MUTATION ANALYSIS OF *PAX9* GENE IN AFFECTED FAMILY OF HYPODONTIA ATTENDING TERTIARY CARE HOSPITAL OF QUETTA

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ABSTRACT

Objective: To identify the phenotype and genotype of hypodontia for a Pakistani family with hypodontia and to map the genes locus responsible for this disease.

Study Design: Descriptive study.

Place and Duration of Study: This descriptive study was performing in human molecular genetics (HMG) laboratory of Baluchistan University of information technology, engineering and management sciences (BUITEMS). The study was of 4 months duration.

Material and Methods: Blood samples (5ml) were collected from all 15 families' members (35participant). Genomic DNA was extracted by using inorganic method. All the three coding exons of *PAX9* (NM_006194) were amplified and sequenced. Sequencing of the *PAX9* coding exons and splice sites showed a homozygous misses substitution in exon 3 (c. 718G>C; p.Ala240Pro) in the affected individuals of the family.

Results: Intra-oral and panoramic radiographs revealed that the proband (II-1) and her father (I-1) have hypodontia denoted by the complete absence of teeth in maxillary arch, while all other family members maintained normal dentitions. The missing teeth are both upper lateral incisors (12, 22 FDI numbering) and third molars (18, 28). Mandibular arch show; retained deciduous teeth and no teeth permanent teeth missing. Pedigree construction indicated that phenotypes in this family showed an autosomal recessive segregation pattern. The sequencing of coding exons and splice sites of *PAX9* gene showed a homozygous missense mutation in exon number 3 (c. 718G>C; p.Ala240Pro) in the affected individuals of the family.

Conclusion: We identified a missense mutation (p.Ala240Pro) in gene *PAX9* coding exon 3 in Pakistani family with hypodontia.

Keywords: Congenital, Hypodontia, Missense mutation, PAX9 gene.

INTRODUCTION

Tooth agenesis usually known as hypodontia is the congenital absence of one or more teeth. It may occur in primary or secondary dentition and is among the most common craniofacial anomalies[1]. Hypodontia occurs in both sporadic and familial forms and can be classified as either non-syndromic (isolated) or syndromic based on the presence of other inherited abnormalities[2,5]. Permanent dentition is more frequently affected than primary dentition[5-6]. Clinical classification of this anomaly occurs according to the number of missing teeth. Hypodontia is defined as the absence of one to six permanent teeth (excluding third molars), Oligodontia occurs when more than six permanent teeth are lacking (excluding third molars), Anodontia refer for complete absence of permanent dentition[7,9]. The prevalence of hypodontia (excluding the third molars) is 1.3% for males and 4.7% for females and third molar agenesis is the most common with an incidence of 20% in general population of Pakistan[9]. The etiology of hypodontia is mainly genetics and environmental factors[3,10-12]. To date, more than 300 genes have been found to be involved in tooth development, but only a few of these genes, such as *MSX1, PAX9, AXIN2, WNT10A, TGFA, IRF6, MMP1, MMP20* and *EDA* are directly involved in tooth genesis[2,13,14]. *PAX9*, in chromosome 14 (14q21-q13) have been identified in families with oligodontia of most molars[15]. *PAX9* encodes a member of another transcription factor protein family, characterized by the presence of a DNA-binding paired-box

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domain[16]. The morphogenesis of teeth is under strict genetic control. The most important events during the regulation of tooth development are inductive interactions between the epithelial and mesenchymal tissues[17]. In the early stage of tooth development, the paired domain transcription factor Pax9 is expressed at the prospective sites of all teeth prior to any morphological manifestations or expression of other known transcription factors and signaling molecules[18]. A high level of *PAX9* expression is maintained throughout the initiation, bud stage and cap stage, and the expression of *PAX9* is down regulated at the bell stage[19]. In the process, Pax9 PAX9 mediates its tooth-specific function through its interaction with other proteins. In Pax9 (-/-) mice, the development of the tooth organ ceases at the cap stage when compared with wild type and the expression of Msx1 and Bmp4 in the mesenchyme is significantly reduced[19]. In view of the important role of *PAX9* in tooth development, researchers have chosen *PAX9* (MIM: 167416) as a candidate gene for hypodontia and identified18 distinct disease-causing mutations[20,23]. They range from missense mutations that change just one amino acid in the entire protein to premature stop codons that result in truncation of the protein products. Some researchers analyzed the function of the *PAX9* mutants in vitro and suggested that the tooth agenesis phenotype may result from the haploin sufficiency of *PAX9*[23-24]. The purpose of the present research study is to identify the phenotype, genotype of hypodontia for the first time in Pakistan and to map the genes locus responsible for this disease. In Pakistan only epidemiological data including prevalence of hypodontia have been published[9].

