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The role of MSX1 in tooth agenesis in Iranians

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Introduction. MSX1 gene has a critical role in craniofacial development, the aim of this case–control study is to test the hypothesis that MSX1 mutation contributes to congenital tooth agenesis in Iranians.
Materials and methods. The study group consisted of 20 affected individuals with tooth agenesis of lower second premolars or upper lateral incisors with mean age of 24.6. The control group consisted of 20 unaffected individuals. DNA was extracted from all 40 individuals; the polymerase chain reaction (PCR) for MSX1 was carried out with Phenol: Chloroform: Isoamylalchol (PCI) extraction method. Ban II restriction digest and agarose gel electrophoresis of the 20 affected individuals verified the presence of mutation in all 20 affected individuals. The unaffected controls did not show any mutation. Statistical analysis performed by the chi-squared method.
Results. Ban II did not digest PCR product (DNA) in the control group (195 bp band on electrophoresis gel) but digested the affected allele (106 bp and 89 bp bands). There is a statistically significant correlation between tooth agenesis and MSX1 mutation ($P < 0.001$).
Conclusion. The results indicated that MSX1 gene mutation contributes to tooth agenesis in Iranian individuals. As the timing of tooth calcification can vary, radiographic finding of congenital tooth agenesis can be confirmed by this molecular method during different dental ages to achieve certainty.

Introduction

Advances in molecular biology, epidemiology, quantitative analysis, and developmental biology have made it possible to identify genes involved in traits important in dental disorders. Heredity is one of the possible factors associated with congenital missing mandibular incisors[1]. A missense mutation in the homeodomain of MSX1 gene has been associated with hypodontia of second premolars and third molars in humans[2]. Recently, a similar pattern of tooth agenesis was found to have a MSX1 Ser105Stop mutation[3]. Some affected individuals also had cleft lip or cleft palate, extending the phenotypes associated with MSX1 mutations in humans and supporting previous associations reported between MSX1 and nonsyndromic cleft lip and cleft palate[4]. However, two studies excluded this gene as causative locus for hypodontia of incisors and premolars[5,6]. More recently, a MSX1 Ser202Stop mutation was reported to be associated with the Witkop syndrome, which includes tooth agenesis and nail dysgenesis[7]. It seems that MSX1[2,3,7–10], PAX9[9–12] and TGFA[10] contribute to tooth agenesis.

In the Iranian population, the incidence of tooth agenesis varies with tooth class. The third molar is the most frequent (20%). Absence of the upper/lower second premolars (3.5%) followed by the maxillary lateral incisors (2.3%) are other frequent findings. Absence of a single premolar occurs frequently. Maxillary lateral incisors are the most frequent missing teeth when only one or two teeth are absent, whereas second premolars are the most frequent missing teeth when more than two teeth are absent.

Demographics of Iran

Iran is a diverse ethnic country. Persians, the founders of Ancient Persia, constitute the majority of the population. Seventy per cent of present-day Iranians are Iranic native speakers of Indo-European languages who are descended from

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the Aryan (Indo-Iranians) tribes that began migrating from Central Asia into what is now Iran in the second millennium BC. The majority of the population speak one of the Iranian languages, including the official language, Farsi. To test the hypothesis that MSX1 mutations are a common cause of congenital tooth agenesis in Iranians, we screened 20 affected individuals and 20 unaffected individuals.

Materials and methods

Patient and control samples

This is a case–control study. A total of 40 individuals, 20 unaffected individuals with 32 teeth, and 20 affected individuals with congenital tooth agenesis of lower second premolars or upper lateral incisors with mean age of 24.6 were recruited from The Shaheed Beheshti University of Medical Sciences, Department of Orthodontics. Convenient sampling method was applied. All individuals had Iranian ancestors. All participants have signed an Institutional Review Board-approved informed consent. The inclusion criteria was congenital agenesis of at least one permanent tooth, not including third molars, as verified by radiographs and clinical examination. Tooth agenesis adjacent to a cleft site were excluded, because the absence of such teeth is likely to be the consequence of local developmental anomalies at the cleft site. Third molar agenesis was not characterized in all subjects, as some individuals were too young for this trait to be determined. The Universal Tooth Numbering System was used to designate which teeth were missing[13]. Medical, birth defect, and family histories were gathered to identify possible associated anomalies. All cases had the history of familial tooth agenesis.

