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Ameloblastin gene polymorphisms in healthy Japanese

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Abstract Ameloblastin is one of the extracellular matrix proteins in tooth enamel and may be responsible for autosomal amelogenesis imperfecta (AI), since it plays a significant role in enamel crystal growth. We investigated polymorphisms of the human ameloblastin gene by polymerase chain reaction, DNA sequencing and single-strand conformational polymorphism (SSCP) analysis using genomic DNA from 50 Japanese subjects with sound dentition. One single sequential trinucleotide deletion and 3 single-nucleotide polymorphisms (SNPs) were identified in the translated region. The nucleotide deletion results in the lack of an amino acid residue and 2 of the SNPs cause nonsynonymous substitutions of amino acid residues. These results provide important background information for the investigation of autosomal AI in Japanese patients.

Key words
Ameloblastin,
Amelogenesis imperfecta,
Gene polymorphisms,
Japanese

Introduction

Ameloblastin[1], also known as amelin[2] or sheathlin[3], is one of the non-amelogenin proteins present in tooth enamel. *In situ* hybridization and immunohistological studies have shown that ameloblastin is present in the enamel prism sheath[4] and principally expressed by enamel-forming ameloblast cells[5,6]. Hence, it is considered to play an important role in the control of enamel crystal growth and determination of the prismatic structure[7]. The human ameloblastin gene has been cloned from tooth germs[8] and shown to be located on chromosome 4q13.2[9].

Amelogenesis imperfecta (AI), a hereditary disorder that causes abnormalities in the quantity and/or quality of dental enamel, is generally classified into 14 distinct subtypes based on the mode of inheritance and clinical manifestation[10]. Further, it is generally classified into 2 forms, autosomal and X-linked, based on inheritance. The autosomal forms of AI are the most prevalent, representing over 95% of all reported cases, and genetically heterogeneous[11]. Four distinct mutations of the human enamelin gene and one of kallikrein 4, which cause autosomal AI, have recently been reported[12–17], while mutations in the X locus of the amelogenin gene is considered to cause the defects seen in X-linked AI patients.

The ameloblastin gene, which is closely linked to the locus of enamelin, is also thought to be responsible for autosomal AI, though its linkage to the disease has not been proven[9]. Hereditary diseases are generally caused by mutations in functional genes, including substitutions or deletions, however, mutations do not always induce a pathogenic condition. Such non-pathogenic mutations are called polymorphisms and are either neutral or functionally significant[18]. Thus, nucleotide mutations found in ameloblastin genes may lead to not only pathogenic mutations causing autosomal AI, but also non-pathogenic polymorphisms. To evaluate whether the ameloblastin gene is responsible for AI, it is necessary to investigate polymorphisms of ameloblastin

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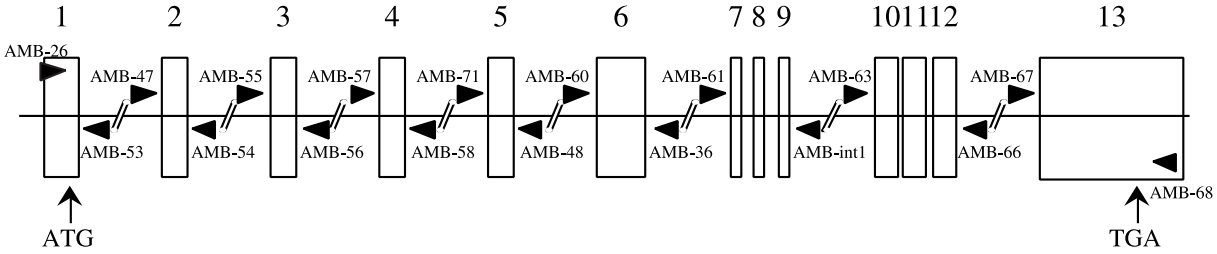

Fig. 1 Schematic representation of the ameloblastin gene showing relative positions of the oligonucleotide primers used for PCR and DNA sequencing. Boxes show exons and arrow heads indicate primer orientations.

Table 1 Primer sequences used to amplify the coding region of the ameloblastin gene

Primers names	Exon numbers	Nucleotide sequences	Orientation	Product size (bp)
AMB-26	1	5′-ATCTTGGTTGGCATCATCAGGC-3′	sense	164
AMB-53	1	5′-ATTCAGCTACTGGTGACAAATGAAGG-3′	antisense	
AMB-47	2	5′-CGGTGGTTTTTGTAAGAGCAGAGACT-3′	sense	310
AMB-54	2	5′-TTAAATCAAGTGAGTCTATGCGTGGAC-3′	antisense	
AMB-55	3	5′-GGGCATTGAAGGAAGTTTTGTACCA-3′	sense	204
AMB-56	3	5′-GCTAGGAAAACTGAGAAGCACACGATTA-3′	antisense	
AMB-57	4	5′-TTCCACCTTTCAGTGATGATTTGTGTC-3′	sense	243
AMB-58	4	5′-CACTACCACCACCATGAATACTTGCA-3′	antisense	
AMB-71	5	5′-AAGGAAAAGGAAAACCAAATATAACCAATG-3′	sense	300
AMB-48	5	5′-TGTTAATGCTAGGACTTGGCTGTTTCT-3′	antisense	
AMB-60	6	5′-TGGTGCTTGCTATGTAAACTCAACTTC-3′	sense	431
AMB-36	6	5′-GGTTAGCTGGTGATTCTGATCTG-3′	antisense	
AMB-61	7, 8, 9	5′-CACTTTGTCTATTTTGTTTATTTTTTGACTGA-3′	sense	481
AMB-62	7, 8, 9	5′-TTGCAAGACAGTGTCTCATTGAGA-3′	antisense	
AMB-63	10, 11, 12	5′-ACTGTTATGGGGATGTGCCTGTGAG-3′	sense	774
AMB-66	10, 11, 12	5′-AAGTGACTGTTCTTCCCTGGCCACT-3′	antisense	
AMB-67	13	5′-GGTATAGTTAATAGCATGTGATGATGGCA-3′	sense	776
AMB-68	13	5′-TTGAAAGCAAGAAGGGGACCTACACT-3′	antisense	

from non-AI individuals and compare them with those from autosomal AI patients. In the present study, we investigated the presence of nucleotide polymorphisms of the ameloblastin gene in Japanese subjects who did not show signs of AI, as a method of screening for the mutations causing autosomal AI.

Materials and methods

Sample population and genomic DNA preparation

Venous blood (10 m*l*) was collected using acid-citrate dextrose as an anticoagulant from 50 Japanese volunteers (28 males and 22 females, 25 to 55 years old) and genomic DNA was isolated using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany). All subjects were healthy and had sound dentition, and gave their written informed consent prior to the study. All procedures were carried out in full compliance with the Japanese Public Health Service and Osaka University Health Guidelines Involving Human Subjects, and were approved by our Institute Review Board.

PCR amplification

Isolated genomic DNA ($<1\mu$g) was amplified by a PCR procedure in 50μl of PCR buffer using a TaKaRa LA PCR kit, (version 2.1, TAKARA, Otsu, Shiga, Japan), following adjustment as recommended by the manufacturer with a GeneAmp 2400 thermal cycler