

Original Research Article

An Interdisciplinary Research Approach to Identify a Nonsense Mutation in the PAX9 Gene in Molar Tooth Agenesis

Dr. Pranav Vaid (Dental Surgeon)¹, Dr. Pratiksha A Srivastava (PGT)², Dr. Kumar Ravishankar (MDS)³, Dr. Mamta Singh (MDS)^{4*}, Dr. Meenakshi M. Singh (PGT)⁵, Dr. Astha Durgvanshi (MDS)⁶

¹Subharti Dental College and Hospital, Subharti University Subhartipuram, Delhi Meerut Haridwar Bypass, Pin Code-250005

²Department of Prosthodontics, Crown and Bridge Subharti Dental College and hospital, Swami Vivekanand Subharti University, Subhartipuram, Delhi-Meerut-Haridwar Bypass, Pin Code: 250005

³Assistant Professor, Department of Periodontology, Aditya Dental College, Beed- 431122

⁴Assistant Professor Department of Periodontics, Kothiwal Dental College & Research Centre, Mora Mustaqueem, Kanth Road, Moradabad, 244001, India

⁵Department of Pedodontics and Preventive Dentistry Kothiwal Dental College & Research Centre, Mora Mustaqueem, Kanth Road, Moradabad, 244001, India

⁶Associate Professor Department of Oral Medicine, Diagnosis and Radiology Kothiwal Dental College & Research Centre, Mora Mustaqueem, Kanth Road, Moradabad, 244001, India

***Corresponding Author**

Dr. Mamta Singh (MDS)

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Abstract: *Background:* Development of dentition is controlled by numerous genes, proven by experimental animal studies and mutations that have been identified by genetic studies in man. *Purpose:* In the present study a nonsense mutation has been identified in the PAX9 gene that was associated with molar tooth agenesis (hypodontia or oligodontia). *Methodology:* The proband of family 1 originally came for treatment to the Department of Pedodontic and Preventive Dentistry. The proband of family 2 was an orthodontic patient of the Department of Orthodontics and Maxillofacial Orthopedics. All family members were clinically examined by one of the authors and agenesis of teeth was also verified with panoramic radiographs. DNA was extracted from venous blood samples using standard methods. The coding region of the PAX9 gene were amplified using four sets of primers Amplification was performed in 100 ml with DNA concentration of 1.5 ng/ml, 1.5 mM MgCl₂ and 1 mU polymerase. *Results:* The A340T transversion creates a stop codon at lysine 114, and truncates the coded PAX9 protein at the end of the DNA- binding paired-box. All the affected members of the family were heterozygous for the mutation. The tooth agenesis phenotype involves all permanent second and third molars and most of the first molars and resembles the earlier reported phenotype that was also associated with a PAX9 mutation [1]. *Conclusion:* The phenotype is presumably a consequence of haplo insufficiency of PAX9. In other family with molar tooth agenesis, similar sequence changes in PAX9 could not be found.

Keywords: Tooth agenesis; Proband, oligodontia; hypodontia; PAX9; nonsense mutation.

INTRODUCTION

Hypodontia or oligodontia is a common phenotypic feature encountered by dentists on a regular basis. Such features appear due to agenesis of permanent successors. It's common to find hypodontia of one or a few permanent maxillary lateral incisors or second premolars in otherwise healthy subjects. This prevalence rate ranges from 6-8%. Wisdom tooth or third molar has been reported to show the highest incidence of agenesis [2-4].

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Missing permanent teeth or agenesis of several teeth is referred to as oligodontia. Mostly such conditions are associated with some syndromes. Apart from wisdom teeth, agenesis is rarely seen in permanent molars.

In vitro studies conducted on experimental animals have reported several genes that find their role in tooth development through gene expression [5, 6]. In human beings, tooth agenesis has mostly been associated with some kind of mutations seen in these gene expression. They include hereditary conditions like ectodermal dysplasias and other syndrome associated phenotypes [7]. Oligodontia is considered as an isolated trait caused by mutations. Literature review is suggestive of two different gene mutations that encode for transcription factor MSX1. Such mutations are mainly seen in families who inherit agenesis of permanent second premolars and third molars [8, 9]. More recently, a different kind of mutation has been reported in gene encoding for transcription factor PAX9. Such mutation mainly is responsible for dominant oligodontia affecting mainly permanent molars [1, 10].

