

Polymorphism in the *Msx1* gene associated with hypodontia in a Brazilian family

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Abstract: Tooth development is regulated by a reciprocal series of epithelial-mesenchymal interactions. With the large number of genes involved in the odontogenesis process, the opportunity for mutations to disrupt this process is high. Mutational analysis has revealed genes that are major causes of non-syndromic hypodontia. The most common permanent missing teeth are the third molars, second premolars, and maxillary lateral incisors. Although hypodontia does not represent a serious public health problem, it may cause masticatory and speech dysfunctions and esthetic problems. *Msx1* (*Muscle Segment Box*) is believed to play an important role in tooth development. To further investigate the role of the gene in human hypodontia, we analyzed genotypes in a family with hypodontia using the SSCP assay. Examinations of all affected and unaffected members of the family studied indicated that 5 of the 10 family members had hypodontia, and it was possible to observe polymorphisms/mutation by SSCP as bands with an anomalous migration pattern in individuals with hypodontia. Our data suggest that *Msx1* gene polymorphism is associated with hypodontia. (J Oral Sci 51, 341-345, 2009)

Keywords: hypodontia; *Msx-1* gene; polymorphism.

Introduction

In the past few years much progress has been made in our understanding of the mechanisms that control tooth

morphogenesis. Epithelial-mesenchymal interactions govern the development of all epidermal organs, including teeth, hair follicles, and mammary glands (1-6). Interestingly, the initial morphological development of these organs is similar; the epithelium undergoes local thickening followed by local condensation of the underlying mesenchyme. The epithelium then invaginates into the condensing mesenchyme until it has attained a characteristic bud structure. After this stage, the development of these organs diverges in order to give rise to specialized organs with vastly different morphologies, cell types, and functions (3,5,7). The epithelial-mesenchymal interactions are reciprocal and sequential, and each component may play important roles in organogenesis, depending on the organ system and the developmental stage.

Studies of odontogenesis at the molecular level, mostly using mouse teeth as models, have indicated that tooth development is under strict genetic control, determining the position, number, size and shape of teeth. More than 200 genes have been identified in developing teeth, and mutations in several of these genes cause arrested tooth development in mice (8-10). The direct participation of several genes in tooth development has been evidenced by tooth absence in mutant knockout mice models (10).

Some of these genes belong to the Hox family, which contain a homeobox, an evolutionarily conserved DNA sequence motif that was found initially in *Drosophila* genes (11-13). The homeobox encodes a homeodomain, a DNA-binding motif, and such homeotic genes encode transcription factors (14-15). The regulatory modules usually comprise multiple binding sites for transcription factors that can be located near the transcription start site, or thousands of base pairs distant from it (16). Mutations within individual modules can enhance or repress gene transcription in a tissue-specific manner, allowing a mutation to exert its effect on a few or even a single

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morphogenetic field (17).

In this regard the study of genetic polymorphisms in hypodontia is an especially appropriate model for understanding the association of gene polymorphisms and changes in morphology. Teeth are serially homologous structures, and the effects of gene variations on the development of these structures can be easily quantified (18-20). Individuals with distinct polymorphic alleles may exhibit slight and specific phenotypic variations in dental patterning. In this sense, association studies between gene polymorphisms and hypodontia as well as other mild malformations that reflect qualitative defects of embryogenesis (21), may help to reveal the molecular mechanisms responsible for the phenotypic variations that may occur in distinct human populations and ethnic groups.

There is some controversy regarding the importance of homeobox gene *Msx1* in hypodontia. The human *Msx1* gene is located at chromosome 4p 16.1 (22). Research groups have identified several *Msx1* mutations in families with non-syndromic forms of autosomal dominant posterior hypodontia (22). *Msx1* has been associated with hypodontia in mice and humans, but interestingly in humans, this gene is associated with specific missing teeth. On the other hand, another report has excluded *Msx1* as the gene responsible for hypodontia (23). Thus this condition may represent a more complex multifactorial trait influenced by a combination of gene function, environmental interaction and developmental timing.

In order to further investigate the role of the *Msx1* gene in human hypodontia, we analyzed a family with this condition. Our data suggest that *Msx1* gene polymorphisms are associated with hypodontia.

Materials and Methods

Subjects

Ten individuals of one family, five with maxillary lateral incisor hypodontia without signs of other disorders, were interviewed and documented. Congenital absence of teeth was confirmed by X-ray analysis. No other dental anomalies were observed in the subjects.

This *in vivo* study was deemed to be ethical according to the Brazilian Guidelines (Resolution 196 of the National Health Council, 1996), and the protocol was approved by the Research Ethics Committee of Federal University of Uberlândia – UFU (process No. 109/2004). Signed consent was obtained from patients after they had received a thorough explanation about the study.

