Research Article

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Evaluating the Significance of PAX9 Gene in Oligodontia: A Prospective Genetic Clinical Study

Vinayak S Kabadi[1] Nausheer Ahmed[2] Rajalakshmi S J[3] Lohit N Naik[4*]

[1]Private Consultant, Orthodontics, Bangalore, Karnataka, India.
[2]Associate Professor, Department of Orthodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.
[3]Assistant Professor, Department of Orthodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.
[4]PG Student, Department of Orthodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.

ABSTRACT

Background: This prospective and clinical genetic study evaluates the significance of the G6R and S43K mutations in exon 2 of the paired box 9 (PAX9) gene in patients with multiple congenital missing teeth (MCMT).

Materials and methods: The samples were taken from 30 Indian subjects (15 women and 15 men) with MCMT. The mean subject age was 16.85 years. Photographs and radiographs were taken. Saliva samples were collected from all subjects and sent to a genetics laboratory for single nucleotide polymorphism (SNP) analysis for evaluating G6R and S43K in exon 2 of the PAX9 gene.

Results: SNP analysis of the subjects showed no transversion of either G6R or S43K in exon 2 of the PAX9 gene.

Conclusions: That no sample showed transversion of either G6R or S43K in exon 2 of the PAX9 gene indicates that the phenotypic pattern of MCMT varies with race and ethnicity.

Keywords: Congenitally missing teeth, exon 2, PAX9, genetic evaluation.

INTRODUCTION

The Pax genes, a family of paired homeobox genes, code for transcription factors that play a major role in the early development of a variety of multicellular organisms. The presence of a 128-amino acid (aa) deoxyribonucleic acid (DNA) paired domain that makes sequence-specific contacts with DNA defines the Pax proteins. In mammals, nine Pax genes (PAX1- PAX9) have been found. The PAX9 gene has five exons and is expressed in pharyngeal pouches, the developing vertebral column, and the mesenchyme of maxillary and mandibular arches, thereby contributing to palate and tooth formation derived from the neural crest [1].

In humans, both non-syndromic hypodontia and oligodontia are associated with mutation of exons 2 and 4 of PAX9. Selective agenesis of the teeth has been reported to be because of mutation or deletion of one PAX9 allele [2]. Non-syndromic tooth agenesis in a Chinese population has been associated with two novel missense mutation in the paired domain of the PAX9 gene: Gly6Arg (G6R) and Ser43Lys (S43K) [3]. Individuals with the G6R mutation were missing two mandibular incisors and maxillary premolars while there were peg-shaped upper lateral incisors and missing molars, premolars, and canines in individuals with the S43K mutation [3]. There are limited studies about mutations in exon 2 of PAX9 in the South Indian population. Therefore, this study was undertaken. This study aims to evaluate the significance of the S43K and G6R positions in exon 2 of PAX9 in patients with multiple congenital missing teeth (MCMT) in a non-syndromic South Indian population.

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*Correspondence Dr. Lohit N Naik.
Department of Orthodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.
Email: drlohith.naik@gmail.com

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MATERIALS AND METHODS

Thirty randomly selected individuals (15 women and 15 men) with non-syndromic MCMT were recruited from the Department of Orthodontics and Dentofacial Orthopaedics, Government Dental College and Research Institute, Bangalore. Ethical clearance was obtained from the institutional ethical committee. Inclusion criteria were six or more congenitally missing teeth, no history of extraction, and being non-syndromic. Exclusion criteria were syndromic patients, supernumerary teeth, or the absence of the third molars. A complete past dental and medical history were recorded for all subjects. Extraoral and intraoral photographs (Figure 1 and Figure 2) and orthopantomogram radiograph (Figure 3) were taken.

