ARCHIVES OF ORAL BIOLOGY 52 (2007) 209–217

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Phenotype and enamel ultrastructure characteristics in patients with *ENAM* gene mutations g.13185–13186insAG and 8344delG

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ARTICLE INFO

Article history:
Accepted 7 October 2006

Keywords:
Dental enamel
Enamel ultrastructure
Amelogenesis imperfecta
Enamelin mutations

ABSTRACT

Objective: The main clinical manifestations of amelogenesis imperfecta (AI) include alteration in the quality and quantity of enamel. AI is associated with different mutations in four genes: enamelin (*ENAM*), amelogenin (*AMGX*), kallikrein (*KLK4*) and enamelysin (*MMP-20*). Seven different mutations have been identified in the enamelin gene (*ENAM*).

Design: In this paper, we describe the phenotype and ultrastructure of enamel observed using scanning electron microscopy (SEM) in patients with two autosomal dominant (AD) mutations in the *ENAM* gene: g.13185–13186insAG and g.8344delG, each in one of two unrelated families. Mutations were confirmed by sequence analysis of PCR amplified products of all 10 exons and exon/intron boundaries of the *ENAM* gene.

Results: Phenotypic diversity was observed in patients with *ENAM* gene mutations g.13185–13186insAG with consecutive protein alteration designated as p.P422fsX488 within family 1. In the proband, the enamel of his entire dentition was chalky white with only mild local hypoplastic alteration, while the phenotypic appearance of his father's dentition was that of local hypoplastic AI. In patients with the *ENAM* gene mutation g.8344delG from family 2 with consecutive protein alteration designated as p.N197fsX277, generalised hypoplastic AI was observed.

Conclusions: Ultrastructural enamel changes in the patient with the autosomal dominant *ENAM* g.13185–13186insAG mutation, described for the first time in this study, were less pronounced compared to ultrastructural changes in patients with the autosomal dominant *ENAM* mutation 8344delG. Ultrastructural characteristics of the g.13185–13186insAG mutation revealed deformed prisms, an oval shape on the cross-section and wider interprism spaces, while enamel with the *ENAM* mutation 8344delG was laminated, but prismless.

1. Introduction

Amelogenesis imperfecta (AI) is an inherited tooth disorder solely affecting tooth enamel formation, with widely varying phenotypes. Although the AI enamel defects can be broadly divided into hypoplastic and hypomineralised phenotypes, there are many subtypes of these two main entities. The classification most used worldwide distinguishes 14 subtypes of AI based on various phenotypic criteria and mode of inheritance (autosomal dominant (AD), autosomal recessive (AR) or X-linked).[1]

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doi:10.1016/j.archoralbio.2006.10.010

Enamel is the most mineralised tissue in the human body. The process of crystal initiation and growth is tightly controlled by temporal and locational regulation of secretion, procession and degradation of unique enamel proteins and proteinases that compose the extracellular matrix (ECM). The major enamel matrix proteins contributing to enamel formation are amelogenin, enamelin and ameloblastin.[2] The formation of unique enamel ECM proteins requires the expression of multiple specific genes.[3] Only then will a mature enamel structure, with its highly ordered prismatic pattern packed by hydroxyapatite crystallites, be formed.

Enamelin is the largest enamel ECM protein (180–190 kDa), with about a third of its apparent molecular weight coming from glycosylation. It is present in comparatively small amounts, constituting only 1–5% of the total matrix protein.[3] Enamelin plays an important role in several different stages of enamel formation, for example, in the initiation of mineralisation and regulation of crystal growth.[4–6] Enamelin is the protein product of the enamelin gene (*ENAM*), located on chromosome 4q21[7], which has 10 exons, 8 of which are coding.[3]

The AI-associated gene mutations identified to date involve mutations in the enamelin gene (*ENAM*) (Table 1), the amelogenin gene (*AMGX*),[8] the serine proteinase kallikrein gene (*KLK4*)[9] and the metalloproteinase enamelysin gene (*MMP-20*).[10,11] However, many of the mutations responsible for the majority of types of AI are still unknown.[12]

A correlation between different phenotypes of AI and genotypes of specific mutations in the *AMGX* gene[13] and the *ENAM* gene[14] is proposed. However, phenotypic diversity associated with some cases of AI may represent environmental influences, pleiotropic effects of the underlying gene mutation or modifying gene effects.[5]

In this study, the phenotypic characteristics and detailed ultrastructure of the enamel in two autosomal dominant mutations in the *ENAM* gene: g.13185–13186insAG and g.8344delG, with consecutive protein alteration designated as p.P422fsX488 and p.N197fsX277, respectively, each in one of two unrelated families, are reported. In both families affected members for the *ENAM* mutations were heterozygous. To the best of our knowledge, this is the first report of enamel ultrastructure in the *ENAM* gene g.13185–13186insAG mutation.

