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

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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.

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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.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Left Primer (5’→3’)</th>
<th>Right Primer (5’→3’)</th>
<th>Product Size</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSX1</td>
<td>CTGGCCTCGCTT ATTAGC</td>
<td>GCTACTCAG</td>
<td>765</td>
<td></td>
</tr>
<tr>
<td>MSX1a</td>
<td>CCGCTATTAGCA GCAAAGAAGTC</td>
<td>ATGTCAGAC</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>MSX1b</td>
<td>CGGTGTCAAAGTG CCGGGGTCTCG</td>
<td>AGAGAGACAG</td>
<td>454</td>
<td></td>
</tr>
<tr>
<td>MSX1</td>
<td>TGATCATGCTCCA ACCAGGCGGGG</td>
<td>AGGAGAATC</td>
<td>552</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Polymerase chain reaction reagents and there quantity.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Chemical</th>
<th>Quantity per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5x PCR Buffer</td>
<td>4 µl</td>
</tr>
<tr>
<td>2</td>
<td>dNTPs</td>
<td>1.2 µl</td>
</tr>
<tr>
<td>3</td>
<td>Primer forward</td>
<td>0.6 µl</td>
</tr>
<tr>
<td>4</td>
<td>Primer Reverse</td>
<td>0.6 µl</td>
</tr>
<tr>
<td>5</td>
<td>Red Taq Polymerase</td>
<td>0.8 µl</td>
</tr>
<tr>
<td>6</td>
<td>DNA Template</td>
<td>4 µl</td>
</tr>
<tr>
<td>7</td>
<td>PCR Water</td>
<td>8.8 µl</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Family/individual</th>
<th>Gender</th>
<th>Age</th>
<th>No of teeth missing</th>
<th>Arch</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- II:1</td>
<td>Male</td>
<td>17</td>
<td>7</td>
<td>Maxillary</td>
<td>⭐️</td>
<td>⭐️</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mandibular</td>
<td>⭐️</td>
<td>⭐️</td>
</tr>
<tr>
<td>2- II:3</td>
<td>Female</td>
<td>16</td>
<td>1</td>
<td>Maxillary</td>
<td>⭐️</td>
<td>⭐️</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mandibular</td>
<td></td>
<td>⭐️</td>
</tr>
</tbody>
</table>

Missing teeth are indicated with start (⭐️), conical shape triangle (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).

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


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