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Polymorphism in the MSX1 gene in a family with upper lateral incisor agenesis

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ARTICLE INFO

Article history:
Accepted 22 April 2012

Keywords:
Hypodontia
MSX1 transcription factor
Polymorphism
Tooth agenesis

ABSTRACT

Objective: The MSX1 gene plays a key role in odontogenesis regulation, particularly during early stages. Since only a few genetic variants have thus far been associated with non-syndromic tooth agenesis, we screened for mutations in this gene, aiming to detect a relationship between genotype and phenotype.

Design: The sample consisted of one proband with non-syndromic hypodontia involving upper lateral incisors, three relatives and ten unaffected controls. The proband and two affected relatives showed the same phenotype. DNA was extracted from buccal epithelial cells, and direct sequencing was performed. The two exons of MSX1 were first sequenced in the proband. When an alteration was detected, his relatives were investigated by the same method.

Results: We identified the known polymorphism *6C > T in the homozygous state in all three affected family members. The unaffected father was heterozygous and ten control samples were negative for the *6C > T polymorphism.

Conclusions: The *6C > T polymorphism, when homozygous, may contribute to agenesis of upper lateral incisors. However, since the *6C > T polymorphism is quite common, additional genes must be involved in this phenotype.

1. Introduction

Tooth agenesis, the congenital absence of one or more teeth, is among the most common craniofacial anomalies. It affects one in five people, but its rate is much lower, ranging from 2% to 10% when third molars are not considered.[1] Clinical classification of this anomaly occurs according to the number of missing teeth. Hypodontia is defined as the absence of from one to six permanent teeth (excluding third molars), and its prevalence varies from 1.6% to 9.6%. Oligodontia occurs when more than six permanent teeth are lacking (excluding third molars), and it is found in 0.3% of the general population.[2] Tooth agenesis can be either a non-syndromic disturbance or a feature associated with several syndromes. Inheritance is usually autosomal-dominant, even though a few autosomal-recessive and X-linked inheritance cases have been reported.[3]

The teeth most often absent are third molars, followed by lower second premolars and permanent upper lateral incisors. Bilateral agenesis of upper lateral incisors appears to be more common than unilateral agenesis.[4] Associated dental anomalies such as microdontia, impacted canines and ectopic eruption have also been reported.[2] Frequency of agenesis affecting the same tooth class among relatives is significantly higher than that affecting different tooth classes, indicating that similar genetic factors may be involved. Furthermore, parents and siblings of probands usually show higher frequencies of hypodontia than found in the general population.[5] All these observations suggest that there is an underlying genetic mechanism leading to non-syndromic hypodontia

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0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2012.04.008

even though some environmental factors can be involved in the etiology.

Studies of tooth development in knockout mice have indicated that odontogenesis depends on more than 200 genes. MSX1 and PAX9 are among those most associated with tooth agenesis.[6,7] Both genes encode transcription factors which are involved in activating the expression of signalling factors for the epithelial–mesenchymal interactions which are necessary for early tooth development.[8] Therefore, defects in either gene may affect odontogenesis. MSX1 is directly involved in craniofacial and limb development. Mutations in this gene are known to cause selective oligodontia predominantly affecting permanent posterior teeth.[8–10]

Genetic mutations in MSX1 may also be associated with other abnormalities, such as orofacial clefting[10] and cancer. The relationship between MSX1 and the p53 Tumor Suppressor gene has been investigated. Interestingly, the homeodomain of MSX1 acts mostly as a protein–protein interacting motif, binding to p53 in the cell nucleus, thus stimulating apoptosis of cancer cells.[11] This is a relatively novel role for MSX1, suggesting that tooth agenesis might be an indicator of tumor susceptibility.