2. Material and methods

2.1. Pedigree and diagnosis

Affected members of two unrelated families with different phenotypes of AI were examined clinically and radiographically to determine affection status and to characterise the clinical phenotype. Any unusual oral findings in addition to quality and quantity of enamel changes, malformations or missing teeth and dental malocclusion, were evaluated. Patients were also examined for any metabolic or endocrine defects, generalised diseases, syndromes or fluorosis. Pedigrees of the AI families were constructed. Informed consent was obtained from all patients and/or their parents. The study was approved by the Slovenian Committee for Medical Ethics (Nos. 24/12/04 and 86/02/06).

2.2. ENAM mutation analysis

Genomic DNA was isolated from 10 ml of peripheral blood. All 10 exons and exon/intron boundaries of the *ENAM* gene were amplified using pairs of primers according to Hart and co-workers.[13] PCR was performed in a volume of 50 μl containing: 20 pM each of forward and reverse primers, 200 μM each of dNTPs, 3 mM $MgCl_2$, 5 μl PCR buffer, 0.4 μl Ampli *Taq* Gold™ polymerase (PE applied Biosystems, Norwalk, CT, USA) and 200 μg DNA. PCR was performed in the GenAmp PCR System 9700 (PE Applied Biosystems, Piscataway, NJ, USA) by an initial denaturation at 94 °C for 9 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C (ENAM 1–3, ENAM 4–5, ENAM 6, ENAM 7, ENAM 10a) or 60 °C (ENAM 1, ENAM 8, ENAM 9, ENAM 10b, ENAM 10c, ENAM 10d, ENAM 10e) for 30 s, extension at 72 °C for 40 s, followed by a final extension at 72 °C for 7 min. The PCR products were electrophoresed on 2% agarose gels dyed with 2 μg/ml Et-bromide (45 min, 90 V). The amplicons were extracted using the Qiagen extraction kit (QIAGEN GmbH, Hilden, Germany). After a second electrophoresis of PCR products, the concentration of amplified sequences was estimated. Sequencing PCR was performed in a volume of 20 μl under the following conditions: 25 repeated cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s and extension at 60 °C for 4 min. Extracted amplicons were sequenced using the ABI PRISM® 310 Genetic Analyser (PE Applied Biosystems). Results were compared to normal sequences of the *ENAM* gene accessible on the Internet (http://www3.ncbi.nlm.nih.gov: Acc. No.: AY167999).

2.3. Scanning electron microscopy

For scanning electron microscopy (SEM) analysis, three deciduous molars were prepared immediately after exfoliation: a lower right deciduous second molar (tooth 85) belonging to the boy from family 1, an upper left deciduous first molar (tooth 64) and an upper right deciduous second molar (tooth 55) belonging to each of the brothers from family 2. For comparison, normal enamel of the second left deciduous mandible molar (tooth 75) from a healthy subject was utilised. The teeth were cut in half in the bucco-lingual direction. The halves were embedded in epoxy resin (Araldite, Ciba-Geigy, East Lansing, MI, USA) with the cut side exposed. The exposed axial cross-sections were polished, dehydrated with 70% alcohol, dried and sputter coated in a vacuum with a thin carbon layer (Vacuum Evaporator, Type JEE-SS, JEOL, Tokyo, Japan) and examined with SEM (JEOL JSM—5610, JEOL, Tokyo, Japan). Images were obtained at magnifications between 1000× and 1400×.

3. Results

3.1. Family pedigrees and phenotype determination

No metabolic or endocrine defects, generalised diseases, syndromes or fluorosis were identified in either family in which the *ENAM* mutation was detected. Pedigrees segregating for autosomal dominant amelogenesis imperfecta are presented in Fig. 1.