International Endodontic Journal

doi:10.1111/j.1365-2591.2009.01617.x

Identification of a novel *FAM83H* mutation and microhardness of an affected molar in autosomal dominant hypocalcified amelogenesis imperfecta

H.-K. Hyun[1], S.-K. Lee[2], K.-E. Lee[2], H.-Y. Kang[2], E.-J. Kim[2], P.-H. Choung[3] & J.-W. Kim[1,2]

[1]Department of Pediatric Dentistry, Dental Research Institute and BK21 Program, School of Dentistry, Seoul National University, Seoul; [2]Department of Cell and Developmental Biology, Dental Research Institute and BK21 Program, School of Dentistry, Seoul National University, Seoul; and [3]Department of Oral and Maxillofacial Surgery and Tooth Bioengineering National Research Laboratory, Dental Research Institute and BK21 Program, School of Dentistry, Seoul National University, Seoul, Republic of Korea

Abstract

Hyun H-K, Lee S-K, Lee K-E, Kang H-Y, Kim E-J, Choung P-H, Kim J-W. Identification of a novel *FAM83H* mutation and microhardness of an affected molar in autosomal dominant hypocalcified amelogenesis imperfecta. *International Endodontic Journal*, **42**, 1039–1043, 2009.

Aim To determine the underlying molecular genetic aetiology of a family with the hypocalcified form of amelogenesis imperfecta and to investigate the hardness of the enamel and dentine of a known *FAM83H* mutation.

Methodology Mutational screening of the *FAM83H* on the basis of candidate gene approach was performed. All exons and exon–intron boundaries was amplified and sequenced. A microhardness test was performed to measure the Vickers microhardness value.

Results A novel nonsense mutation (c.1354C>T, p.Q452X) was identified in the last exon of *FAM83H*, which resulted in soft, uncalcified enamel. The affected enamel was extremely soft (about 17% of the normal control), but the underlying dentine was as hard as the normal control.

Conclusions Mutational analysis revealed a novel mutation in *FAM83H* gene. Hardness of dentine was not affected by the mutation, whilst the enamel was extremely soft.

Keywords: amelogenesis imperfect, dentin, enamel, *FAM83H*, microhardness, mutation.

Received 22 March 2009; accepted 12 June 2009

Introduction

Amelogenesis imperfecta (AI) is a heterogeneous group of hereditary enamel defects without any nonoral syndromic phenotypes (Witkop 1988). Enamel defects are categorized as hypoplastic, hypocalcified or hypomatured types. Hypoplastic AI is characterized by thin but hard enamel and is caused by mutations in the amelogenin gene (*AMELX*; OMIM 300391) in an X-linked hereditary pattern and by mutations in the enamelin gene (*ENAM*; OMIM 606585) in an autosomal dominant or recessive hereditary pattern (Wright *et al.* 2006, Hu *et al.* 2007). Hypomatured AI is characterized by hypomineralized enamel with normal thickness and is caused by mutations in enamelysin (*MMP20*; OMIM 604629) or kallikrein 4 (*KLK4*; OMIM 603767) gene in an autosomal recessive hereditary pattern (Hart *et al.* 2004, Kim *et al.* 2005). Hypocalcified AI (OMIM 130900) shows soft enamel with normal thickness and was recently demonstrated to be caused by mutations in *family with sequence similarity, member H* (*FAM83H*; OMIM 611927) gene in an autosomal dominant hereditary pattern (Kim *et al.* 2008, Lee *et al.* 2008, Hart *et al.* 2009).

Correspondence: Dr Pill-Hoon Choung, Department of Oral and Maxillofacial Surgery, School of Dentistry, Seoul National University, 28 Yongon-dong, Chongno-gu, Seoul 110-749, Korea (e-mail: choungph@snu.ac.kr); Dr Jung-Wook Kim, Department of Cell and Developmental Biology, Department of Pediatric Dentistry, Dental Research Institute and BK21 Program, School of Dentistry, Seoul National University, 275-1 Yongon-dong, Chongno-gu, Seoul 110-768, Korea (e-mail: pedoman@snu.ac.kr)

