INNSPUB
International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 1, p. 425-429, 2018

RESEARCH PAPER OPEN ACCESS

The role of *MSX1* gene in affected families of hypodontia attending in Tertiary Care Hospital of Quetta

Muhammad Nawaz*[1], Nasrullah Mengal[1], Agha Muhammad Raza[2], Nisar Ahmed[3], Muhammad Saeed[3], Jamil Ahmad[3]

[1] *Orthodontics Department, Dental Section, Sandeman Provincial Hospital, Quetta, Pakistan*
[2] *Departments of Microbiology, BUITEMS, Quetta, Pakistan*
[3] *Departments of Biotechnology, BUITEMS, Quetta, Pakistan*

Key words: Hypodontia, *MSX1 gene*, Missense mutation, Congenital

http://dx.doi.org/10.12692/ijb/13.1.425-429 Article published on July 30, 2018

Abstract

The purpose of this study is to identify genotype and phenotype of in Pakistani families with hypodontia and to map the genes locus responsible for this disease. Tooth agenesis known as hypodontia, is a tooth developmental anomaly characterized by congenital absence of one or more teeth. It may occur in primary or secondary dentition and is one of the common craniofacial anomalies. Hypodontia prevalence of is 4.7% for females and 1.3% for males. Third molar agenesis is the most common with an incidence of 20% in general population of Pakistan. The etiology of hypodontia is mainly Genetics whereas environmental factors may also play a role in hypodontia. Blood samples (5ml) were collected from all family members. Genomic DNA was extracted by using inorganic method. All the two coding exons of *MSX1* (NM_002448.3) were amplified and sequenced. Sequencing of the *MSX1* coding exons and splice sites showed a homozygous missense substitution in exon 1 (c.119C>G p.Ala40Gly) in the two affected individuals of the two families out of fifteen families. We identified a missense mutation (p.Ala40Gly) in *MSX1* gene coding exon 1 in two Pakistani families with hypodontia.

* **Corresponding Author:** Dr. Muhammad Nawaz ✉ muhammadnawazpanezai41@gmail.com

Introduction

Teeth have a prominent importance to socio-cultural interactions of humans and at an individual level can represent a good or bad life quality. Congenital tooth abnormalities including agenesis is the most common tooth anomaly observed in the craniofacial region development of humans (Paixao-Cortes *et al.*, 2011). The formation of teeth includes sequence of genetic and epigenetic interaction between the ectodermal and mesodermal cells. Any pathology or mutation in genetic sequence may result in hypodontia and other anomaly of oral cavity (Swinnen *et al.*, 2008). Dyanrajani has classified the tooth agenesis i.e. hypodontia on the basis of the severity of the condition. Mild to moderate hypodontia includes the absence of two to five teeth whereas severe hypodontia includes the missing of six or more than six teeth (Dhanrajani, 2002). Congenital tooth absence is also associated with different disease i.e. colorectal cancer, cleft lip, oro-facial clefting and more than 60 Syndromes (H Vastardis, N Karimbux, SW Guthua, JG Seidman, CE Seidman, 1996).

MSX1 is a homeobox gene located on chromosome 4 and encodes a DNA-binding protein (H Vastardis, N Karimbux, SW Guthua, J Seidman, CE Seidman, 1996). The main function of *MSX1* protein is to interact with TATA box-binding protein (TBP) and some transcription factors to increase the rate of the transcription process (SHAHID; Zhang *et al.*, 1997). Like the *PAX9* knockouts, *MSX1* knockouts also lack teeth, and their development is arrested at the bud stage. Mutations in the *MSX1* gene may lead to tooth loss as well as it may also form disturbances in cleft lip or cleft palate and nail dysplasia (Cobourne, 2007; Jumlongras *et al.*, 2001).

The purpose of my study is to identify the phenotype, genotype of hypodontia for Pakistani families and to map the genes locus responsible for this disease. To determine the frequency of mutated *MXS1* gene in affected families of Hypodontia attending Sandeman Provincial Hospital Quetta for dental treatment.

