Case Report

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Dentistry Section

A Nonsyndromic Autosomal Dominant Oligodontia with A Novel Mutation of PAX9-A Clinical and Genetic Report

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ABSTRACT

Oligodontia is congenital absence of one or more teeth which has familial abnormality and attributable to various mutations or polymorphisms of genes often associated with malformative syndromes. The present case reports a rare case of non syndromic oligodontia in an 8-year-old girl with missing 14 permanent teeth excluding third molars in mixed dentition. It is a rare finding which has not been frequently documented in Indian children. Mutations in MSX1 and PAX9 have been described in families in which inherited oligodontia characteristically involves permanent incisors, lateral incisors, premolars and molars. Our study analysed one large family with dominantly inherited oligodontia clinically and genetically. This phonotype is distinct from oligodontia phenotypes associated with mutations in PAX9. Sequencing of the PAX9 revealed a novel mutation in the paired domain of the molecule. The multiple sequence alignment and SNP analysis of the PAX9 exon 2 revealed two mutations.

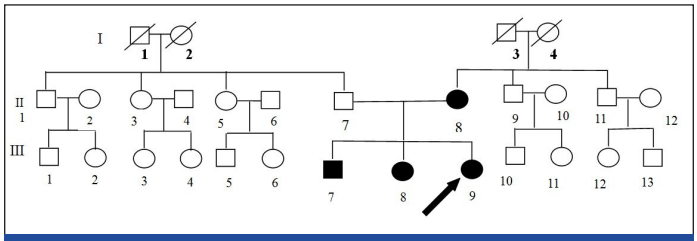
Keywords: MSX1, Tooth development, PCR

CASE REPORT

An 8-year-old female patient (proband) reported to the Department of Paediatric Dentistry with a chief complaint of multiple decayed teeth in lower left quadrant of the jaw. The patients past medical history and the family history were not significant. It was patient's first visit to a dentist. Extra-oral examination revealed no abnormalities. Intraoral examination, revealed grossly decayed teeth in relation to 54, 65, 74 and hypo plastic teeth in relation to 64 and 84. The proband (III: 9) Orthopantomographic (OPG) examination revealed agenesis of 18 teeth including third molars. The missing teeth were 12, 13, 15, 17, 18, 22, 23, 25, 27, 28, 33, 35, 37, 38, 43, 45, 47, 48 [Table/Fig-1]. The panoramic radiograph also revealed a few developing permanent teeth in relation to 14, 24, 16 and 26. The teeth present were reduced in size, shape. Interviews revealed missing teeth were not extracted and were absence since childhood. Clinical examination of her siblings and the mother showed to have a similar problem.

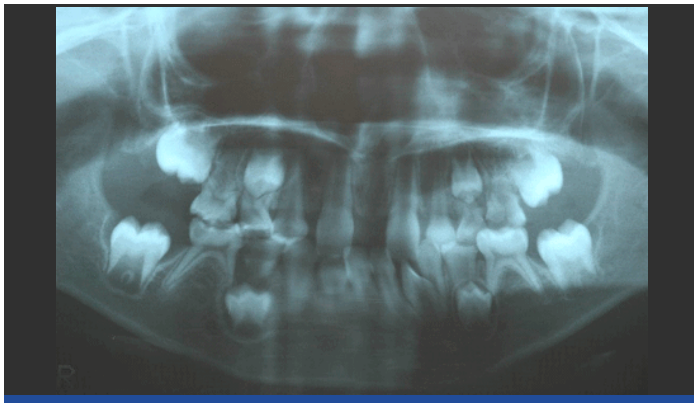
Pedigree chart

Probands three generation familial pedigree chart was drawn. Probands both parents side pedigree chart was drawn separately. Probands grandparents of both side were no longer lived, so history of missing teeth could not be assessed, excluding them the entire members in the family (25members) were examined clinically. Except for the proband, her siblings and her mother, no one else in the entire family had any history of congenitally missing teeth [Table/Fig-2].

