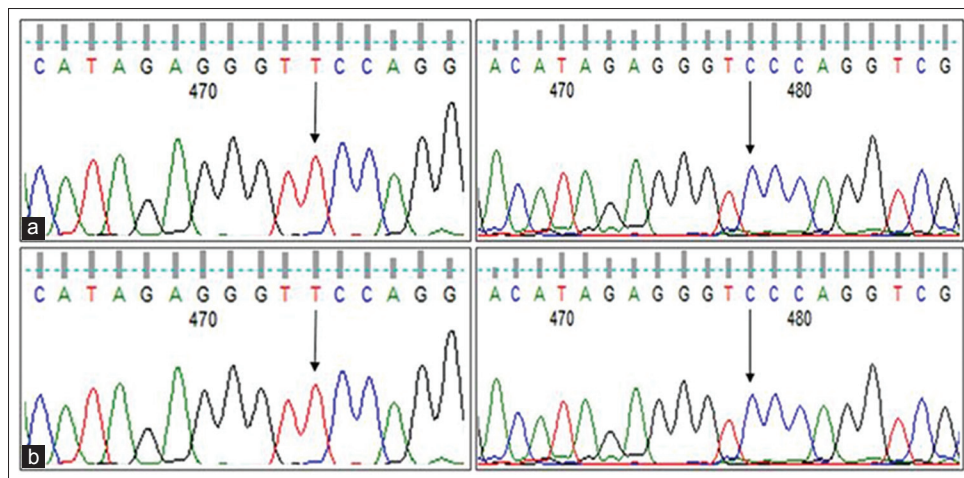

Figure 7: (a) The chromatogram showing homozygote c.*6C > T base change in the 3' UTR region belonging to the individual who was represented by sample number DHD-004. (b) The chromatogram shows the normal nucleotide sequence for the same region

have been reported as environmental factors that cause discontinuance of dental development.

In recent times, there has been an increasing focus on studies aiming to determine gene mutations underlying missing teeth. Tooth agenesis in humans has been noted to be an outcome of genes, either acting separately or in combination with other genes, and leading to a specific phenotype.[81] One of the first genes defined to be involved in missing teeth is *MSX1*, a transcription factor gene.[82] There are studies performed on different populations, which support the association of the *MSX1* gene and missing teeth. Focusing on a literature screen of other mutations found in other populations will be useful to understand the genetic basis of tooth agenesis.

In United States of America, Vastardis *et al.*[82] conducted a study on a family with second premolar

and third molar teeth missing, as an autosomal dominant feature, and searched for a mutation in the *MSX1* gene. In their study, some family members were also reported to have maxillary first premolar, mandibular first molar, one or two maxillary lateral incisors or one mandibular central incisor teeth missing, while showing normal primary dentition. The family members were found to have a missense mutation characterized by arginine-to-proline change at position 31 in the second exon of the *MSX1* gene (Arg31Pro). The *MSX1* gene was noted to have a very important part in the normal dental development.

In Holland, van den Boogard *et al.*[83] studied the *MSX1* gene in a family with frequently second premolar and third molar teeth missing along with different variations of cleft lip or palate. A heterozygotic alteration, reflected by a change from cytosine to adenine at nucleotide 752 (c. 752C > A) and a change from

serine to stop codon at position 105 (Ser105Stop) in the first exon of the *MSX1* gene, was determined. In family members without missing teeth or cleft lip and palate, no mutation was found. It was reported that *MSX1* gene may be associated with tooth agenesis and oro-facial clefts, while the need for further detailed analyses was underscored, as well.

In United States of America, Lidral and Reising[59] performed mutation screening of the *MSX1* gene among 92 individuals from different families. who had at least one missing permanent tooth. excluding the third molars. Seventy-four percent demonstrated one to two missing teeth, whereas, only 10% had five or more missing teeth. The most common missing teeth were the mandibular second premolars followed by maxillary lateral incisors and second premolars, respectively. There was a mutation resulting in a change from thymine to adenine at nucleotide 620 (c. 620 T > A) and a change from methionine to lysine observed at codon 61 in the first exon of the *MSX1* gene in two of the individuals, who were siblings. All the seven other members of the family of those siblings, who exhibited oligodontia and mutation, were found to have the same mutation. Therefore, they concluded that the mutations in the *MSX1* gene played a role in familial tooth agenesis, while having no influence in cases with one or two missing teeth.

Kim *et al.*[68] studied *MSX1* gene mutation in an American family with members having autosomal dominant oligodontia without any systemic disease or syndrome. Two members of this family, who predominantly had second premolars and mandibular central incisors missing, in addition to other missing teeth, were found to have a frameshift mutation (g. 62dupG, p.G22RfsX168) in the first exon of the *MSX1* gene.