Material and methods

Identification and enrolment of families

Fifteen hypodontia families were identified from different private clinics and hospitals, each family with one or more hypodontia effected individuals, ages ranging from 11 to 25 years were selected in present study. Family pedigree and history were collected from the families (Fig. 1). Hypodontia clinical appearance and confirming the absence of missing teeth by digital OPG (orthopantogram) X-ray were considered to be the basic criteria for the study (Fig. 2). Accidental tooth loss, tooth extraction or patients with history of trauma were excluded from the study by conducting complete patient history. Peripheral blood sample of 3.5 to 5ml were extracted intravenously from all affected individual, normal siblings and their parents in 15ml falcon tubes containing 200μl EDTA. Every falcon tube labeled with family credentials. Blood samples were then frozen at -20c. After the 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.

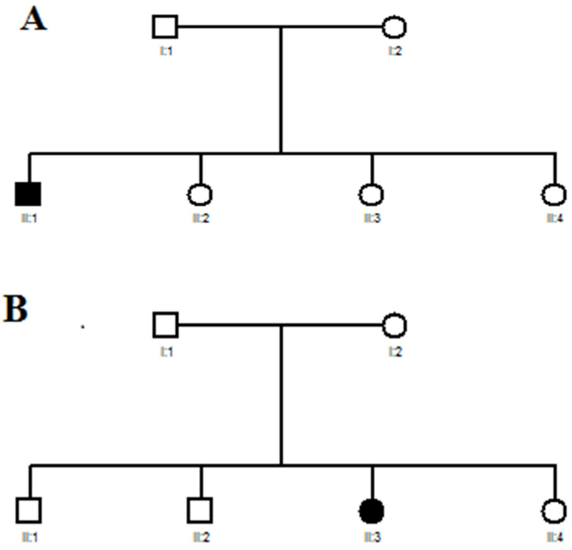

Fig. 1. Pedigree showing phenotypes of family 1 (A), family 2 (B). Squares = males; circle = female; black = hypodontia; white = unaffected.

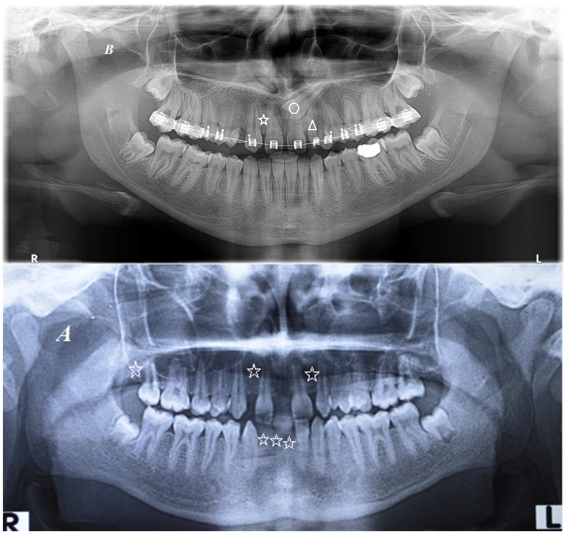

Fig. 2. (A) panoramic radiograph shows that proband (II-1) was missing 7 teeth at the age of 17 years. (B) Panoramic radiograph shows that proband (II-3) was missing 1 teeth at the age of 16 years. The missing teeth are indicated with start (★), conical shape teeth triangle (▲), impacted teeth circle (●).

Mutational analysis of MSX1

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. The final extracted DNA was run in electrophoresis gel to check the quality of the extracted DNA. Primers for coding exons of *MSX1* gene were designed by using computer web program Primer3, UCSC Genome Bioinformatics and Ensembl genome browser (Table 1). After primer was designed, they were assembled from Macrogen Company Korea. Amplification of the exons using pre designed primer was done by polymerase chain reaction (PCR). The polymerase chain reaction (PCR) protocol of BUITEMS Human Molecular Genetic laboratory was followed for this purposed shown in table 2. The amplified DNA was sequenced to check any possible mutations in *MSX1* gene from 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).

Table 1. Primers list for the MSX1 gene.