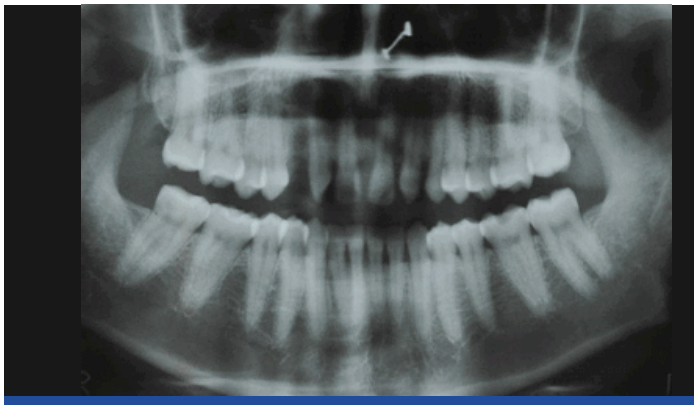


[Table/Fig-2]: Family history screening using three generation pedigree
Darkened symbols represent affected, clear symbols indicate normal unaffected, square indicate male, circles indicate females, (/) indicates deceased, arrow mark showing proband

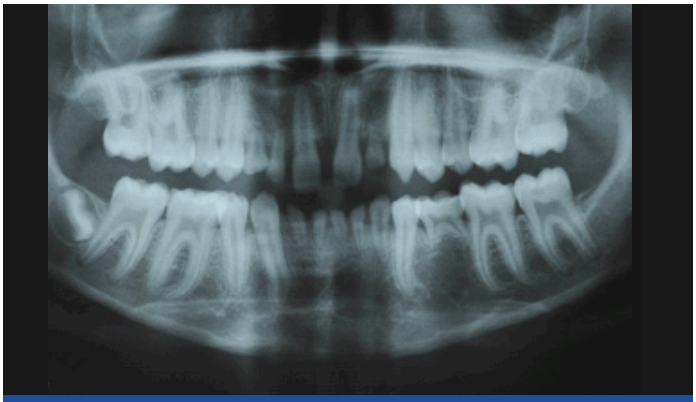
Probands father (II:7) aged 50 years did not have any missing teeth and mother (II:8) of age 38 years had retained teeth in relation to 63, and congenitally missing teeth in relation to 12, 18, 22, 28, 38, 48 [Table/Fig-3]. Probands brother (III:7) of age 17 years was examined clinically and radiographically which revealed retained primary teeth in relation to 52, 53, 62, 71, 72, 73, 75, 81, 82, and congenitally missing permanent teeth 12, 13, 18, 22, 28, 31, 32, 33, 35, 38, 41, 42, 43 [Table/Fig-4]. Probands sister (III:8) of age 13 years was examined clinically and radiographically, which revealed root stumps in relation to 74 and congenitally missing teeth in relation to 12, 18, 22, 28, 38, 48 [Table/Fig-5].



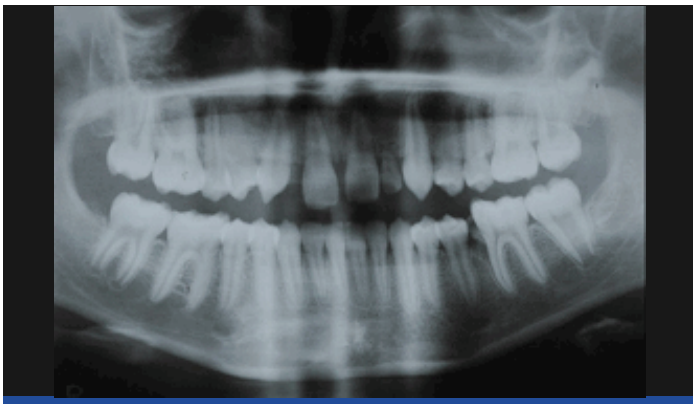
[Table/Fig-1]: OPG of Proband



[Table/Fig-3]: OPG of Proband Mother



[Table/Fig-4]: OPG of Proband Brother



[Table/Fig-5]: OPG of Proband Sister

BCC subtype	Right								Left							
	M			P		C		I	I		C	P		M		
Maxilla	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
Mandible	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
II:8	*						*			*						*
	*															*
III:9	*	*		*		*	*			*	*		*		*	*
	*	*		*		*					*		*		*	*
III:8	*						*			*						*
	*															*
III:7	*					*	*			*						*
						*	*	*	*	*	*		*			*