In Belgium, De Muynck *et al.*[84] investigated the association of the *MSX1* gene with missing teeth and a cleft lip and palate in 55 members from 40 families. In the second exon of the *MSX1* gene, a cytosine-to-thymine change at nucleotide 559 (c. 559C > T) was observed and the glutamine-coding codon at position 187 was found to be replaced by a stop codon (Gln187Stop) for one of the 40 studied families. In this family, this mutation was determined in three members having many missing teeth, with or without cleft lip and palate, and these individuals were found to have an heterozygotic genotype. *MSX1* gene mutation was not found to be a frequently observed factor involved in familial hypodontia or cleft lip and palate.

In Poland, Mostowska *et al.*[85] studied the *MSX1* gene mutation in an individual with 14 missing teeth, including the third molars, and the family members. The patient who had no systemic disease or syndrome and normal primary dentition, was reported to have 18, 15, 14, 24, 28, 38, 37, 35, 34, 31, 41, 45, 47, and 48 numbered teeth missing, 11-21 numbered teeth with a smaller size, and 17-27 numbered teeth with delayed dental development, while the family members were represented to have normal primary and permanent dentition. As the result of the study showed, this individual with heterozygotic genotype and the parents were found to have a mutation resulting in cytosine-to-thymine change at nucleotide 581 (c. 581C > T) and an alanine-to-valine change at codon 194 (Ala194Val) in the second exon of the *MSX1* gene. As for other family members, two previously described polymorphisms in the *MSX1* gene (c. 452-15delT and c.*6C > T) were detected. It was concluded that mutations responsible for oligodontia could present with absence of penetrance and show an oligogenic character, due to simultaneous mutations in different genes.

Chisti *et al.*[86] performed a study in Pakistan and investigated *MSX1* gene mutation in two families related to each other: Five individuals having dental anomalies and autosomal recessive form of oligodontia and eight individuals with no missing teeth. A mutation resulting in guanine-to-alanine change at nucleotide 655 (c. 655G > A) in the second exon of the *MSX1* gene for five individuals with missing teeth was detemined. This heterozygotic variation was found to cause an alanine-to-thyronine change at codon 219 (Ala219Thr), leading to a missense mutation. Via this study, *MSX1* gene mutation was reported for the first time in individuals with autosomal recessive form of missing teeth associated with dental anomalies.

In the study of Xuan *et al.*,[87] in China, *MSX1* gene mutation was investigated in one individual with 17 missing teeth, including the third molars, and the family members. This individual had no systemic disease or syndrome, but had 18, 13, 12, 11, 21, 22, 28, 38, 35, 33, 32, 31, 41, 42, 43, 45, and 48 numbered teeth missing. The number of missing teeth was nine in the grandfather, 13 in the mother, and nine in the uncle. The grandfather had wedge-shaped maxillary lateral incisors. The most common missing teeth were the mandibular second premolars followed by maxillary second premolars, maxillary lateral incisors, and maxillary first premolars, respectively. Mutation screening revealed that in all the family members with missing teeth, there was a missense mutation (c. 662C > A) leading to an alanine-to-glutamic acid change at amino acid 221 in the second exon of the *MSX1* gene (Ala221Glu). Furthermore, a c. 347C > G polymorphism was detected in the *MSX1* gene. This

new heterozygotic change determined in the *MSX1* gene was deemed to have a potential to be the likely cause of various types of missing teeth. Wedge-shaped maxillary lateral incisor teeth were evaluated as a phenotypic expression unlike congenital missing teeth, associated with the same autosomal dominant gene involved in reduced penetrance. The family members without missing teeth showed no such mutation. The detected mutation was thought to be responsible for the phenotype of oligodontia.

In Poland, Pawlowska *et al.*[88] conducted a study, wherein, *MSX1* gene mutation was scanned in three individuals with various missing teeth, other than the third molars: The first patient had the second premolars and mandibular first premolars missing; the second patient had the maxillary lateral incisors, mandibular central incisors, and mandibular second premolars missing; and the third patient had the first premolars, maxillary canines, mandibular central and lateral incisors, and the mandibular left second premolar missing. The families of these three individuals had no missing teeth other than the third molars. The first patient exhibited a homozygous variation resulting in the deletion of 11 nucleotides in the intronic region (del1740-751) and another homozygous 3755A > G change in the second exon. The second patient showed a heterozygous 218G > A variation in the first exon, a heterozygous thymine deletion in the intronic region (3014 delT), and another heterozygous 3485C > T variation in the second exon. Furthermore, similar to the first patient, second patient was also determined to have 11 nucleotide deletions. The third patient demonstrated no mutation. The deletion of 11 nucleotides was found to be capable of reducing the expression of the *MSX1* protein, however, its relationship with oligodontia could not be shown due to requirement for further studies.