Sampling

At least 1 h after tooth brushing, the study subjects were asked to vigorously rinse their mouths with 5 ml of

3% sucrose solution for 60 s, and instructed to rub their tongue on the oral mucosa and teeth. Each individual's mouthwash content was collected in a 15-ml centrifuge tube. Three milliliters of TNE solution [17 mM Tris/HCl (pH 8.0), 50 mM NaCl and 7 mM EDTA] diluted in 66% ethanol was added to the tube, as described previously (24).

DNA purification and PCR procedures

The DNA was extracted using a *Super DNA extraction quick gene kit* (Analytical Genetic, USA) in accordance with the manufacturer's instructions. PCR amplifications were performed in a total volume of 50 µl containing 500 ng of genomic DNA, 10 pmol of each primers 5' ACT TGG CGG CAC TCA ATA TC 3' (forward) and 5' TGT GAG GGT TAA AGG GAA GG 3' (reverse), 200 µM each deoxynucleotide, 1 U Taq polymerase (Promega), 2.5 mM MgCl₂ and 5× KCl buffer. The product size generated after the PCR procedures was 668 bp. Samples were heated initially to 95°C for 5 min. Each cycle comprised denaturation at 95°C for 1 min, primer annealing at 60°C for 1 min and extension at 72°C for 1 min. Samples were subjected to 35 cycles of amplification followed by a final extension at 72°C for 5 min. The PCR products were subjected to SSCP analysis.

SSCP analysis

After the PCR, 5 µl of the amplified PCR product was treated with 5 µl of LIS buffer (95% formamide, 10 mM NaOH, 0.025% bromophenol blue, 0.025% xylene cyanol), then heated at 95°C for 5-10 min and the hot tubes were immediately placed on ice for 2-5 min. Electrophoresis was conducted on 10% acrylamide gel in 1× TBE buffer for 16 h at 120V at room temperature. The DNA bands were visualized by the rapid silver staining method.

Results

Examinations of all members of the family studied indicated that 5 of the 12 family members were affected. Individuals with hypodontia showed no signs of syndromic conditions or systemic illnesses associated with hypodontia.

Figure 1 shows the familial dendrogram with expression of hypodontia, and SSCP analysis demonstrated polymorphism-mutations as bands with an anomalous migration pattern in individuals with hypodontia. Black graphic representation indicates the affected individuals. Importantly, no significant differences were observed in allele frequency between male and female patients. As demonstrated in Fig. 2A individuals numbered as 4, 5, 7, 9 and 12 lacked one band in comparison with patients without hypodontia. In Fig. 2B (higher magnification), it is possible to observe that unaffected individuals possessed

two bands (indicated by arrows) while affected individuals had a single band (indicated by arrow).

Discussion

Many theories regarding the etiology of hypodontia have been suggested in the literature, particularly before the intense genetic studies of the present day, and obviously both genetic and environmental factors are contributory (25). Although hypodontia is occasionally caused by environmental factors, in the majority of cases it has a genetic basis. Gene polymorphisms are a mechanism by which individuals may exhibit variations within the range of what is considered biologically normal. Gene polymorphisms are also known to be associated with disease susceptibility. Most polymorphisms are single-nucleotide exchanges that occur at high frequency in the human genome and may affect the function of genes (23).

Although hypodontia, the congenital absence of one or a few teeth, does not represent a public health problem, it may cause speech and masticatory dysfunction and esthetic disorders (26). Hypodontia is one of the most common alterations of the human dentition. The most common permanent teeth missing are the third molars (20%), second premolars (3.4%), and maxillary lateral incisors (2.2%) (27). One of the major questions in modern biology is how genetic variations interfere with development and are translated into changes in morphology, since small changes in organogenesis can produce large changes in adult morphology (28,29).

Gene expression and experimental studies in mice have implicated numerous different genes in tooth development, and in theory, any of these genes may cause hypodontia (30). Genetic mutations in man indicate that the

development of dentition is under the control of several genes. Initial discoveries in mice indicated that the homeodomain protein *Msx1* and the paired-domain transcription factor *Pax9* are important genes in tooth morphogenesis (22). Mutations in three genes have been identified in human pedigrees with familial hypodontia or oligodontia: *Msx1*, *Pax9* and *Axin2* (31,32). Studies of the expression of *Bone Morphogenetic Protein 4 (Bmp4)*, the HMG box gene *Lef1*, heparan sulfate proteoglycan *syndecan-1* and *Transforming growth factor-beta 1 (Tgfb1)* in mice have indicated the important role of these genes in tooth development (23).