Gene	Left Primer (5 > 3)	Right Primer (5 > 3)	Product Size	Annealing temperature
MSX1_ X1	CTGGCCTCGCCTT ATTAGC	CCTGGGTTCTG GCTACTCAC	765	
MSX1_ X1a	CGCCTTATTAGCA AGTTCTCTG	GCAAAGAAGTC ATGTCAGCAG	300	
MSX1_ X1b	CGGTGTCAAAGTG GAGGACT	CCTGGGTTCTG GCTACTCAC	454	
MSX1_ X2	TGATCATGCTCCA ATGCTTC	ACCAGGGCTGG AGGAATC	552	

Table 2. Polymerase chain reaction reagents and there quantity.

S. No	Chemical	Quantity per sample
1	5x PCR Buffer	4 µl
2	dNTPs	1.2 µl
3	Primer forward	0.6 µl
4	Primer Reverse	0.6 µl
5	Red *Taq* Polymerase	0.8 µl
6	DNA Template	4 µl
7	PCR Water	8.8 µl

Results

Clinical findings

The number of families enrolls for study were fifteen and out of fifteen families only two family show *MSX1* gene mutation. Pedigree diagram of both the families are shows (Fig. 1) that only one member (family 1 proband II-1, family 2 proband II-3) of the families are affected. Intra oral and Xray investigation confirms that the proband (II-1) having a class II skeletal pattern and Class II malocclusion with missing seven permanent teeth in both jaws. These are in maxilla both lateral incisors, third molars and in mandible both central and right lateral incisors missing (Fig. 2 and Table 3). Proband II-1 also have tongue tie with a large mid line diastema 8.5mm present between maxillary central incisors and spacing in mandibular arch. In family two proband II-3 present with class I malocclusion with lacking only maxillary right lateral incisor, conical shape maxillary left lateral incisor, impacted maxillary left canine and retained deciduous left canine (Fig. 2 and Table 3). Examination of the other family members of these patients did not showed any missing tooth or other tooth anomaly. The diagnosis of hypodontia in the both proband was confirmed in an interview, clinical examination and panoramic radiographs.

Table 3. Phenotypes of affected family members with ala119gly mutation.

Family/ individual	Gender	Age	No of teeth missing	Arch	Right								Left							
					8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
1- II:1	Male	17	7	Maxillary	★						★			★						★
				mandibular							★	★	★							
2- II:3	female	16	1	Maxillary							★			▲	●					
				mandibular																

Missing teeth are indicated with start (★), conical shape teeth triangle (▲), impacted circle (●). In dentition: (1) central incisor; (2) lateral incisor; (3) canine; (4) first premolar; (5) second premolar; (6) first molar; (7) second molar; (8) third molar (wisdom tooth). See Fig. 1A, B for location of each individual on the pedigree.

Screening for mutations

Sequence analysis of the coding region of *MSX1* gene was performed through BioEdit, Chromas and Seqman software's and a missense mutation was found. This gene is a homeobox sequence gene (Fig. 4). Here the transition of alanine-to-glycine leads to a substitution at amino acid position 40 (c.119C>G p. Ala40Gly).

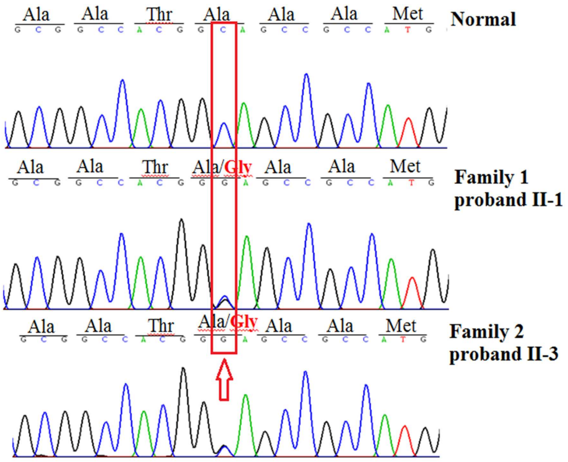

Fig. 3. Homozygous missense mutation in exon 1 of MSX1 gene (c.119C>G p.Ala40Gly) in the affected individual of family 1 (proband II-1) and family 2 (proband II-3).