MATERIAL AND METHODS

This descriptive study was performed in human molecular genetics (HMG) laboratory of Baluchistan university of information tech-nology, engineering and management sciences (BUITEMS). The study was ~~done~~ of ~~-~~4 months duration. Sampling technique was non-probability purpo-sive sampling.

Family selection and pedigree construction

Total 15 families (35 participants) were taken in our study. The proband was 12 years old when she was referred from general dental OPD of Sandeman Provincial Hospital Quetta to the department of Orthodontics for orthodontic consultation. The main chief complaint was spacing in the front teeth due to absence of permanent upper lateral incisors. Since her family had a dental history involving the absence of teeth, the proband and her family member were invited to participate in this study. Diagnosis of the anomaly was verified by clinical intra oral examination detail (Fig: 4A-C), panoramic radiographs (Fig: 3), lateral cephalogram and cast model analysis of all family members, allowing the pedigree to be determined (Fig: 1). After the

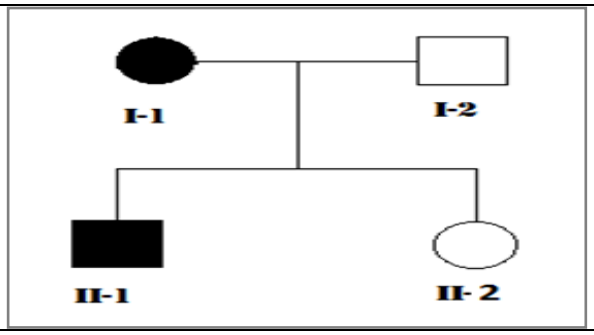

Figure-1: Pedigree showing phenotypes and a autosomal recessive mode of inheritance.
Circle = male, Square = female, Black = hypodontia, White = uneffected

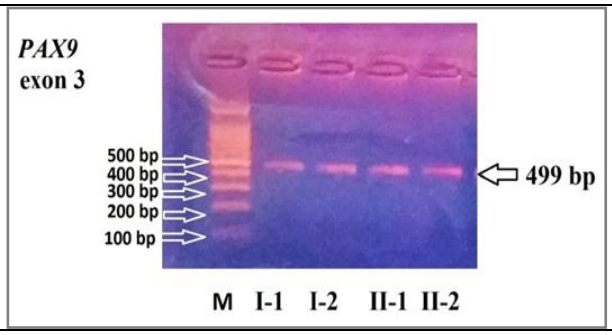

Figure-2: Gel electrophoresis of PAX9 exon 3.

institutional review board (IRB#00007818) approval, at the department of Biotechnology, Baluchistan university of information technology, engineering and management sciences (BUITEMS), Quetta, Pakistan these families were enrolled in current study. The study was conducted according to the tenets of the declaration of Helsinki. Written inform consent was obtain from all participant and their parent.

Table-I: Primers for coding exon of PAX9 gene.????

Exon	Left Primer (5>3)	Right Primer (5>3)	Product size	Ann. Temp
2	CATCCGACCGTGTGACATC	AGGATGTCGGTGACGGAGT	498	61°C
3	TGGAAAGGCCTACTCTGAGG	GAAGGATCTGGCTCGTAGCA	499	61°C
4	TCAGAGCATTGCTGGCTTAC	ATGTGAGACCTGGGAATTGG	443	62°C

Table-II: Sequence of primers used for PCR amplification of human PAX9 exons and PCR conditions.

Relatives	Phenotype	Missing teeth[b]	PAX9 – c718G > C[c]
I-1	Hypodontia	12,18,22,28	G>C
I-2	Unaffected		
II-1[a]	HYpodonita	12,18,22,28	G>C
II-2	Unaffected		

a: Indicates affected daughter, b: Missing teeth numeration follows FDI standards, c: Represent homozygous missense mutation

DNA collection, screening and mutational analysis

Venous blood 5ml was collected from the patients under aseptic conditions. The collected blood was preserved into a collecting tube containing EDTA to avoid clotting of the blood; the samples were stored at -20 degree Celsius. DNA was extracted by using inorganic method from the blood leukocytes (samples) following a standardized protocol already established in human molecular genetics (HMG) laboratory of Baluchistan university of information technology, engineering and management sciences BUITEMS. The final extracted DNA was run in electrophoresis gel to check the quality of the extracted DNA (fig-2). Primers for coding exon of *PAX9* gene were designed by using computer web program Primer[3], UCSC genome bioinformatics and ensembl genome browser (table-I). After primer was designed, they were assembled from macrogen company korea. Amplification of the axons using pre designed primer was done by polymerize chain reaction (PCR). The polymerase chain reaction (PCR) protocol of BUITEMS human molecular genetic laboratory was followed for this purpose. The amplified DNA was sequenced to check any possible mutations in *PAX9* gene from Macrogen Company Korea. Data was analyzed using bioEdit, chromas, Segman softwares.