The first identified mutation in the MSX1 gene associated with tooth agenesis was described in 1996 in a family with autosomal-dominant agenesis of all permanent second premolars and third molars; several incisors, upper first premolars and lower first molars were also missing.[12] Despite the high prevalence of tooth agenesis, mutational screening for MSX1 has thus far returned only a few causative mutations (Table 1). Most of these mutations were found within the homeobox domain, which is a highly conserved sequence enabling MSX1 to bind to DNA-specific sites as well as to other proteins. The mutations lead to haploinsufficiency by diminishing the amount of functional protein to 50%,[13] particularly when nonsense and frameshift mutations are involved,[9,10,15,17] and cause the arrest of tooth development at the bud stage.

In the present research, we analyzed a Brazilian nuclear family affected with non-syndromic hypodontia involving upper lateral incisors. The family was screened for mutations in the MSX1 gene to investigate the relationship between genotype and phenotype.

2. Materials and methods

2.1. Family selection and pedigree construction

The proband was 11 years old when he was referred by his general dentist for orthodontic consultation in a private office. The main complaint was a possible lack of permanent upper lateral incisors, since the patient still had deciduous upper lateral incisors. Since his family had a dental history involving the absence of teeth, the proband and his relatives were invited to participate in this study. Family members are Caucasians of Italian descent and live in a community of immigrants from the region of Veneto, Italy. Diagnosis of the anomaly was verified by anamnesis, clinical examination and panoramic radiographs of all family members, allowing the pedigree to be determined (Fig. 1). Customized questionnaires were administered for better assessment of the familial medical history. Associated dental anomalies were also registered. The affected members of the family were reported to have had normal deciduous dentition. All four individuals in the family, including minors, signed consent forms. The protocol for this research was approved by the Research Ethics Committee of the University of Caxias do Sul, Rio Grande do Sul, Brazil, according to Resolution 196/96 of the National Health Council.

2.2. DNA collection, screening and mutational analysis

A sample of buccal epithelial cells was collected from all family members by means of cytology brushes. For DNA extraction, a Wizard® Genomic DNA Purification Kit (ProMega, Madison, WI, USA) was used according to the manufacturer's recommendations. The amplification process by polymerase chain reaction (PCR) was performed with two sets of primers covering the two exons and boundaries of the MSX1 gene, as follows: MSX1x1F 5′-GCTGGCCAGTGCTGC-3′; MSX1x1R 5′-ACGGGGTCCTCTCGGGCTTC-3′; MSX1x2F 5′-ACTTGGCGG-CACTCAATATC-3′; and MSX1x2R 5′-AAGCTATGCAGGAGA-CATGG-3′. All primers were obtained from Primer-BLAST software (www.ncbi.nlm.nih.gov/tools/primer-blast). PCR was carried out in an Eppendorf Mastercycler® Gradient Thermal

Table 1 – Identified mutations in MSX1 that have been associated with tooth agenesis.

Gene	Exon	Intron	Mutation		References	Phenotype	Type
			Nucleotide	Residue*			
MSX1			–	–	Nieminen et al.[13]	Oligodontia/Wolf-Hirschhorn syndrome	Gene deletions
	1		62–63insG	Gly22ArgfsX168	Kim et al.[9]	Oligodontia	Insertion
	1		182T > A	Met61Lys	Lidral et al.[14]	Oligodontia	Missense
	1		314C > A	Ser105X	van den Boogard et al.[10]	Oligodontia/Cleft lip-palate	Nonsense
		•	740–751del	–	Pawlowska *et al.*[8]	Oligodontia	Intronic deletion
	2		559C > T	Gln187X	De Muynck et al.[15]	Oligodontia/Cleft lip-palate	Nonsense
	2		581C > T	Ala194Val	Mostowska et al.[16]	Oligodontia	Missense
	2		587G > C	Arg196Pro	Vastardis et al.[12]	Oligodontia	Missense
	2		605C > A	Ser202X	Jumlongras et al.[17]	Oligodontia/Witkop syndrome	Nonsense
	2		655G > A	Ala219Thr	Chishti et al.[18]	Oligodontia/dental anomalies	Missense
	2		662C > A	Ala221Glu	Xuan et al.[19]	Oligodontia	Missense

* Change of residues referred by the authors or obtained from sequence alignment.