MSX1 mutation screen and sequencing

DNA was extracted from all 40 individuals[14]. The polymerase chain reaction (PCR) for MSX1 (NCBI accession number XM_048684) was carried out with the use of Fail Safe (Epicentre, Madison, WI, USA) buffer under the following conditions: 10-min 95 °C activation/premelt step, followed by 35 cycles of 30-s 94 °C melt, 30-s 60 °C anneal, and 30-s 72 °C extension. PCR product purification was performed by ExoSAP-IT® (USB, Cleveland, OH, USA), followed by sequencing with ABI Big Dye® terminator reagents (Applied Biosystems, Foster City, CA, USA) with the use of an ABI PRISM 377 DNA sequencer (AME Bioscience, Toroed, Norway). Ban II restriction enzyme digestion was performed to detect the MSX1 mutation.

Results

Clinical diagnosis

A total of 40 Iranian natives in the age range of 14–27 years old were selected. Review of the medical history did not reveal significant medical problems in affected individuals. There were no abnormalities of the toenails, fingernails, hair, or sweat glands. A clinical examination by the treating orthodontist was initially performed to determine the status of the dentition for individuals. We examined dental radiographs to confirm the diagnosis of oligodontia for affected individuals (Fig. 1).

The patterns of missing teeth among family members showed considerable variability. Some individuals reported the congenital absence of primary teeth, but dental records were not available for verification.

Agarose gel electrophoresis. Ban II restriction digest and agarose gel electrophoresis of the 20 affected individuals verified the presence of mutation in all 20, but none of the 20 controls had the mutation (Fig. 2).

The results indicated that MSX1 gene contributes to tooth agenesis in Iranian

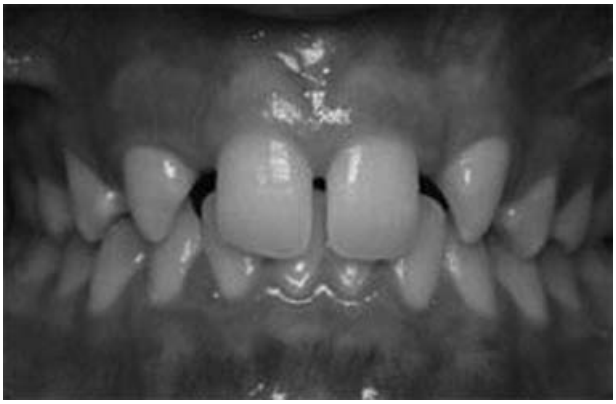

Fig. 1. An individual with missing upper lateral incisors.

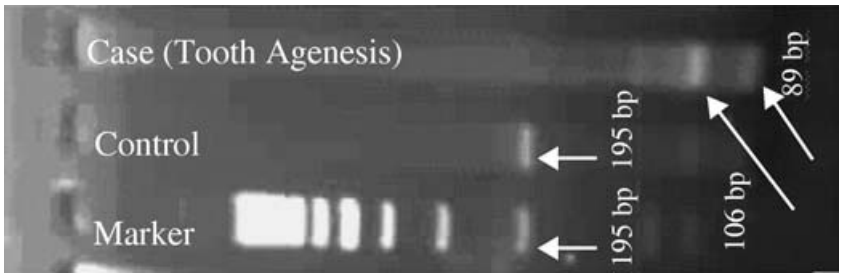

Fig. 2. Restriction-enzyme analysis of PCR-amplified DNA fragment of MSX1. PCR fragments 195 bp long were digested with Ban II in Case group.

individuals. There is a statistically significant correlation between tooth agenesis and MSX1 mutation ($P < 0.001$).

