A MISSENSE MUTATION OF MSX1 GENE IN PAKISTANI FAMILIES WITH HYPODONTIA

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ABSTRACT

Objective: To understand the role of *MSX1* gene in Pakistani families with hypodontia.
Study Design: Descriptive study.
Place and Duration of Study: This descriptive study was performed in Human Molecular Genetics (HMG) Laboratory of Baluchistan University of Information Technology, Engineering and Management Sciences (BUITEMS). The study was of 5 months duration.
Material and Methods: Peripheral blood sample of 5 ml was extracted intravenously from all affected individuals, normal siblings and their parents in 15ml falcon tubes containing 200µl EDTA. Human genomic 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 BUITEMS. Two coding exons of *MSX1* (NM_002448.3) were amplified and sequenced. Sequence analysis of the coding region of *MSX1* gene is reveal through Bio Edit, Chromas and Seqman software's.
Results: Hypodontia of permanent teeth of each affected family member was confirmed from history, clinical and radiographic examination. The clinical examination of Family 1 (proband I) revealed missing maxillary teeth 12, 18, 22, 28 and mandibular teeth 31, 41, 42 (FDI number system). The proband I-1 has also shown dental malformation of teeth and tongue tie. Family 2 (proband II) had missing maxillary lateral incisor 12, 22 (FDI number system). Cephalometric analysis showed that proband I and proband II both had mild skeleton class-II malocclusion (ANB-6°, ANB-5° respectively) with normal vertical pattern. Dental cast analysis showed class-II dental relation with large midline diastema and anterior spaces in both affected proband. The coding region of *MSX1* exons were sequenced and analyzed through Bio Edit, Chromas and Seqman software's and a missense mutation was found. Here the transition of alanine-to-glycine lead to a substitution at amino acid position 40 (c.119C>G p. Ala40Gly).
Conclusion: This genetic study revealed missense mutation (c.119C>G p.Ala40Gly) in exon 1 of *MSX1* gene in Pakistani families with upper lateral hypodontia along with other dental anomalies including microdontia and tongue tie.

Keywords: Congenital, Homeobox gene, Hypodontia, Missense mutation, *MSX1* gene.

INTRODUCTION

Hypodontia is congenital absence of one or more than one deciduous and permanent teeth[1]. Other terms used in the literature to define the number of missing teeth: aplasia of teeth, anodontia, oligodontia, absence of teeth, congenitally missing teeth, lack of teeth and agenesis of teeth[2]. Human teeth have importance in esthetic, function, stability of oral facial structure and socio-cultural interaction, which represent at individual level as a good or bad life quality. Hypodontia is the most common anomaly seen in the human orofacial region[3-5]. Diagnosis of hypodontia is confirmed by using history, clinical examination and panoramic radiograph at age range from 3-14 year[6]. It is also classified on the basis of number of missing teeth into three types: hypodontia (the lack of one to five teeth, excluding third molars), severe hypodontia or oligodontia (the lack of six or more teeth, excluding third molars) and anodontia (the complete absence of teeth)[7]. Hypodontia is

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associated with other dental anomalies such as enamel hypoplasia, failure of eruption of teeth and dental malformation which lead prematurely to the edentulous state[8]. The most common sequence of missing teeth is mandibular 2nd premolar followed by maxillary lateral incisor, maxillary 2nd premolar, and then mandibular first premolar[9].

In Pakistani orthodontic patients, the prevalence of hypodontia (excluding the third molars) has been reported 4.7% for females, 1.3% for males, and 6.08% for both sexes combined[9]. The number of missing teeth has been greater in mandibular arch as compared to maxillary arch. Hypodontia is most common on left side. Most study has shown that hypodontia of deciduous and permanent dentition has strong correlation. The children with hypodontia in the deciduous dentition predominantly show hypodontia of the its permanent successor teeth[6]. The female population is more affected than male[9,10].

The etiology of hypodontia is complex and yet not clearly understood, but generally it can be credited to genetic factors and environmental factor. In genetic factor tooth agenesis can be either non-syndromic or syndromic and the genes responsible for hypodontia are *MSX1*, *PAX9*, *WNT10A, AXIN2, TGFA, IRF6, MMP1, MMP20* and *FGF3*[11-13]. Environmental factor are endocrine tissue disorders, developmental anomalies, trauma to head and neck region, oral pathologies, medical therapies, radiotherapy in early age, high fever and rubella-type diseases.