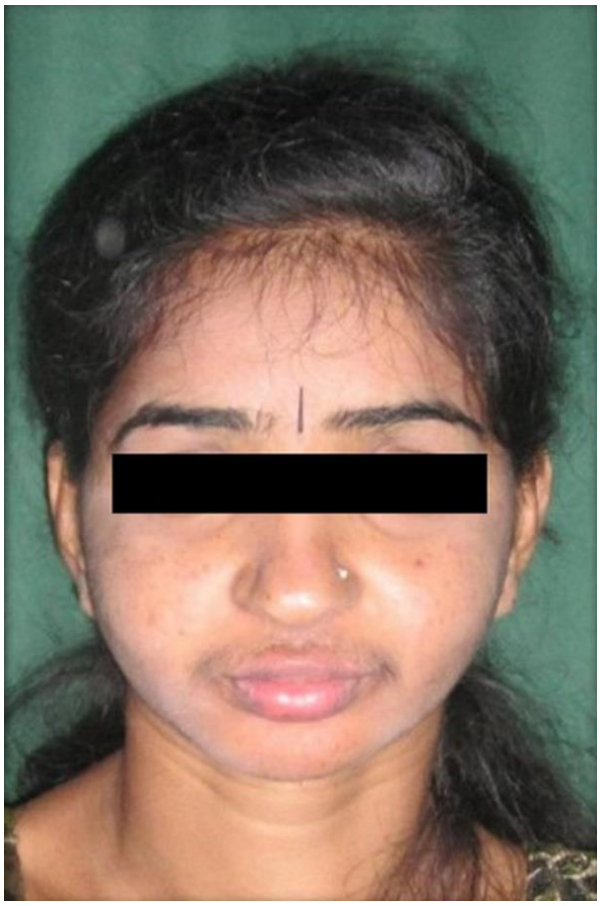


Fig 1: Extra oral photograph

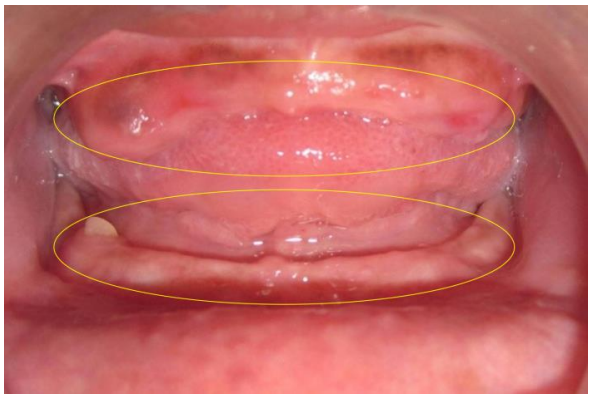


Fig 2: Intra oral photograph

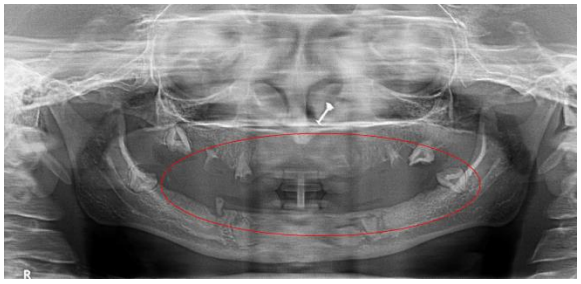
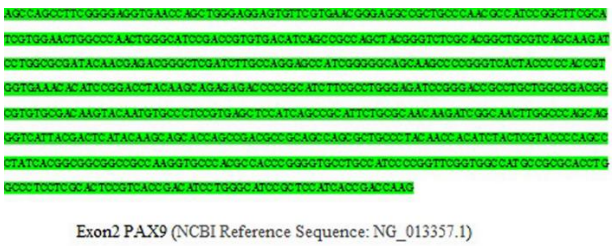


Fig 3: Orthophantomograph radiograph

Genetic evaluation

All 30 affected individuals were subjected to genetic evaluation for mutational analysis of the sixth and 43rd position of exon 2 of the PAX9 gene (Chromos Biotech Ltd.) using genomic DNA (gDNA) isolated from saliva samples collected in sterile tubes. The polymerase chain reaction (PCR) products obtained were loaded on a 2% agarose gel. The DNA concentration was determined by visualization under spectrophotometer ultraviolet light to indicate the amplification of the required segment of the gene of interest. Primers were designed by using the reference sequence of the human PAX9 gene (Figure 4).