Affected enamel shows reduced thickness or poor mineralization, and thus, excessive attrition and/or sensitivity to thermal changes are frequent findings in AI patients (Hart *et al.* 2003, Kim *et al.* 2004). However, many patients display a tendency of openbite or Class III occlusion regardless of their AI subtype (Kim *et al.* 2006). It is still unclear whether genes involved in AI have a role in the development of the malocclusions. It is not uncommon for affected individuals to need extensive prosthodontic and endodontic treatment because of thin or weak enamel with hypersensitivity to thermal changes. However, it is not yet known how soft the hypocalcified enamel and dentine are in these patients. In this study, the microhardness of an affected tooth in an AI patient was investigated and a novel *FAM83H* nonsense mutation in autosomal dominant hypocalcified AI family was identified.

Materials and methods

The protocols used in this study were reviewed independently and approved by the Institutional Review Board at the Seoul National University Dental Hospital. The experiments were performed with the understanding and written consent of each subject according to the Declaration of Helsinki.

Mutational analysis

Genomic DNA was extracted from the peripheral blood cells of participating family members using the QuickGene DNA whole blood kit S with QuickGene-Mini80 equipment (Fujifilm, Tokyo, Japan). Purity and concentration of the DNA was measured by spectrophotometry (Nanodrop, Thermo, Wilmington, DE, USA) using the OD_{260}/OD_{280} ratio. PCR conditions and primer pairs used for PCR and sequencing the *FAM83H* gene were previously described (Kim *et al.* 2008). PCR amplification was performed using HiPi DNA polymerase premix (ElpisBio, Taejeon, Korea) and the products were purified with a PCR Purification Kit (ElpisBio). DNA sequencing was performed at the DNA sequencing centre (Macrogen, Seoul, Korea). All nucleotide numbering was determined by counting from the A of the ATG translation initiation codon of the human *FAM83H* reference sequence (NM_198488.3).

Microhardness test

A partially impacted permanent right mandibular third molar was extracted from an affected individual with a known *FAM83H* mutation (c.973C>T, p.R325X). The extracted tooth was sectioned using a diamond saw after embedding using expoxy resin. The specimen was then polished using a series of SiC papers. The microhardness score was measured at six points (enamel; near surface, middle, near dentinoenamel junction (DEJ), dentine; near DEJ, middle, near pulpal). The measurement was repeated four times at each point. Vickers microhardness was measured using a microhardness tester (HMV-2, Schimadzu, Tokyo, Japan) applying a 4.903N load for 10 seconds.

Scanning electron microscopy

After the measurement of the microhardness, the tooth was fractured for scanning electron microscopy. The fractured tooth sample was dried and carbon-coated.

Results

Mutation analysis

Mutational analysis revealed a novel nonsense mutation in the *FAM83H* gene. The identified mutation was a C to T nucleotide change resulting in a CAG to TAG (c.1354C>T, p.Q452X) in the last exon (exon 5) of the *FAM83H* (Fig. 1a,b).

Clinical findings

The proband was a 5-year and 8-month-old Korean girl whose deciduous teeth (III:3) showed hypoplastic enamel with brown discoloration. There were several islands of normal-looking enamel, especially in the cervical part of the mandibular deciduous anterior teeth. Soft uncalcified enamel appeared to be worn by masticatory force (Fig. 1c). Panoramic radiography showed developing permanent teeth with normal enamel thickness and reduced mineralization (Fig. 1d). The proband's teeth were moderately sensitive to thermal changes. The affected mother (II:4) had full coverage restorations in almost all teeth.

Microhardness test

The microhardness test revealed extremely soft enamel associated with the *FAM83H* mutation. There was a tendency for decreasing microhardness values towards the dentinoenamel junction in normal enamel ($P < 0.001$, ANOVA). In contrast, the surface of the affected enamel was mostly soft throughout the