Immediately after initiation of tooth development, the aforementioned transcription factors (MSX1 and PAX9) are expressed in primitive dental mesenchyme in response to epithelial signals [11, 12]. Null mutations in experimental mouse have reflected that both are indispensable for tooth and craniofacial development [12, 13]. Lack of PAX9 also leads to malformations of limbs and pharyngeal pouch derivatives [12]. Paralogues of PAX9 in the family of 'paired-box' transcription factors have crucial roles in organogenesis of various organs. Interestingly, while the mouse null mutations of several genes including MSX1 and PAX9 cause a complete lack of teeth, the mouse heterozygotes usually have normal dentition.

This is probably explained by simple histodifferentiation of mouse dentition. It thus emphasizes the relevance of identifying the gene defects in different cases of tooth agenesis in humans.

MATERIALS AND METHODS

The proband of family 1 originally came for treatment to the Department of Pedodontic and Preventive Dentistry. The proband of family 2 was an orthodontic patient of the Department of Orthodontics and Maxillofacial Orthopedics. A proband is usually the first affected individual in a family who brings a genetic disorder to the notice of the medical community. The study was conducted under consent of all family members and approved by the Ethics Committee of the Institute. All family members were examined clinically by one of the authors and agenesis of teeth was also verified with panoramic radiographs.

DNA was derived from venous blood samples using standard methods. The coding region of the PAX9 gene was amplified using four sets of primers. Amplification was performed with DNA concentration of 1.5 ng/ml, 1.5 mM MgCl₂ and 1 mU polymerase in 100 ml.

The primers and PCR conditions were as follows (numbering indicates the position of the first base of the primer in the coding sequence): exon 2, forward primer 1 AGGCAGCTGTCC- CAAGCAGCG (exon start 758), reverse primer 1 GGAGGG- CACATTGTA CTGTGCGC (357), annealing T 648C, 32 cycles; exon 2, forward primer 2 ATCCGACCGTGTGACATCAGCC (109), reverse primer 2 GAGCCCCTACCTTGGTCTGGTG (exon end +10), annealing T 648C, 30 cycles; exon 3, forward primer GGGAGTAAACTTCACCAGGC (exon start 7197), reverse primer CCACCTGGCCTGACCCTC (exon end +28), annealing T 618C, 32 cycles; exon 4, forward primer GGAGAGTAGAGTCAGAGCATTGCTG (exon start 7121), reverse primer GAGACCTGGGAATGGGGGA (stop +74), annealing T 618C, 32 cycles. PCR products were purified by agarose gel electrophoresis and Qiaquick gel extraction kit. Both strands of the PCR products were sequenced using the BigDye terminator chemistry and analysed on an ABI 377XXL DNA sequencer.

RESULTS

Thorough clinical evaluation of the members of recruited family didn't reveal any significant systemic condition except for the lack of several teeth (oligodontia) in three individuals in one family and two in the other family (Table 1 and Figure 1a).

Remaining family members had normal dentition. The affected individuals in family 1 had missing all second and third permanent molars as well as both maxillary lateral incisors (the proband was too young (pediatric subject) for the definitive diagnosis of the missing third molars to be ascertained). The proband as well his mother had missing all first permanent molars and several second premolars.

In the primary dentition of the proband all second molars were missing. The affected brother had all primary teeth but the second primary molars were submerged. The mother and the affected brother had a malposition of upper permanent canine.

In addition, some permanent teeth appeared smaller than normal in affected patients. The oligodontia phenotype was more severe in family 2. The proband lacked eight permanent molars, including all third molars, and several other permanent teeth. The affected father had only three permanent teeth: upper central incisors and one lower lateral incisor.

Both of them had all primary teeth. The small size of the families does not allow complete segregation analyses to be made, but occurrence of oligodontia in families fits into an autosomal dominant mode of inheritance (Figure 1b).

Table-1: Oligodontia phenotypes

	right				incisors				left							
	molars	prem.	c.	molars	prem.	c.	incisors	prem.	c.	molars	prem.	c.	molars			
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
Family 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Family 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Family 1	?	*	*	*	*	*	*	*	*	*	*	*	*	*	*	?
Family 2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Family 2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Sequencing of the PAX9 gene (exons 2, 3 and 4 that include the coding region) revealed three nucleotide changes as compared to the published [14] sequences. All changes could be verified from both strands. A nucleotide transversion A340T present in heterozygous state of exon 2 in all three affected members of family 1 produces a stop codon at lysine 114 in the end of the DNA-binding 'paired box' of PAX9. The premature termination of translation creates a truncated protein that lacks the last a-helix (a6) of the paired box and the entire C-terminal region.