Exon2 PAX9 (NCBI Reference Sequence: NG_013357.1)

Fig 4: Primers designed using the reference sequence of the PAX9 gene of the human.

Designed with the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

In our study, PCR and gel electrophoresis has successfully amplified the exon 2 region of PAX9 gene in all the 30 samples. Further DNA sequencing of the PCR products was carried out in all the 30 samples. Single nucleotide polymorphism (SNP) analysis was done by comparing the sequence data obtained from the samples against the reference data (Figure 4) by using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to identify mismatches if any. To detect an SNP or point mutation, the nucleotide sequence of a given sample and the standard reference sequence of the respective exon from NCBI were entered into NCBI BLAST which then aligns the nucleotide sequence of the sample and the reference sequence (query) head to head with a vertical line between them (Figure 5). If any of the vertical lines are missing, it indicates a difference (mutation) at that position.

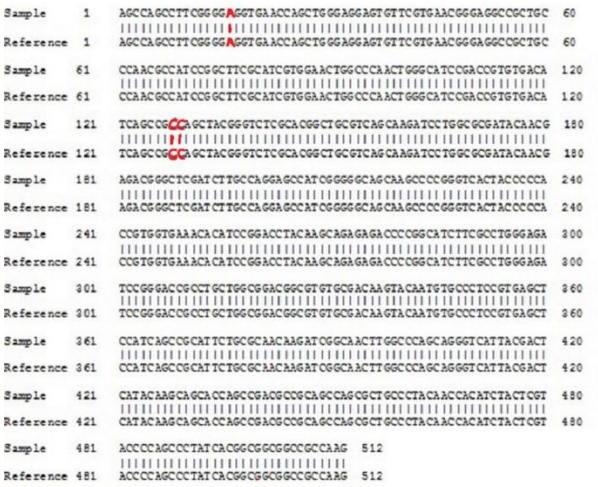


Fig 5: Alignment of exon 2 of a sample and the NCBI reference sequence using NCBI BLAST

The nucleotides highlighted in red color indicates their position (16, 128, 129) in exon 2. There is no mismatch of nucleotides (16, 128, 129) in sample 8 when aligned with their respective nucleotides (16, 128, 129) from the NCBI reference exon 2. Abbreivations: NCBI, National Center for Biotechnology Information; BLAST, Basic Local Alignment Search Tool.

RESULTS

Bidirectional sequence analysis of the PCR amplified products of exon 2 of the PAX9 gene in all samples, using forward and reverse primers, revealed no SNPs in exon 2 at positions 16, which would cause substitution of glycine to arginine at amino acid 6 (G6R), or 128 or 129, which would replace serine with lysine at amino acid 43 (S43K). All 30 samples were negative for any SNPs in exon 2 (Figure 5).

DISCUSSION

This study was done to evaluate the significance of the positions of G6R and S43K SNPs in exon 2 of the PAX9 gene in MCMT in a non-syndromic South Indian population. In humans, within each quadrant of the jaws, the dentition is further divided into four dental fields (incisors, canines, premolars, and molars). In these dental fields, when a tooth is missing, all posterior teeth within these dental fields are likely to be absent. Similarly, when a single tooth is missing, it is usually the most posterior tooth within its dental field [4]. Studies have shown that the patterns of missing teeth were confined to both anterior and posterior teeth in patients with MCMT [5,6]. In the present study, the number of missing teeth in our subjects were equally distributed in both the anterior and posterior fields (missing anterior teeth: 74; missing posterior teeth: 78). Additionally, the absence of the second premolars and third molars in most samples supports posterior dental field theory (Butler's field theory) [4].