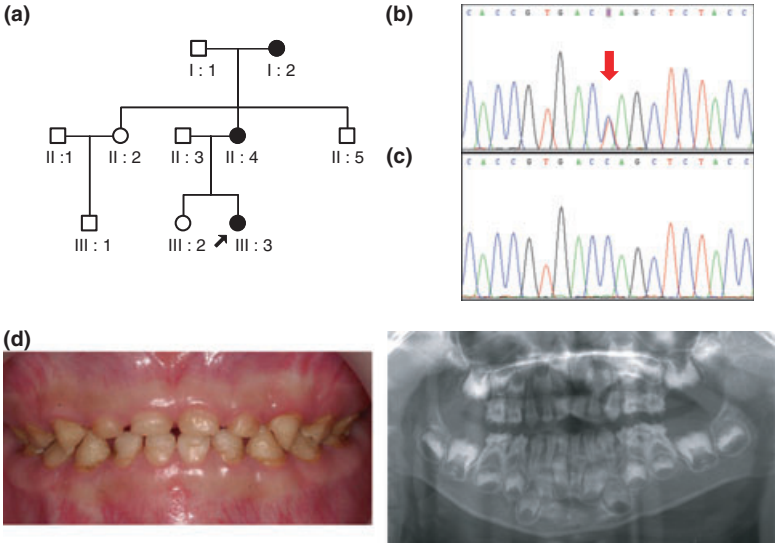

Figure 1 Pedigree and mutational analyses of the kindred. (a) Pedigree of the kindred. Black arrow indicates proband. (b) DNA sequencing chromatogram of the affected individual III-3. Red arrow indicates the position of the mutation. (c) DNA sequencing chromatogram of a normal control. (d) Left: frontal view of affected individual III-3 at age 5 years 8 months. Right: panoramic radiograph of affected individual III-3 at the same age.

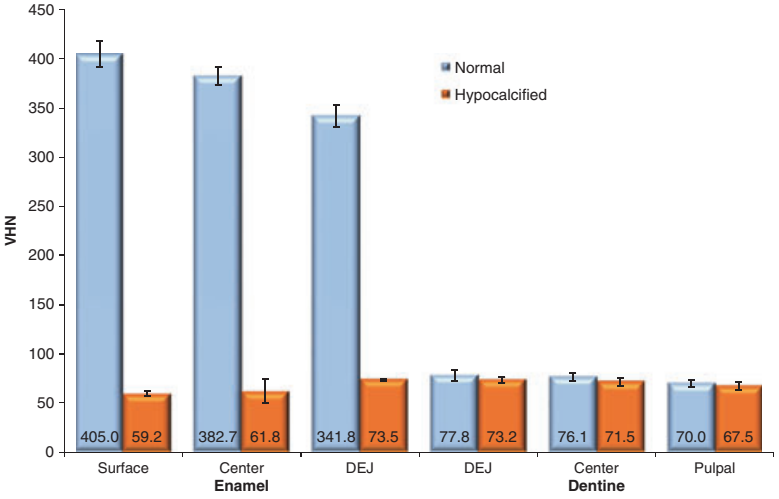

Figure 2 Microhardness of normal and hypocalcified teeth. The Vickers microhardness number is indicated in the box. The 95% confidence interval is indicated in the box for the normal teeth. Standard deviation of the hypocalcified tooth is indicated in the box.

enamel. However, the microhardness values of the affected dentine were similar to the values of normal controls (Fig. 2).

Scanning electron microscopy

Fractured enamel sample showed normal-looking prismatic structure (Fig. 3). However, there were crevices between enamel prisms.

Discussion

The hypocalcified form of AI is the most common subtype in the United States (Witkop 1988). The affected enamel is so soft that it can easily be affected by attrition, leaving thin residual enamel attached to underlying dentine. Islands of normal-looking enamel, especially in the cervical and/or cusp tip area, have been reported, as in this case (Lee *et al.* 2008). It is uncertain whether this phenomenon occurs by other calcification mechanisms, which are not related to the function of *FAM83H* or to the nature of the mutation (genotype–phenotype relation).