RESULTS

Out of 15 families (35 participants) only one family has shown *PAX9* gene mutation. The diagnosis of non syndromic hypodontia was confirmed in the effected proband (II-1) and her father (I-1) by intra oral clinical examination (fig-4) and radiographic analysis (fig-3). Diagnosis confirms congenital absence of permanent upper lateral incisors and third molars. Preoperative panoramic X-ray shows; in maxillary arch

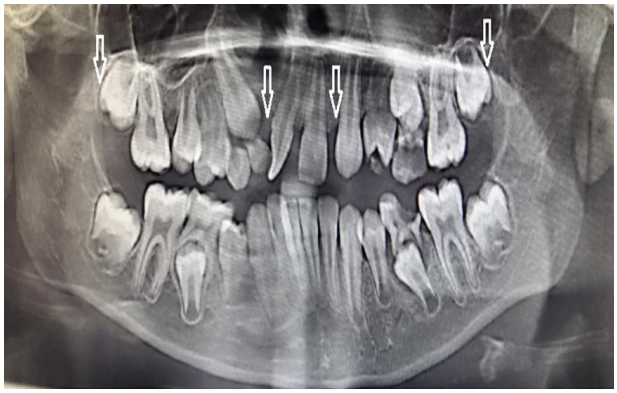

Figure-3: Preoperative panoramic x-ray of the effected daughter (II-1). Arrow represent agenesis of permanent upper lateral incisors and upper third molars (hypodontia). Retained deciduous upper right caniner; diciduous first premolar and upper left second deciduous molar.

retained deciduous teeth are (FDI numbering) 54, 53 and 65, erupting permanent teeth are 17, 13, 25 and 27, maxillary arch also shows missing both upper lateral incisors (12, 22) and third molar (18,28). Mandibular arch show; retained deciduous teeth are 35 and 45, both, erupting permanent teeth are 35 and 45 and no teeth permanent teeth missing. Pedigree construction indicated that phenotypes in this family showed

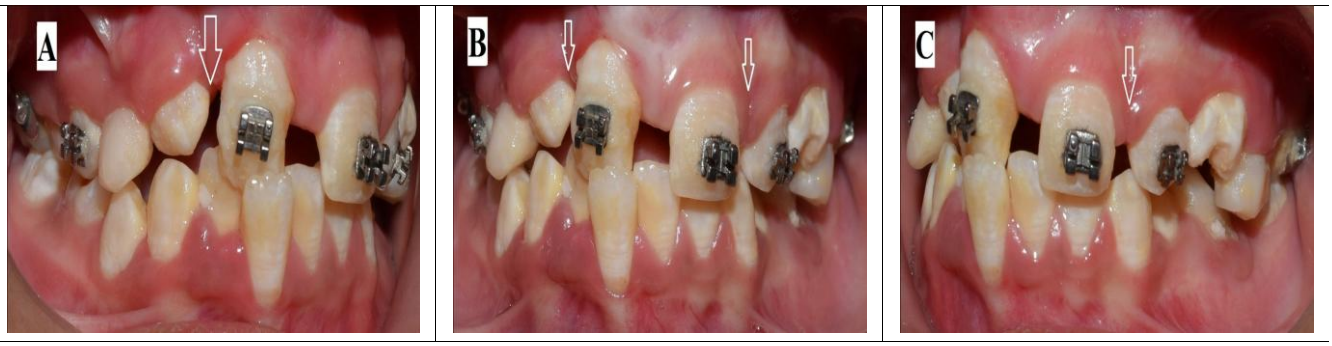

Figure-4: Intraoral photo gragh of affected daughter (II-1), (A) Right occlusal view, (B) Front occlusal view, (C) Left occlusal view and arrow denote congenitally missing tooth.

follicles or sweat glands. Associated dental anomalies were identified in affected father (I-1) of the patient (II-1). The sequencing of coding exons and splice sites of *PAX9* gene showed a homozygous missense mutation in exon number 3 (c. 718G>C; p.Ala240Pro) in the affected individuals of the family (Fig: 5).

