Clinica Chimica Acta 461 (2016) 135–140

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

A novel *MSX1* intronic mutation associated with autosomal dominant non-syndromic oligodontia in a large Chinese family pedigree

Jinjie Xue [a,b], Qingping Gao [c], Yanru Huang [a], Xiaoyu Zhang [c], Pu Yang [a], David S. Cram [b,d], Desheng Liang [a], Lingqian Wu [a,*]

[a] State Key Laboratory of Medical Genetics, Central South University, Changsha, Hunan 410078, China
[b] Children's Hospital of Shanxi, Women Health Center of Shanxi, Taiyuan, Shanxi 030013, China
[c] Department of Stomatology, Xiangya Hospital, Central South University, Changsha, Hunan 410078, China
[d] Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia

ARTICLE INFO

Article history:
Received 19 July 2016
Accepted 28 July 2016
Available online 30 July 2016

Keywords:
Tooth agenesis
Permanent teeth
Genome-wide scan
Short tandem repeat
Intronic mutation
MSX1 homeobox gene

ABSTRACT

Background: Tooth agenesis is a common developmental dental anomaly. The aim of the study was to identify the causal genetic mutation in a four-generation Chinese family affected with non-syndromic autosomal dominant tooth agenesis.
Methods: Genome-wide scanning was performed using the Illumina Linkage-12 array. Genotyping of short tandem repeat markers was used to finely map the causative locus. Haplotype analysis and Sanger sequencing was performed to precisely locate the position and nature of the gene defect.
Results: Clinical examination of the available 23 family members showed variable tooth agenesis in 10 subjects, ranging from oligodontia to mild hypodontia. Genome-wide scanning and haplotype analyses identified the 4p16.1–p16.3 region with a maximum multi-point LOD score of 3.50, which overlapped with the *MSX1* gene. A single heterozygous point mutation IVS1–5 G>A in the *MSX1* gene was exclusively detected in the 10 family members affected with tooth agenesis. Sequencing of *MSX1* cDNA revealed that the intronic mutation did not affect the normal splicing pattern of the pre-mRNA. However, real-time qPCR analysis of lymphocyte RNA showed that the level of *MSX1* mRNA was significantly decreased in individuals heterozygous for the mutation.
Conclusions: We identified and characterized a novel intronic mutation in the *MSX1* gene in a large Chinese pedigree, adding to the small repertoire of *MSX1* mutations associated with autosomal dominant tooth agenesis. We hypothesize that the variable degree of tooth agenesis observed in each affected individual may be due to suboptimal levels of MSX1 expression during critical stages tooth development.

1. Introduction

The most frequently observed developmental dental anomaly is tooth agenesis, manifesting in 1.6 to 9.6% of the population [1]. Tooth agenesis, excluding the lack of the third molars is classified into two types, namely; oligodontia defined as agenesis of six or more permanent teeth and hypodontia defined as agenesis of less than six teeth. Tooth agenesis is a highly heterogeneous genetic disorder which can show an autosomal recessive, autosomal dominant or X-linked inheritance pattern. To date, DNA variants in *MSX1*, *PAX9*, *WINT10A*, *LTBP3*, *AXIN2* and *EDA* are strongly associated with tooth agenesis [2,3].

Heterozygous mutations in the *MSX1* gene (formerly *HOX7*) located at 4p16.2 are a common cause of teeth agenesis [2]. The *MSX1* gene comprising two exons encodes a 303 amino acid protein which is a member of the muscle segment homeobox gene family [4]. MSX1 is involved in multiple epithelial-mesenchymal interactions and functions as a transcriptional repressor during embryogenesis through interactions with components of the core transcription complex and other homeoproteins, and appears critical for normal tooth development [5]. Numerous family pedigree studies have shown that specific *MSX1* mutations cause either non-syndromic hypodontia or oligodontia, predominantly affecting the formation of second premolars and third molars [2,6–15]. In addition, patients with Wolf-Hirschhorn syndrome caused by 4p terminal deletions show tooth agenesis when the deletion involves the *MSX1* gene [16]. Further, rare *MSX1* mutations cause Witkop Syndrome which is associated with tooth agenesis and nail abnormalities [17,18]. Paradoxically, other *MSX1* mutation types cause cleft lip and palate with [19,20] and without [20–24] tooth agenesis.