[Table/Fig-6]: Clinical phenotypes of affected proband, mother and siblings. Star represents congenitally missing teeth

Clinical phenotypes of affected proband, mother and siblings are tabulated [Table/Fig-6].

On clinical and radiographical examination it was diagnosed that the proband and elder brother were affected with severe oligodontia, elder sister and mother with hypodontia. Once it was clinically and radiographically confirmed of congenitally missing multiple permanent teeth, we were curious to find out genes which were responsible for agenesis of teeth. So in this case all the family members of proband were subjected to genetic evaluation.

Genetic Evaluation

The entire procedure was explained to the family members and informed consent was obtained. Venous blood samples of 5ml were with-drawn from all the family members of proband to extract DNA for genetic evaluation. The evaluation was carried at Vittal Mallya Scientific Research Foundation Bengaluru, India.

Blood DNA extraction: Whole blood DNA was extracted using red cell lysis buffer method [1]. Blood sample was incubated with red blood cell lysis buffer for 1.5 hours followed by centrifugation at 8000 rpm for 10 minutes. The supernatant was taken and purifies using phenol chloroform extraction and precipitated with absolute alcohol. The DNA was spun down at 12,000 rpm for 10 minutes followed by 70% ethanol wash, drying and dissolving in nuclease free water.

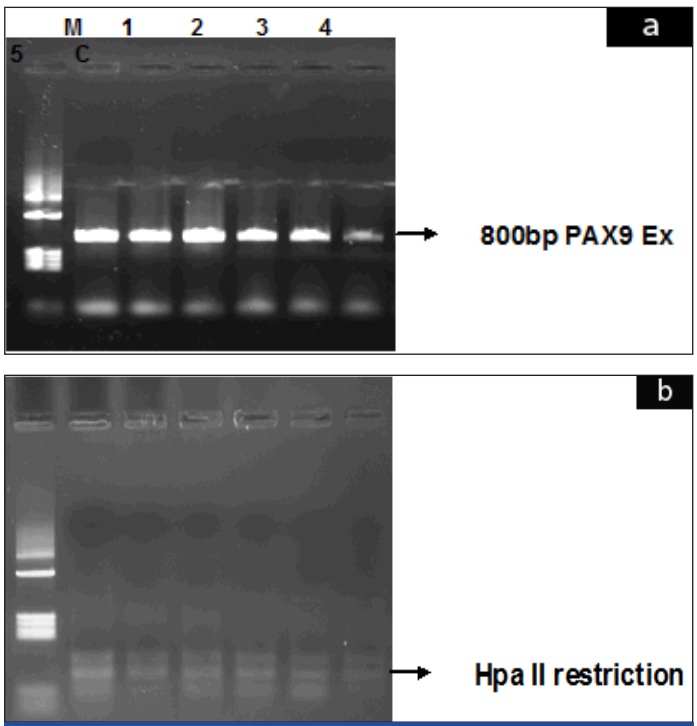
SEQ and RE analysis:

Total genomic DNA was used for amplification of the PAX9 exon 2 using a forward primer with (5' CTGTCCCAAGCAGCGGGTGC3') and reverse primer with sequence (5' ACTGGGACCTCCGCGAGAGTG 3'). The PCR conditions were 94^{0}C for 5 minutes followed by 30 cycles each of 94^{0}C for 30 seconds, 52^{0}C for 45 seconds and 72^{0}C for 40 seconds and a final extension of 5 minutes at 72^{0}C. The PCR products were evaluated by running on a 2.0% agarose gel and visualizing under UV trans-illuminator. The products were purified using Qiagen Gel purification kit and sequenced by automated sequencer (Eurofins Ltd). The PCR product was profiled via restriction digestion using HpaII restriction endonuclease [2]. The sequences obtained were confirmed using nucleotide BLAST search and aligned using Clustal W2 and mutations were recorded. The sequences were translated to protein sequences using ExPASy translate tool and the protein sequences were aligned using ClustalW2. The SNPs (single nucleotide polymorphisms) were detected in these sequences using the tools [3].