The Muscle Segment Homeobox[1] (*MSX1*) gene is expressed in both dental follicle and dental papilla stages of tooth development as transcription factors[5]. It is located on chromosome 4p16.1. In early odontogenesis it is expressed in mesenchymal tissue and has important role in the pathways of odontogenesis[14]. *MSX1* homeobox gene mutation cause tooth agenesis in human[5].

Targeted null mutation of *MSX1* results in multiple craniofacial abnormalities that include arrested tooth development at the bud stage, small mandible with alveolus defect, and cleft palate with 100% phenotype penetrance[15,16]. *MSX1* is a key signaling mediator during tooth morphogenesis between the dental mesenchyme and epithelium tissue.

MSX1 is appreciated as an interesting candidate gene for envelopment in both selective tooth agenesis and cleft-palate in human with possible monogenetic cause of orofacial clefting in combination with hypodontia. The objective of present study was to determine the role of *MSX1* candidate gene genotype and phenotype of hypodontia families and to determine the mutation in *MSX1* gene of affected families of hypodontia.

MATERIAL AND METHODS

This descriptive study was performed in Human Molecular Genetics (HMG) Laboratory of Baluchistan University of Information Technology, Engineering and Management Sciences (BUITEMS). The study was of 5 months duration using non-probablity consecutive sampling. The study group consisted of 35 individuals from fifteen hypodontia families from different private clinics and government hospitals identified and enrolled for the study. In each family three probands were selected which included hypodontia affected individual with one sibling and one of parents, either mother or father. The inclusion criterion was diagnosis of hypodontia patient in each family with ages ranging from 11 to 25 years. The exclusion criteria included accidental tooth loss, tooth extraction and history of trauma in orofacial region. Each hypodontia patient was diagnosed through taking history, intra oral clinical examination and dental panoramic radiograph (fig-1). Additional record of hypodontia proband included lateral cephalometric radiograph, dental cast analysis, dental extra oral and intra oral photograph (fig-1). Peripheral blood sample of 3.5 to 5 ml was extracted intravenously from all affected proband, normal siblings and their parents in 15ml falcon tubes containing 200µl EDTA. Every falcon tube was labeled with family credentials.

Blood samples are then frozen at -20°C. After the approval of Institutional Review Board (IRB# 00007818), at the department of Biotechnology, Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Quetta, Pakistan the families were enrolled for current study. The study was conducted according to the tenets of the declaration of Helsinki. Written inform consent was obtained from all adult participants and their parents in case of minors.