In this study, we investigate the molecular basis of autosomal dominant tooth agenesis in a four-generation Chinese family and characterize a novel *MSX1* intron 1 mutation that reduces the normal basal level of mature mRNA.

* Corresponding author at: State Key Laboratory of Medical Genetics, Xiangya Hospital, Central South University, 110 Xiangya Road, Changsha, Hunan 410078, China.
E-mail address: wulingqian@sklmg.edu.cn (L. Wu).

http://dx.doi.org/10.1016/j.cca.2016.07.025
0009-8981/

2. Methods

2.1. Patient and control samples

The study was approved by the University Ethics Committee and written informed consent was obtained from the 23 living family members. Orthodontic examinations and panoramic dental radiography was used to confirm the diagnosis of tooth agenesis. Genomic DNA for linkage and mutation analysis was extracted from peripheral blood leukocytes of all available family members (10 affected and 13 unaffected) and 200 unrelated healthy individuals.

2.2. SNP array genotyping

Genotyping was performed using the Linkage-12 Human DNA Analysis Kit (Illumina, San Diego, USA). The array contains 6090 single-nucleotide polymorphism (SNP) markers, with an average 0.58 cM genetic map spacing and an average 441 kb physical map spacing. Genomic DNA samples were labeled and hybridized to the array according to the manufacturer's recommended protocol. Fluorescence signals were scanned using Illumina Bead-studio (version 2.1.15). Whole genome linkage was then performed by a parametric two-point analysis using the MERLIN program (version 1.01).

2.3. Fine-mapping and linkage analysis

Short tandem repeat (STR) markers D4S3023, D4S2925, D4S2285, D4S431, D4S3007, D4S2366, D4S2935, and D4S394 were selected from the Human Genome Database and the Marshfield Database (http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp) and used for fine mapping of the causative locus. PCR amplifications were performed using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) in a 5 μl reaction mixture with a touchdown procedure. One of each pair of primers was labeled with a phosphoramidite fluorescent tag. PCR products were analyzed on an ABI 3730 sequencer. GS500 size standards run in the same capillary, were used as internal standards. Allele identification and sizing was performed with GeneScan software version 3.0 and GenoTyper software version 3.7 (Applied Biosystems, Foster City, CA, USA). Two-point LOD scores were calculated using the MLINK subprogram of the LINKAGE package (version 5.1; http://linkage.rockefeller.edu/soft/) applying an autosomal-dominant model of inheritance with 95% penetrance. We assumed a disease allele frequency of 0.024 and no sex difference in the recombination rates. Recombination frequencies between male and female were assumed equal. Cyrillic software (http://www.cyrillicsoftware.com/) was used to finally map the pedigree and reconstruct haplotypes.

2.4. MSX1 mutation screening

MSX1 was the only gene associated with tooth agenesis that mapped to the region showing evidence of linkage. The two *MSX1* coding regions and the intron/exon boundaries were amplified by PCR using previously designed primers [9]. A 20 μl reaction mixture included 30 ng genomic DNA, 2 μM of forward and reverse primers and 10 μl Premix Ex Taq TM Hot Start Version (Takara, Dalian, China). PCR amplifications were performed in a Perkin-Elmer thermal cycler (PTC-200; Bio-Rad, Hercules, CA, USA). Following a 4 min initial denaturation at 94 °C, 30 PCR cycles were performed consisting of 30 s at 94 °C, 30 s at 60 °C to 65 °C and 40 s at 72 °C with a final elongation step for 10 min at 72 °C. After verifying successful PCR amplification by polyacrylamide gel electrophoresis, products were directly sequenced using the ABI Big Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI 3100 sequencer. Sequencing results were analyzed using the DNASTAR® software program package. The NatGene2 gene program (http://www.cbs.dtu.dk/services/NetGene2/) was used for splice site prediction.