Family studies show that, as an isolated form, hypodontia

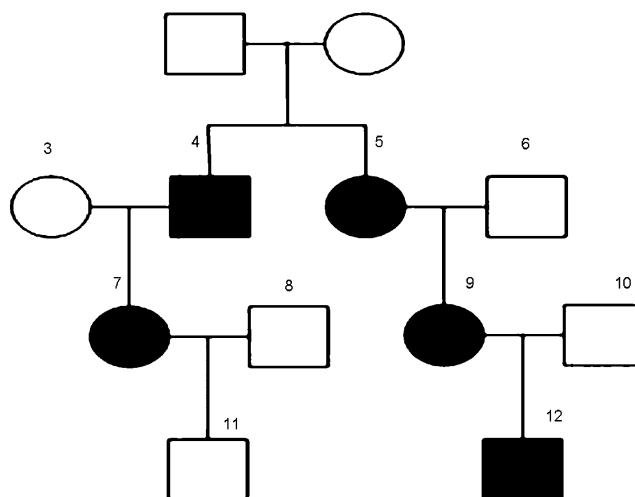


Fig. 1 Heredrogram showing the subjects of the analyzed family. Black graphics represent the affected individuals.

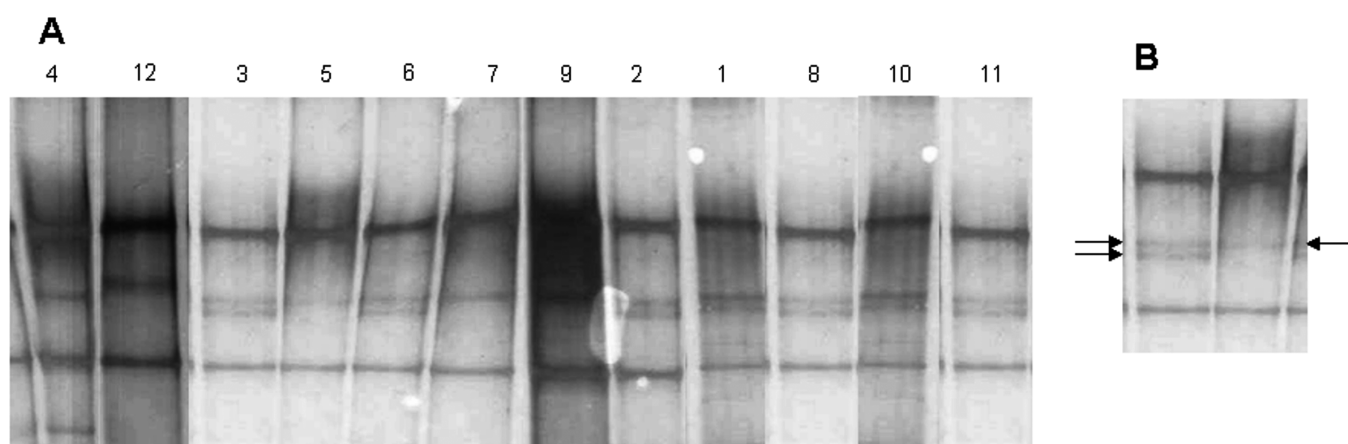


Fig. 2 (A) SSCP analysis of *Msx-1* in individuals with normal dentition (Lanes 1, 2, 3, 6, 8, 10 and 11) and tooth hypodontia (Lanes 4, 5, 7, 9 and 12). (B) Note the absence of one band in SSCP analysis of the subject with hypodontia, as indicated by the arrows.

is inherited as an autosomal dominant trait with incomplete penetrance and variable expression (33). An autosomal recessive model has been described in one family (34). The obscure mechanism underlying congenital lack of teeth and the differing results of genetic studies have drawn attention to the phenotypic and genotypic variation in this phenomenon.

There is some controversy regarding the importance of *Msx1* in hypodontia. Our present results showed a missense mutation in the homeodomain of the *Msx1* gene in chromosome 4 (4p16) in all affected members of a family with hypodontia. A previous study of a Dutch family demonstrated a nonsense mutation in the *Msx1* gene associated with hypodontia and various combinations of cleft lip and/or palate (35). On the other hand, another report has excluded *Msx1* as the gene responsible for hypodontia (36).

We hypothesized that the *Msx1* gene is important for tooth development, since experimentally induced mutations in transcription factor genes have revealed a role of *Msx1* in the regulation of tooth development (37,38). In humans, the role of the *Msx1* gene in craniofacial development has been highlighted by the identification of *Msx1* mutations that are associated with various alterations. G-C transversion in the homeobox region of the *Msx1* gene results in an arginine to proline substitution in the conserved domain of the protein. This is the cause of specific patterns of hypodontia (27). Thus, our present results have confirmed the importance of the *Msx1* gene in tooth development, since subjects who possessed an abnormal pattern in this gene exhibited hypodontia.

In conclusion, the present study of a family in Brazil has shown that *Msx1* polymorphism/mutation is associated with hypodontia of the maxillary lateral incisors.

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