The other nucleotide changes found were in exon 3, and interestingly, in adjacent nucleotides. The silent change C717T in the codon of His239 was present in heterozygous state in the healthy mother and affected daughter of family 2. A G718C transversion causes a conservative change Ala240- Pro. The latter change was present in heterozygous state in all members of family 2 and in homozygous or heterozygous state in some of the members of family 1.

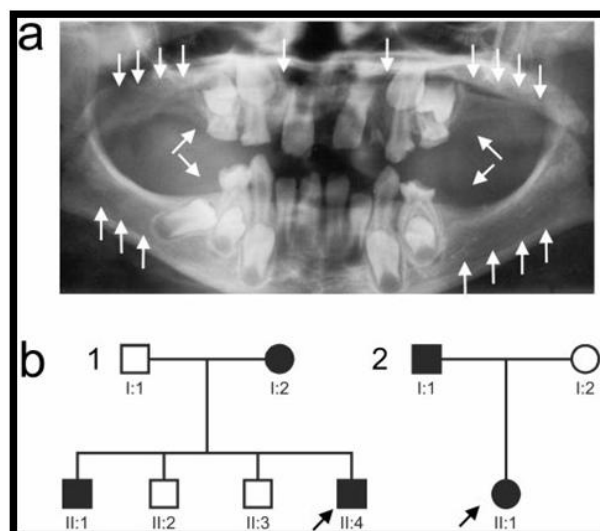


Fig-1(a,b) Oligodontia phenotype and families (a) Panoramic radiograph of proband of family 1 (II:4) in age of 6. Missing teeth are indicated by arrows. (b) Pedigrees of molar oligodontia families 1 and 2. Squares, males; circles, females; darkened, affected; arrows, probands

DISCUSSION

The present research has mentioned a second inactivating mutation PAX9 and has been a determining factor for oligodontia for permanent molar. In the two families recruited for study having PAX9 mutations, second premolars were missing only when a neighboring first molar was absent. The A340T mutation creates a stop codon and truncates the PAX9 protein in the C-terminal subdomain of the DNA-binding paired box. The insG219 mutation [1] destroys the paired box 121 nucleotides earlier, between the N- and C- subdomains. The lack of the C-terminal region that follows the paired box most likely disrupts the normal function of both mutant proteins. The mildness of the phenotypes as compared to the PAX9 mouse null mutant suggests that both mutations are loss-of-function and exert their effect mainly through inactivation of one copy of protein, leading to haplo insufficiency.

A probable rationale might be the mutant proteins that retain some of the DNA binding capacity. Especially the slightly more severe phenotype of family 1 may be attributed to the dominant negative effect due to the more complete paired box of the Lys114 Stop mutant protein.

The identification of a second mutation in the PAX9 gene that segregates with oligodontia of molar teeth supports the idea that PAX9 is especially important for the development of the most distal teeth, i.e. molars. Instead, the consensus phenotype for a MSX1 haplo sufficiency includes agenesis of second premolars and third molars and in only some cases of first and second molars [8, 9]. In mouse, both PAX9 and MSX1 are induced in dental mesenchyme in response to epithelial signals, and subsequently regulate the reciprocal signals from the mesenchyme. Both genes are also indispensable for tooth organogenesis, as shown by mouse null mutants where tooth development is arrested at bud stage [12, 13]. The differences in the consequences of the haplo sufficiency most likely reflects the varying relative importance of the two genes in the development of different tooth families (incisors, premolars and molars).

In different types of hypodontia or oligodontia, those teeth in each tooth family that develop latest are most vulnerable for agenesis. It is believed that agenesis follows from inability to overcome a critical threshold at an early stage of development. The MSX1 haploinsufficiency appears to affect all tooth families, while reduced amount of PAX9 protein may be especially critical for molars. As lack PAX9 of mouse leads to decreased expression of MSX1 [12] it is possible that the effect of PAX9 haploinsufficiency on some incisors and premolars are realized through reduced expression of MSX1.

CONCLUSION

Lack of evidence of coding region mutation in the second family may be attributed either to a presence of a mutation in the non-coding regulatory regions of the PAX9 gene, or a mutation in another gene, perhaps acting upstream or downstream of PAX9. In present paper recombination in terms of both MSX1 and PAX9 has been detected in case of milder phenotype (with only missing premolars and incisors). However, these milder phenotypes may also be caused by genes acting in the same signaling pathways with MSX1 and PAX9.

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