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Clinical and genetic evaluation of a Chinese family with isolated oligodontia

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ABSTRACT

Objectives: Oligodontia is defined as the congenital absence of 6 or more permanent teeth excluding the third molar. Tooth agenesis may be classified as syndromic/non-syndromic and as familial/sporadic. To date, more than 300 genes have been found to be involved in tooth development, but only a few of these genes, such as *MSX1*, *PAX9* and *AXIN2*, are related to the condition of non-syndromic oligodontia. The objective of the present work was to investigate the disease-causing gene of non-syndromic oligodontia in a Han Chinese family and analyse the pathogenesis of mutations that result in oligodontia.

Design: We examined all individuals of the oligodontia family by clinical and radiographic examinations. Based on the clinical manifestations, the candidate genes *MSX*, *PAX9* and *AXIN2* were selected to analyse and screen for mutations.

Results: The clinical evaluation suggested that the family might show non-syndromic oligodontia. DNA sequencing of the *MSX1* gene revealed two mutations in the two patients with oligodontia: a heterozygotic silent mutation, c.348C > T (P.Gly116=), in exon 1 and a homozygotic deletion of 11 nucleotides (c.469 + 56delins GCCGGGTGGGG) in the intron. However, the silent mutation and the deletion mutation were thought to be known polymorphisms (rs34165410 and rs34341187) by bioinformatics analysis. We did not detect any mutations in the *PAX9* and *AXIN2* genes of oligodontia patients.

Conclusion: Our finding suggests that identified polymorphisms (c.348C > T and c.469 + 56delins GCCGGGTGGGG) may be responsible for the oligodontia phenotype in this Chinese family, but the association requires further study.

1. Introduction

Tooth development is a complex process that involves a series of reciprocal interactions between the epithelium and underlying mesenchyme. Any disturbance in this tightly balanced process may result in tooth agenesis or other dental defects.[1,2] Tooth agenesis affects approximately 20% of the world's population. If third molars are excluded, more than 5% of the population is still afflicted. Dental abnormalities can provide some of the earliest warning signs of some systemic disorders, such as bulimia[3] and the consequences of antineoplastic treatments.[4] The dentist could be the first professional to notice these symptoms, which in turn can lead to early detection, faster treatment, and a higher survival rate.

Oligodontia is defined as the congenital absence of 6 or more permanent teeth, excluding the third molar. Tooth loss appears as both a feature of multi-organ syndromes and as a non-syndromic isolated characteristic. People with missing teeth have many problems with aesthetics, phonetics, and mastication. Many of the genes that underlie dental defects have been identified,[5,6] but the occurrence of the

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non-syndromic forms still remains poorly understood. Previous work has shown that *MSX1*, *PAX9* and *AXIN2* are key regulators of tooth development. Some different *MSX1* defects have been reported to cause multiple congenitally missing teeth or oligodontia. Patients with MSX1-associated oligodontia preferentially miss the maxillary and mandibular second bicuspids and maxillary first bicuspids.[7,8] *PAX9* mutations responsible for oligodontia were identified, and patients with PAX9 defects are more likely to be missing the maxillary first molars, maxillary second molars, and mandibular second molars.[9,10] Lammi et al.[11,12] recently suggested that oligodontia may be caused by mutations of the axis inhibition protein 2 (*AXIN2*) gene, which is localised on chromosome 17q21–q25. In humans, mutations in *AXIN2* cause tooth agenesis that affects permanent teeth predominantly, including the permanent molars, lower incisors, and upper lateral incisors. Asians, in particular the Chinese, have a high prevalence of oligodontia. However, reported mutations underlying non-syndromic oligodontia in Chinese patients are limited. Thus, the objective of the present study was to identify the mutations responsible for oligodontia in our family. In addition, we wanted to reveal genotype–phenotype correlations that could improve our current understanding of different mutations based upon the pattern of tooth agenesis.