DISCUSSION

We have identified a *PAX9* homozygous

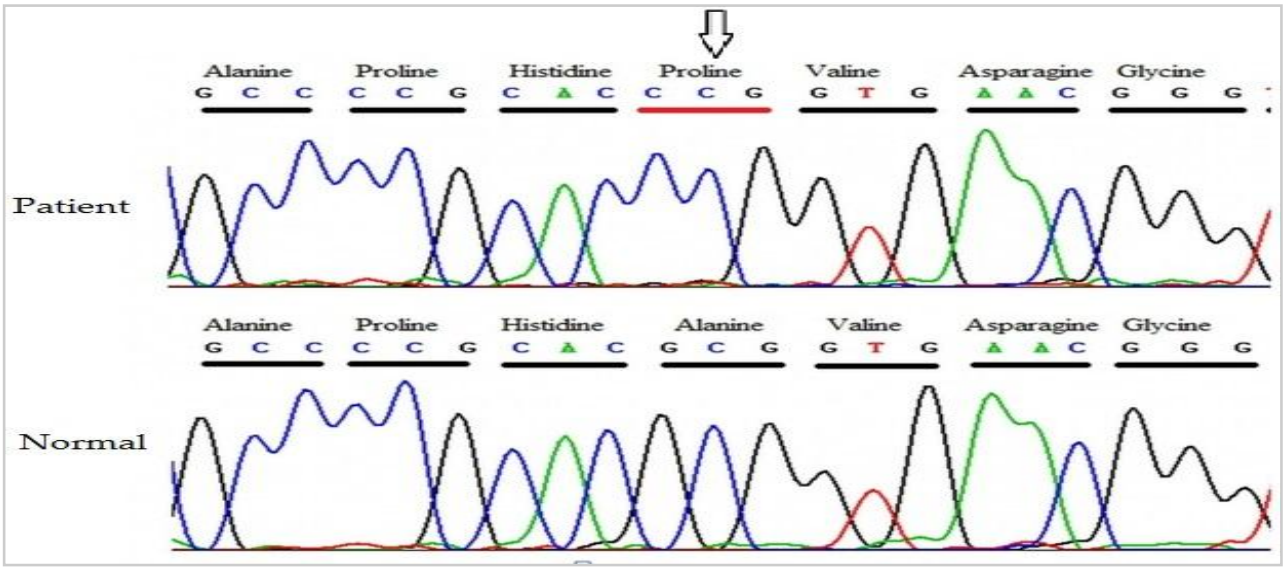

Figure: 5 Homozygous missense mutation in exon 3 of PAX9 gene (c. 718C>G; p.Ala240Pro) in the affected individual (II-1).

an autosomal recessive segregation pattern (fig-1). Mutational analyses of the *PAX9* gene, phenotypes and missing teeth observed in the studied family are shown in (table-II).Clinical examination and medical records did not disclose health problems or disorders related to nails, hair missense mutation in individuals with hypodontia from a Pakistani family. The mutation results in the substitution of proline for alanine in exon 3 of PAX9 gene. This mutation appeared in affected members of family, whereas all unaffected family members were negative for

this variant. In humans, thirty two *PAX9* mutations have been identified including 21 missense/nonsense, 6 insertion/deletions and 2 for complex re-arrangements (www.hgmd.cf.ac.uk). Many genes, including *MSX1, PAX9, WNT10A, EDA* and *AXIN2*, have been involved in non-syndromic tooth agenesis. *PAX9* mutations cause molar agenesis whereas *MSX1* mutations cause second premolar agenesis[25]. Whereas *WNT10A* aberrations usually cause autosomal recessive or isolated tooth agenesis[26]. *EDA* mutations are more likely to cause anterior teeth agenesis[27]. In addition, *AXIN2*-associated tooth agenesis is often accompanied with predisposing to colorectal cancer[28]. For this study we have selected *PAX9* prior to the other candidate genes. Up to date, more than thirty *PAX9* mutations have been found and all of these mutation were differentiated into two subsets[29]. The first subset, in-frame mutations, includes missense mutations and in-frame insertions or deletions. The second subset, truncating mutations, includes nonsense mutations, out-of-frame insertions or deletions, initiation codon mutations and deletion of the entire gene. Now, we focused on functional studies of the mutant *PAX9* to explain the oligodontia phenotype. The study of Mensah indicated that the 219 insG *PAX9* mutant could not function properly because it stayed in the cytoplasm, while the wild-type *PAX9* was trans located to the nucleus[23]. Cytoplasmic localization was not found in eight other tooth agenesis causing Pax9 mutations in the study of Wang *et al*[30].

CONCLUSION

The sequencing of the *PAX9* gene showed a homozygous missense substitution in exon 3 (c. 718G>C; p.Ala240Pro) in the affected individuals of the family. Due to this mutation the maxillary lateral incisors along with maxillary third molars were congenitally absent.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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