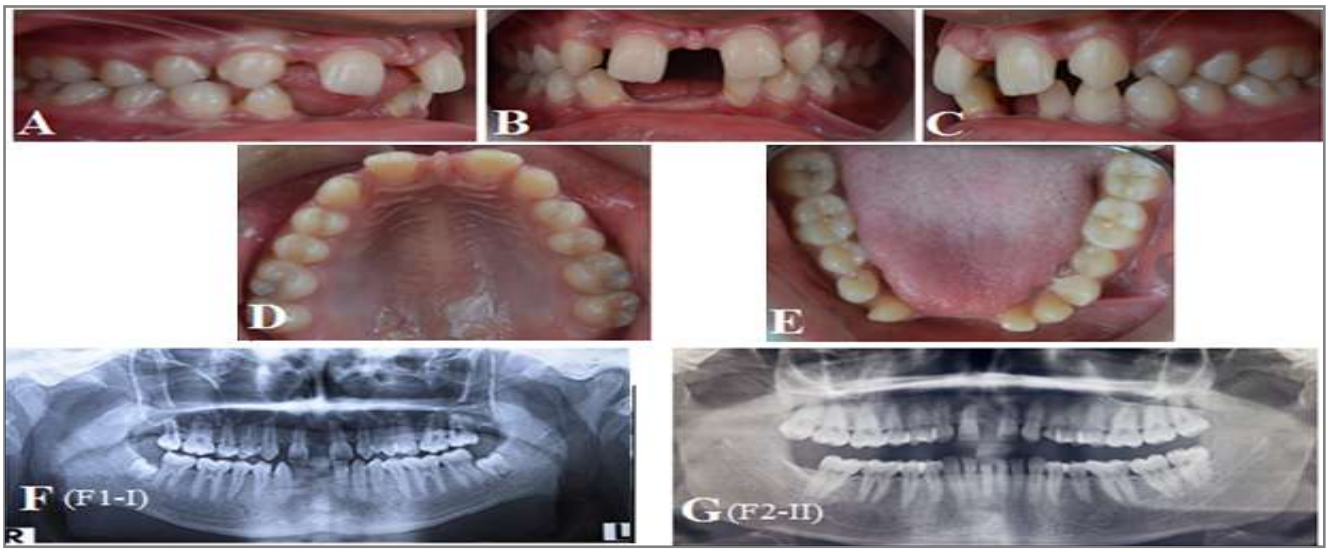

Figure-1: Phenotype of family 1(F1) and family 2 (F2), Intra oral photograph (A) Right occlusal view, (B) Front occlusal view, (C) Left occlusal view, (D) Upper occlusal view, (E) lower occlusal view, (F) Panoramic radiograph of proband (F1-I) show missing teeth 12, 18, 22, 28,31, 41, 42 (FDI number system) (G) Panoramic radiograph of proband (F2-II) show missing teeth 12, 22, 48.

Figure-2: Agarose gel electrophoresis of exon 1 of MSX1_1b gene of family 1 (F1) and family 2 (F2).

HMG Laboratory of BUITEMS was selected for lab procedures. The human genomic DNA were extracted by using inorganic method from the blood leukocytes following a standardized protocol already established in HMG laboratory of BUITEMS. The extracted DNA was subsequently run in Agarose gel electrophoresis to check its quality. Four sets of primers for coding Exons (Exon 1 and 2) of *MSX1* gene were designed by using computer web program Primer[3], UCSC Genome Bioinformatics and Ensembl genome browser (table-I). The designed primers were synthesized from Macrogen Company Korea. Amplification of the exons using predesigned primer was accomplished by polymerase chain reaction (PCR). The polymerase chain reaction (PCR) protocol of BUITEMS'HMG

laboratory was followed for this purpose. The amplified DNA of each exon was then run in Agarose gel electrophoresisas shown in fig-2. The extracted DNA was sequenced to check any possible mutations in *MSX1* gene.

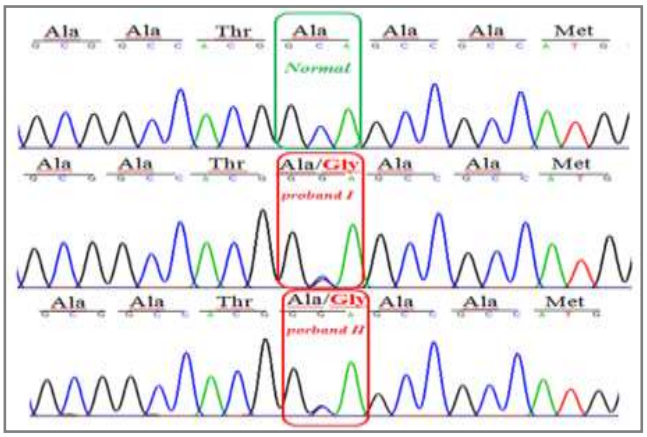

Figure-3. DNA- sequencing Chromatograms of exon 1 of proband (I) and proband (II), a missense mutation (c.119C>G p. Ala40Gly) is indicated.

The primer assembly and DNA sequenceing were out sourced to Macrogen Company, Korea. The identified mutation was checked in public databases, namely dbSNP (www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes database (www.browser.1000genomes.org/index.html), NHLBI Exome Variant Server (www.evs.gs.washington.edu/EVS/) and the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php).

RESULTS

The medical history of all family members revealed no health problems or disorders related to nails, hair or sweat glands. Hypodontia of permanent teeth of each affected family member was confirmed from history, clinical and radiographic examination as shown in fig-1 and table-II. The clinical examination of Family 1 (proband I) as shown in intraoral photograph, panoramic radiograph (fig-1A to F) revealed a missing maxillary teeth 12,18,22,28 and mandibular teeth 31,41,42 (FDI number system). The proband I-1 also showed dental malformation of teeth and tongue tie. The tongue tie was treated surgically through glossectomy prior to orthodontic treatment. Family 2 (proband II) as shown in panoramic radiograph (fig-1G) had missing maxillary lateral incisor 12, 22 (FDI number system). Cephalometric analysis showed that proband I

Table-I: Sequence of primers used for amplification of different exons of MSX1 gene with forward primer and reverse primer.