PCR and sequence analysis

The PCR using Exon 2 specific primers gave identical 800bp products in all the proband's sample [Table/Fig-7a&b]. In order to detect previously reported mutations by Mu et al., & Lammi et al., in our proband's sample we restricted the PCR products with a diagnostic restriction enzyme HpaII. The restriction pattern of all the Probands was similar. The multiple sequence alignment and SNP analysis of the PAX9 exon 2 revealed two mutations (T to C) at 48th and 64th nucleotide [Table/Fig-8]. These two mutations alter the translated protein sequence (data not shown).

Genetic analysis was done and there was a mutation in the PAX9 gene. Based on the clinical, radiographic and genetic evaluation, the present case was diagnosed as non syndromic autosomal dominant form of oligodontia. A comprehensive treatment was planned based on the diagnosis.



[Table/Fig-7a]: Gel electrophoresis of PAX9 Exon 2 800bp PCR product. The sample codes are M- DNA Marker, 1- RV7, 2-RV9, 3- RV10, 4- RV11, 5-RV-12, 6- Control (normal) **[Table/Fig-7b]:** HpaII restriction digestion of the PCR products

[Table/Fig-8]: Nucleotide sequences of PAX9 exon in proband's sample. The mutations are highlighted

DISCUSSION

Agenesis of six or more teeth excluding the third molars is described as oligodontia and it has population prevalence of 0.3% [4]. In our sample 14 permanent teeth were missing excluding third molars. Oligodontia can be syndromic or nonsyndromic. Proband showed nonsyndromic form of oligodontia. The genetic factors may be dominant or recessive [5]. Family of proband showed autosomal dominant oligodontia. Several genes are responsible for congenitally missing teeth. Mutations of MSX1 and PAX9 genes have been associated with agenesis of teeth [6,7]. PAX9 is a paired domain transcription factor that plays a critical role in odontogenesis and the locus of mutation is seen in paired box domain (exon 2) of the PAX9 [8]. A frame shift mutation identified within the paired domain of the transcription factor PAX9, has been linked to a unique form of oligodontia in a single multigenerational family. The literature reports eight families in whom PAX9 mutations segregate with nonsyndromic autosomal-dominant inherited oligodontia [7]. Proband showed a novel mutation with nonsyndromic form of autosomal dominant oligodontia.

The present case report shows developmental agenesis of 14 permanent teeth excluding the third molars with unknown aetiology. In this case genetic analysis was done and it was found that mutation in PAX9 gene was responsible for agenesis of multiple permanent teeth. Mutation analysis in the exon 2 region of PAX9 transcription factor in our proband's sample shows two mutations at 48th and 64th nucleotide plausibly causing missing teeth in the Indian population. Our findings are distinct from the previous observations recorded on Finnish [7] and Mexican subjects [9]. Our findings suggest that PAX9 mutations in oligodontia patients vary across populations [9,10] and mapping all the mutations in this transcription factor within the Indian population requires a study with higher number of subjects.

CONCLUSION

Most of the case reports published till date has not evaluated the genetic factors that could have led to the agenesis of the teeth. With advancements in the field of genetics, such evaluations may help the clinicians in understanding the basis of such conditions, so that the apprehension associated with these in the families can be addressed to accordingly. As future prospective, disease causing mutation represents an initial step in explaining the pathologic mechanisms underlying PAX9 mediated tooth agenesis. As more mutations are analysed, it will be possible to correlate the position of a mutation, and the relative effect on function, with the observed developmental defects.

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