Furthermore, there are studies reporting no relationship between mutation in the *MSX1* gene and missing teeth.[60,89-97]

In our study, we detected two missense mutations (Pro155Gln and Ala32Val) that were determined in a patient group for the first time, two polymorphisms in the coding region (Ala40Gly and Gly116Gly) that were not previously determined in patient groups, and one known polymorphism in the 3' UTR region. Detailing the characteristics of those amino acids involved in the mutation and polymorphisms should contribute to revealing the relationships between missing teeth and the *MSX1* gene.

Alanine, valine, and glycine aminoacids are of a hydrophobic character and they differ with regard to molecular size. Valine is an aliphatic amino acid with a branched chain. It has a more effective role than alanine in intramolecular and intermolecular hydrophobic interactions. Glycine is the smallest amino acid that is particularly placed over the folds of protein structures. In the alanine-glycine replacement, glycine exhibits a lower steric interaction than that of alanine. We believe that these aminoacid changes may have an influence over the phenotype by causing changes in the three-dimensional structure and folding of the *MSX1* protein.

Proline is a non-polar and aliphatic amino acid that is particularly responsible for the rigidity and coiling of the protein structure. Glutamine is a nonpolar amino acid with a side chain. The hydrophilic functional group (R group) in the glutamine amino acid can form hydrogen bonds with water and other polar molecules. The strong hydrophilic feature of glutamine enables this amino acid to either rise to the surface of the protein or submerge deep into the protein. Although proline–glutamine change appears to be ineffective in intramolecular or intermolecular covalent formations, it is thought that it may affect the three-dimensional structure of the protein due to differences arising from the hydrophobicity. Such a change in the three-dimensional structure of *MSX1*, which acts as a transcription factor, may have a role in its activation, by influencing its bonding with the target DNA.

In literature, many of the mutations in the *MSX1* gene have been reported in individuals with oligodontia within the same family. However, in our study, we investigated individuals with varying number of missing teeth who were not relatives, and this allowed us to better understand and evaluate the effects of the *MSX1* gene on tooth agenesis.

The *MSX1* gene has been mainly associated with missing third molars and second premolars.[82,84,98] In the present study, all of the individuals that we have detected with mutation or polymorphism, had at least one second premolar missing. However, when patients with other missing teeth (hypodontia or oligodontia) are considered, the opinion proposing that the *MSX1* gene affects the second premolars more often, appears to be misleading. Similarly, missense mutations determined in the DHD-022 and DHD-040 samples, may be thought to be associated with the agenesis of 15, 25, and 35 numbered teeth as well as taurodontism and dens invaginatus (common characteristics of these individuals). Nonetheless, the absence of a mutation in individuals with the same characteristics, indicates that such a proposition would not be correct. Unless familial studies are performed and other genes associated with the missing teeth

are investigated in such individuals, determining the phenotypic characteristics of nucleotide changes in the *MSX1* gene appears to be impossible. In Group I, which included individuals with hypodontia, the nucleotide change in the sample DHD-012, obtained from the individual who had only the mandibular right second premolar tooth missing, was not supportive of the report by Lidral and Reising,[59] who argued that mutations in the *MSX1* gene did not have any role in cases with one or two missing teeth. This may be a consequence of the genetic-based differences between populations.

It has been emphasized that genetic studies focusing on tooth agenesis must perform a thorough evaluation for characteristics of missing teeth and dental anomalies in the present teeth in syndromic or non-syndromic tooth agenesis forms.[81] Many of the individuals in our study had various dental anomalies associated with tooth agenesis. Detection of taurodontism by mutation screening in patients with nucleotide change both in Group I and II suggests that taurodontism may be sharing the same genetic etiology with tooth agenesis. As a result of clinical and radiographical examination, individuals who are shown to have missing teeth and taurodontism, can be recognized as candidates for mutation screening in the *MSX1* gene.

The nature of tooth agenesis is regarded as a heterogenous event, both genotypically and phenotypically. It is supposed that the wide variation observed in tooth agenesis and the association between tooth agenesis and other dental anomalies can be explained by the fact that different genes including the interaction of different molecular pathways lead to different phenotypic forms.[81]

CONCLUSIONS

Positive and negative results obtained from studies on genetic researches are important to determine the mutation incidence in patients with missing teeth. Furthermore, investigating other candidate genes out of the commonly studied genes and researching the interactions between these genes will provide a better understanding of the various models of tooth agenesis.

Tooth agenesis, a multifactorial condition that presents morphological differences and developmental delays on dentoalveolar structures, should not be regarded as just a number anomaly, and it should be borne in mind that it has also an importance with regard to the diagnosis of other dental anomalies and syndromes. Additionally, patients presenting with dental anomalies should be suspected of having missing teeth. This approach can allow early diagnosis of tooth agenesis and application of a multidisciplinary approach.

We hope that our study, which evaluated missing teeth in a comprehensive way, will be a step toward determining the genetic profile of missing teeth.

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