In a study by Wang et al., a group of 14 unrelated Chinese individuals with different levels of non-syndromic hypodontia had two missense mutations in the paired domain of PAX9 [2]. Sequencing analysis of exon 2 of PAX9 revealed two mutations (a double nucleotide mutation), G128A and C129A, resulting in the substitution of serine by lysine (S43K) in familial cases, and a novel heterozygous missense mutation, G16A, that had caused a change from glycine to arginine at amino acid 6 (G6R) [2]. The patient carrying the G6R mutation had only two mandibular incisors and one maxillary premolar, while patients from the family with the S43K mutation had many teeth missing, including molars, maxillary premolars, and mandibular canines [2].

In the present study, all the 30 samples had a positive nucleotide band after PCR amplification on gel electrophoresis. When the amplified PCR

products of exon 2 of the PAX9 gene of these samples were subjected to bidirectional sequencing analysis using forward and reverse primers, no single nucleotide polymorphisms at positions 16, 128, or 129 of exon 2, related to the S43K and G6R mutations, were revealed. This is in disagreement with a study done by Wang Y et al., in a group of 14 unrelated Chinese individuals, even though all 30 samples in our study had six or more teeth missing congenitally (oligodontia) [2]. One possible explanation for this observation may be that the subjects who underwent genetic examination in our study were from a South Indian population, differing in genetic ethnicity than the Chinese subjects in a study by Wang Y et al. This finding suggests that the location of SNP sites responsible for multiple congenitally missing teeth may vary in position in different races and ethnicities. The mutation site responsible for multiple congenitally missing teeth may be located in different exons of the same PAX9 gene or could be located in a different gene entirely, such as MSX1. However, to confirm the SNP site responsible for MCMT, the entire PAX9 gene sequence would be required.

A study by Frazier-Bowers et al. sought to describe the clinical features, inheritance, and mutational analysis of familial mandibular incisor agenesis in people of Vietnamese descent and found unique forms of hypodontia, wherein the mutation analysis failed to identify mutations in the coding regions of either MSX1 or PAX9 [7]. This observation supports our findings that an entirely different gene may be responsible for multiple congenitally missing teeth. However, further studies are needed to track down the responsible gene.

Some of the previous human studies done on exon 2 of the PAX9 gene, such as that by Lammi et al., in a Finnish family affected with oligodontia, found a novel mutation, C76T [8]. Another study, by Nieminen et al., revealed a nucleotide transversion of A340T that created a truncated protein that lacked the last a-helix (a6) of the paired box and the entire C-terminal region [9]. A direct sequencing study by Kapadia et al. revealed a heterozygous A to T transversion mutation at nucleotide 259, substituting phenylalanine for isoleucine at position 87 in the predicted protein sequence [10]. In another study, DNA sequencing revealed a cytosine insertion at nucleotide 793 in exon 2 for all affected individuals analyzed [11]. Mostowska et al. described a novel mutation of PAX9, 619_621delATCinsTACCGACCAAGGTAGGGCATCCCT, located 13 base pairs (bp) upstream of the 3' end of exon 2 [12].

However, in the present study, while all cases subjected to direct sequencing had severe hypodontia (especially case 8, in which the patient had only four teeth present), none of our samples demonstrated any SNPs (mutation) along the entirety of exon 2 of the PAX9 gene. Also, none of the samples in our study appeared to have either a deletion, duplication, or insertion mutation in exon 2 of PAX9.

The limitations of the study were samples are from single ethinicity, the use of a single method for analyzing the sample data for mutation detection, and financial constraints.

CONCLUSION

In the present study, none of the 30 samples showed a transversion of G6R and S43K in exon 2 of the PAX9 gene, indicating that the phenotype of MCMT pattern may vary with race and ethnicity. Genetic evaluation by direct sequencing found no SNPs within the whole of exon 2 of the PAX9 gene. Therefore, we concluded that exon 2 of the PAX9 gene was not the cause for MCMT in the subjects in the present study. Further studies are required to investigate different exons of the PAX9 gene and other genes involved in MCMT with a larger sample size to determine the contribution of the respective genes.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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