Discussion

In our current research, the mutation in the *MSX1* gene that was found in two Pakistani families enrolled for anterior teeth hypodontia of permanent dentition. 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 result in different abnormalities of the oral and facial regions (Mostowska, Biedziak, & Jagodzinski, 2012).

In the present study proband II-1 of family 1 show hypodontia with tongue tie. The tongue tie was surgically corrected before orthodontics treatment started. While proband II-3 of family 2 show hypodontia with other tooth malformation, such has conical shape maxillary lateral incisor and tooth abnormality, such has impacted maxillary left canine. The association of hypodontia with this tooth malformations and abnormality also has been reported before (Cobourne, 2007; Han *et al.*, 2008; Pinho, Silva-Fernandes, Bousbaa, & Maciel, 2010; Xuan *et al.*, 2008).

The *MSX1* Mutations causes tooth agenesis, tooth malformations including conical shape tooth and impactions and at the same time it may also play its role in associated syndrome 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 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.

Conclusion

We identified a missense substitution in exon 1 of *MSX1* gene at (c.119C>G p.Ala40Gly) in two Pakistani families with hypodontia.

Acknowledgments

We are grateful for the participation and contribution of probands and their families.

References

Cobourne MT. 2007. Familial human hypodontia--is it all in the genes? Br Dent J **203(4),** 203-208.
DOI: 10.1038/bdj.2007.732

Dhanrajani PJ. 2002. Hypodontia: Etiology, clinical features, and management. Quintessence international **33(4)**.

Han D, Gong Y, Wu H, Zhang X, Yan M, Wang X, Song S. 2008. Novel EDA mutation resulting in X-linked non-syndromic hypodontia and the pattern of EDA-associated isolated tooth agenesis. European journal of medical genetics **51(6),** 536-546.

Jumlongras D, Bei M, Stimson JM, Wang WF, DePalma SR, Seidman CE, Olsen BR. 2001. A nonsense mutation in MSX1 causes Witkop syndrome. The American Journal of Human Genetics **69(1),** 67-74.

Mostowska A, Biedziak B, Jagodzinski PP. 2012. Novel MSX1 mutation in a family with autosomal-dominant hypodontia of second premolars and third molars. Arch Oral Biol **57(6),** 790-795.
DOI: 10.1016/j.archoralbio.2012.01.003

Paixao-Cortes VR, Braga T, Salzano FM, Mundstock K, Mundstock CA, Bortolini MC. 2011. PAX9 and MSX1 transcription factor genes in non-syndromic dental agenesis. Arch Oral Biol **56(4),** 337-344.
DOI: 10.1016/j.archoralbio.2010.10.020

Pinho T, Silva-Fernandes A, Bousbaa H, Maciel P. 2010. Mutational analysis of MSX1 and PAX9 genes in Portuguese families with maxillary lateral incisor agenesis. Eur J Orthod **32(5),** 582-588.
DOI: 10.1093/ejo/cjp155

Shahid, M. Genetic Anomalies and Tooth Agenesis: Review Article.

Swinnen S, Bailleul-Forestier I, Arte S, Nieminen P, Devriendt K, Carels C. 2008. Investigating the etiology of multiple tooth agenesis in three sisters with severe oligodontia. Orthodontics & craniofacial research **11(1),** 24-31.

Vastardis H, Karimbux N, Guthua SW, Seidman J, Seidman CE. 1996. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. Nature genetics **13(4),** 417-421.

Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. 1996. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. Nat Genet **13(4),** 417-421.

Xuan K, Jin F, Liu YL, Yuan LT, Wen LY, Yang FS, Jin Y. 2008. Identification of a novel missense mutation of MSX1 gene in Chinese family with autosomal-dominant oligodontia. Arch Oral Biol **53(8),** 773-779.
DOI: 10.1016/j.archoralbio. 2008.02.012

Zhang H, Hu G, Wang H, Sciavolino P, Iler N, Shen MM, Abate-Shen C. 1997. Heterodimerization of Msx and Dlx homeoproteins results in functional antagonism. Molecular and cellular biology **17(5),** 2920-2932.