2.5. Sequence analysis of MSX1 cDNA

Total RNA was extracted from peripheral blood leukocytes using TRIzol reagent (Invitrogen, Carlsbad, CA) in accordance with the recommended protocol. Reverse transcription was performed in a 20 μl reaction containing 500 ng of purified RNA using the RT Kit Reagent (Invitrogen, Carlsbad, CA). A 2 μl aliquot of first strand product was used as a template for subsequent PCR analysis of *MSX1* cDNA sequences using an exon 1 forward primer (5′-ACTCCTCAAGCTGCCAGAAGAT-3′) and an exon 2 reverse primer (5′-CCTCCCTCTGCGCCTGGGTTC-3′). The PCR products were separated on 2% agarose gels, purified and Sanger sequenced.

2.6. Analysis of MSX1 mRNA expression levels by real-time quantitative PCR

For real-time quantitative PCR (qPCR), 1 μg total RNA was reverse transcribed to first strand cDNA in a 20 μl reaction using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). First strand products were further diluted 1:3 with RNase free H_2O. Quantitative PCR was performed on an Bio-Rad CFX96 Touch Deep Well Real-Time PCR Detection System (Bio-Rad) in a 20 μl reaction containing 10 μl of 2× Maxima SYBR Green qPCR Master Mix with no ROX (Thermo Scientific), 5 μl of SYBR Green, 0.2 μl of forward primer (10 μM), 0.2 μl of reverse primer (10 μM), 3.8 μl of diluted cDNA and 0.8 μl of RNase free H_2O. The relative standard curve method using Ct values was used to measure *MSX1* mRNA levels. Expression levels were normalized to *HPRT1* mRNA levels in the same sample. For each sample, *MSX1* mRNA was measured in three independent replicates. Two different qPCR primer pairs *MSX1*-E1/2a (forward 5′-ACTCCTCAAGCTGCCAGAAGAT-3′; reverse 5′-TTACGGTTCGTCTTGTGTTTGC-3′) with a product size of 156 bp and *MSX1*-E1/2b (forward 5′-AACACAAGACGAACCGTAAGC-3′; reverse primer 5′-GAACCATATCTTCACCTGCGT-3′) with a product size of 158 bp were used to amplify *MSX1* cDNA sequences. The *HPRT1* primer pair (forward 5′-GACTTTGCTTTCCTTGGTCAGG-3′ and reverse 5′-AGTCTGGCTTATATCCAACACTTCG-3′) with a product size of 101 bp was used as the control.

3. Results

3.1. Clinical assessment of the family pedigree

We analyzed 23 of 27 members of a four-generation family with tooth agenesis that segregated as an autosomal-dominant trait (Fig. 1). One of the family members IV:1 (designated as the proband) initially presented for dental assessment. Clinical and radiographic examinations revealed the proband lacked 15 permanent teeth, including all premolars, maxillary central incisors, the maxillary right canine, the mandibular right central incisor and lateral incisor and, the mandibular left first and second molars (Fig. 2, Table 1). Orthodontic treatment was recommended by the dental surgeon.

Further examination of the other family members showed that nine other individuals also lacked permanent teeth with numbers varying significantly between affected individuals (Table 1). Patterns of tooth agenesis were unique between affected family members. In total, the 10 affected individuals lacked 90 permanent teeth altogether (excluding third molars), including 10 central incisors, 5 lateral incisors, 7 canines, 17 first premolars, 16 second premolars, 12 first molars and 23 second molars. All examined individuals had normal primary dentition and did not reveal abnormalities of lip, palate, nails, skin, hair or sweat glands. Family records confirmed that the founding great grandfather (I:1) and his first daughter (II:2) also had tooth agenesis. Examination of the remaining 13 unaffected family members showed a full set of normally developed teeth.