2. Materials and methods

2.1. Patient and controls

The present study was reviewed and approved by Institutional Review Board and the Ethics Committee and was conducted under the written consent of all participants. The female proband was a patient of the Department of Stomatology, The First People's Hospital of Lianyungang City. We collected a family in which oligodontia was segregating in an autosomal-dominant manner to define the clinical features of oligodontia and to localise the gene locus behind this anomaly. A pedigree of this family was constructed by extended interviews (Fig. 1). Retrospective data were reviewed and the diagnosis of oligodontia was verified by panoramic dental radiographs for all available family members. Eight members of this family were studied, with two members being affected and six unaffected. In this present study, all individuals had presented with normal physical development and normal intelligence, and the clinical examination for other ectodermal abnormalities of the nails, hair, skin and sweat glands as well as for craniofacial and ocular malformations, including orofacial clefts and glaucoma, did not reveal any defects in any of the family members. Thus, we suggested that the family might show non-syndromic oligodontia. Furthermore, 100 unrelated individuals, who were not affected with tooth agenesis (excluding third molars), were used as controls.

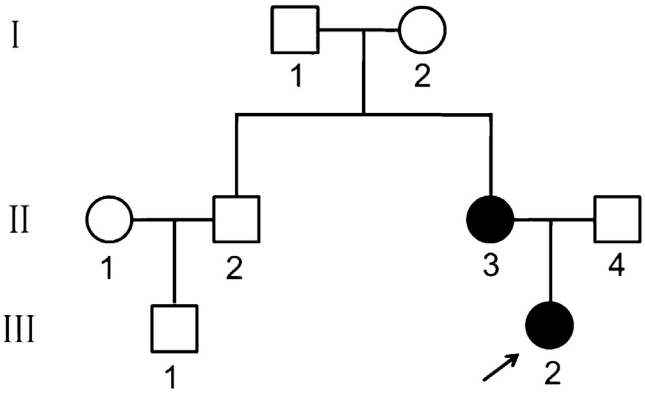

Fig. 1 – Pedigree of the family.

2.2. DNA extraction and PCR of candidate genes

Blood samples were collected from all members of the family and the controls. Genomic DNA was extracted from these samples with the QIAamp DNA Blood Mini Kit (Qiagen, USA). To screen for putative mutations, two exons of *MSX1* (GenBank accession number M97676), four exons of *PAX9* (GenBank accession number AJ238381) and ten exons of *AXIN2* (GenBank accession number AE006463), in addition to their exon–intron boundaries, were PCR amplified with the use of primers that were previously reported,[13] or designed using the Primer 3 online application (http://frodo.wi.mit.edu/primer3/input.htm). The primer sequences and optimal annealing temperature for each primer pair are available upon request (Tables 1–3). The PCR amplifications used the GC-rich PCR system (Roche, USA) and PrimeSTAR PCR system (Takara, JAPAN). The amplified fragments of *MSX1*, *PAX9* and *AXIN2* from individuals of the affected family and controls were gel-purified, with the MinElute Gel Extraction Kit (Qiagen, USA) according to the manufacturer's protocol. The sequencing analyses were performed with an ABI BigDye™ terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase on an ABI PRISM™ 377XLDNA sequencer (Applied Biosystems, USA). Finally, we analysed the mutations and polymorphisms in *MSX1, PAX9* and *AXIN2* at the genomic and protein levels using MegAlign 5.01 software (DNASTAR, USA), Polyphen-2 software (USA) and Exonic splicing enhancer (ESE) Finder 3.0 software (USA).

3. Results

3.1. Clinical finding

The proband was an 11-year-old girl, who first visited the paediatric dental clinic due to the late eruption of her permanent teeth. Oligodontia, segregated in her family in

Table 1 – MSX1 primer sequences.

Region	Forward primer (5′–3′)	Reverse primer	Size (bp)	Annealing temperature (°C)
Exon 1	GGCTGCTGACATGACTTCTTTGC	TTGGAACCTTCTTCCTGGGTG	642	65
Exon 2	CCAGAAGCAGTACCTGTCCAT	TCAGGGATCAGACTTCGGAGA	506	65