The target frament (exons)	Sequence (5′~3′)	Product Size (bp)	Annealing Temperature (°C)
MSX1_1F MSX1_1R	CTGGCCTCGCCTTATTAGC CCTGGGTTCTGGCTACTCAC	765	61
MSX1_1aF MSX1_1aR	CGCCTTATTAGCAAGTTCTCTG GCAAAGAAGTCATGTCAGCAG	300	61
MSX1_1bF MSX1_1bR	CGGTGTCAAAGTGGAGGACT CCTGGGTTCTGGCTACTCAC	454	61
MSX1_2F MSX1_2R	TGATCATGCTCCAATGCTTC ACCAGGGCTGGAGGAATC	552	61

Table-II: Dentition profile of affected family members of proband (I) and proband (II) with p.Ala40Gly mutation.

Family/ Proband	Jaws	Right Quadrant								Left Quadrant							
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
1:I	Upper	X						X			X						X
	Lower							X	X	X							
2:II	Upper							X		X							
	Lower	X															

Missing Teeth (X), Dentition: (1) Central Incisor; (2) Lateral Incisor; (3) Canine; (4) first premolar; (5) second premolar; (6) first molar; (7) second molar; (8) Third molar.

and proband II both had mild skeleton class-II malocclusion (ANB-6°, ANB-5° respectively) with normal vertical pattern. Dental cast analysis show class-II dental relation with large midline diastema and anterior spaces in both affected probands. Examination of the other family members did not show any missing tooth or other tooth anomaly.

The coding region of *MSX1* exons was sequenced and analyzed through BioEdit, Chromas and Seqman software's and a missense mutation was found as shown in (fig-3). Here the transition of alanine-to-glycine lead to a substitution at amino acid position 40 (c.119C>G p. Ala40Gly).

DISCUSSION

The missense mutation in Pakistani family with hypodontia of permanent tooth is reported in the present study. The mutation at CDNA level (c.119C>G) causes the substitution amino acid Alanine instead of Glycine at protein position 40 (p.Ala40Gly). This site is a highly conserved site for *MSX1* protein, where the mutations may cause potential variations in the protein form which results in different abnormalities of the orofacial regions[17].

The dental microdontia was found in prob and II of family 2. Studies have shown that clinical investigation of affected family members demonstrated hypodontia with dental anomalies i.e. malformation, enamel hypoplasia, delay eruption of teeth, premature eruption and reduce size of teeth[18-21].

In the present study only three families out of fifteen families have shown oligodontia (more than six teeth missing), all other have hypodontia (less than six teeth missing).

The importance of *MSX1* gene is during the bud and cap stages of the tooth development, *MSX1* along with its family homeobox gene (*MSX1*, *MSX2* and *MSX3*) expresses its interaction especially where epithelial mesenchymal inter-action takes place.

The mutations of *MSX1* gene causes tooth agenesis, tooth malformations including conical shaped tooth and impactions at the same time it may also play its role in associated syndromes like Witkop syndrome, cleft lip and cleft palate and Wolf-Hirschhorn syndrome. Other research reports indicate that polymorphism of *MSX1* might be a risk factor for multiple phenotypic isolated or syndromic tooth absentia.

The etiology of non-syndromic tooth agenesis have been identified mostly in *MSX1* gene mutations. Many of them are missense mutations that change only one amino acid in the highly conserved homeodomain sequence (amino acids 167–225)[13]. These mutations have been described in families with severe hypodontia inherited in an autosomal-dominant fashion. Moreover, a mutation identified in the Polish population (Ala194Val) showed incomplete penetrance. In our study Pakistani family shows mutation at (p.Ala40Gly) at amino acid 119.

The incidence of tooth agenesis has been observed to be increasing during the 20th century. So in the coming years more affected individuals are expected for tooth malformations. The detail studies should be carried out by researchers to analyze the gene networks underlying this anomaly.

The research should be carried out at molecular genetic level along with increased sample size for better diagnosis of the role and prevalence of these genes associated with mutations.

CONCLUSION

This genetic study revealed missense mutation (c.119C>G p.Ala40Gly) in exon 1 of *MSX1* gene in Pakistani families with hypodontia along with other dental anomalies include microdontia and tongue tie.

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CONFLICT OF INTEREST

This study has no conflict of interest to be

declared by any author.

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