Discussion

The 100% concordance in Iranian study and the fact that we did not find mutation on 20 normal controls confirms the linkage of MSX1 mutations and hereditary tooth agenesis. This is in contrast to two studies that did not find MSX1 linkage or mutations in families or subjects with tooth agenesis[5,6], but in accordance with another study that recently was carried out on 92 individuals that constituted 82 nuclear families[3]. Mutations in PAX9[9–12] and TGFA[10] are also associated with isolated tooth agenesis.

Association of MSX1 mutation and tooth agenesis was reported with mild maxillary anterior–posterior hypoplasia[2]. Others have postulated that congenital absence of teeth may result in decreased mesenchymal tissues required for normal growth of the maxilla[15]. The MSX1 homeobox gene is expressed at diverse sites of epithelial–mesenchymal interaction during vertebrate embryogenesis, and has been implicated in signalling processes between tissue layers[16]. Inductive interactions mediated by the MSX genes are essential for normal craniofacial, limb, and ectodermal organ morphogenesis; also essential to survival in mice, as manifested by the phenotypic abnormalities shown in knockout mice and in humans[17]. However, cephalometric analysis initially identified two affected siblings in this family revealed normal facial proportions. The Met61Lys mutation may alter normal MSX1 function by a variety of mechanisms. MSX and DLX proteins have been shown to form dimeric complexes[18]. DLX proteins have also been shown to be important in dental development[19].

The overlapping expression patterns of MSX and DLX genes and their involvement in epithelial–mesenchymal signalling cascades of murine odontogenesis suggest that MSX and DLX proteins form heterodimeric complexes *in vivo* that provide a mechanism for transcriptional regulation via functional antagonism. It is possible that the Met61Lys may interfere with dimerization of MSX1 with DLX proteins. MSX1 has also been shown to interact with TBP and other components of the core transcriptional complex[20,21]. However, both the interactions with DLX proteins and TBP have been shown to be mediated by the homeodomain, specifically amino acids in the N-terminal region, which are located distal to the Met61Lys mutation, suggesting interference with these interactions may not be a likely explanation[3]. Alternatively, a likely explanation may involve a highly conserved 10- to 12-amino-acid region (53-LPFSVEALMA-62) in MSX1, MSX2, and MSX3 across many species[22]. This region, which is quite hydrophobic, has also been suggested to be similar to the engrailed homology (EH-1) repression domain[23]. Repression by the EH-1 domain is mediated by Groucho, a basic helix-loop-helix protein that interacts with a variety of motifs in other transcription repression proteins[24]. Thus, this conserved MSX1 region may also have repression activity and interact with Groucho. Previous studies did not correlate this region with MSX1 repression[20,25]. However, the Met61Lys mutation may be affecting an uncharacterized repression domain in the protein[3].

The hypodontia pattern observed with MSX1 mutations[2,7,8] suggests a threshold level of MSX1 function is critical for development of only selected teeth, and MSX1 functions in pattern of dentition[3]. This corroborates the hypothesized odontogenic homeobox code proposed by Sharpe[26]. The variation observed

average of 11.0/person[2], 8.4/person[8], 16.4/person[7], and 12.2/person[3] suggests that other factors modulate the effects of MSX1 mutations. In fact, Necdin, a potent growth suppressor that is expressed predominantly in post-mitotic cells such as neurones and skeletal muscle cells, and MAGE-D1 (NRAGE, Dlxin-1) cooperate to modulate the function of Dlx/MSX homeodomain proteins in cellular differentiation[27]. Furthermore, the complexity of the genetic network that governs tooth development can change during ontogenetic trajectory, and these changes may be related to macroevolutionary changes[28]. The variation in tooth number in the affected members of human families bearing mutations in the MSX1 and PAX9 genes can help to understand how genetic variations within a population can modulate evolutionary changes in dental patterning. MSX1 mutations result in a specific pattern of inherited tooth agenesis. The cause for the more common cases of tooth agenesis, where only one or two teeth are missing, is not explained by MSX1 mutations[3]. To our knowledge, this is the first report of a MSX1 and tooth agenesis in Iranians. Our results indicate MSX1 contributes to tooth agenesis in Iranian individuals.

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