Eight mutations in the *FAM83H* gene have been identified (Table 1). However, the novel mutation identified in this study brings the number of mutations resulting in hypocalcification of the enamel to nine. It is very interesting that all identified mutations

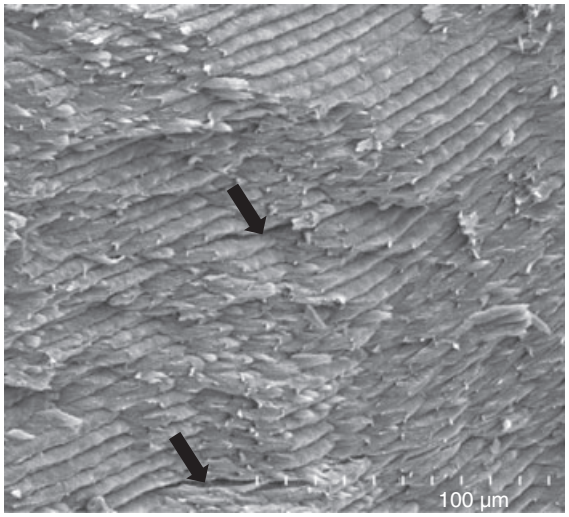

Figure 3 Scanning electron microscopy of the fractured hypocalcified enamel. Black arrows indicate crevices between enamel prisms.

are nonsense mutations occurring in the last exon. The *FAM83H* gene has five exons and the last exon encodes most of the protein (933 out of total 1179 amino acids). Although it has been reported that some nonsense mutations, specifically near the 3′ region of the last exon, cause nonsense-mediated decay (NMD) of mutated mRNA (Tan *et al.* 2008), nonsense mutations reported in *FAM83H* do not seem to induce NMD because these mutations span from the beginning (p.Y297X) to the middle (p.Q667X) of exon 5. This means that, truncated protein product lacking the C-terminal are most likely synthesized. It is still unknown if the hypocalcification of the enamel is a result of a dominant-negative effect or haploinsufficiency, but it is clear that wild-type *FAM83H* is essential for proper calcification of tooth enamel. Further mutational analysis and functional study will help to understand genotype–phenotype correlation and the mechanism of tooth enamel formation.

Thickness of the hypocalcified enamel is usually normal, which may indicate that the secreted amount of enamel matrix proteins at least amelogenin is not affected by the *FAM83H* nonsense mutations. The crevices found in fractured enamel samples may represent increased content of nonmineral component.

From this study, it is clearly shown that the affected enamel of hypocalcified AI because of a *FAM83H* nonsense mutation is extremely soft compared with the normal control. The microhardness values of the affected enamel were similar to or less than the normal values of dentine. However, microhardness values of the dentine of hypocalcified AI were similar to the values of normal dentine. Therefore, root canal preparation in hypocalcified AI patients could be performed as in healthy patients without concerns about lateral stripping or perforation because of excessive dentine softness.

Conclusion

A novel nonsense mutation in the last exon of *FAM83H* was identified in a family of autosomal dominant hypocalcified AI. The microhardness test of a hypocalcified tooth with a known mutation showed that the enamel was extremely soft but the dentine was as hard as the normal control. Further study on the function of *FAM83H* and genotype–phenotype correlation will help the clinician and geneticist understand the mechanism of normal and pathologic enamel formation.

Table 1 Summary of mutations in *FAM83H* resulting in hypocalcified amelogenesis imperfecta

Location	cDNA[a]	Protein	Ethnicity	Heredity	Reference
Exon 5	c.891T>A	p.Y297X	Korean	*de novo*	Lee *et al.* (2008)
	c.973C>T	p.R325X	Korean	AD	Kim *et al.* (2008)
	c.1192C>T	p.Q398X	Korean, Turkish	*de novo*, AD	Kim *et al.* (2008), Hart *et al.* (2009)
	c.1243G>T	p.E415X	Hispanic	AD	Lee *et al.* (2008)
	c.1330C>T	p.Q444X	Turkish	AD	Hart *et al.* (2009)
	c.1354C>T	p.Q452X	Korean	AD	This report
	c.1366C>T	p.Q456X	Turkish	AD, *de novo*	Hart *et al.* (2009)
	c.1380G>A	p.W460X	Caucasian	AD	Lee *et al.* (2008)
	c.2029C>T	p.Q677X	Caucasian	AD	Lee *et al.* (2008)

AD, Autosomal dominant.
[a]Numbering assumes the A of the ATG start codon as nucleotide 1 based on reference sequence NM_198488.3.

Acknowledgements

The authors would like to thank all of the proband's family members for their cooperation. This research was supported by the Korea Science and Engineering Foundation (KOSEF) through the National Research Lab. Program funded by the Ministry of Education, Science and Technology (No. M1060000283-06J0000-28310) and the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. R01-2008-000-10174-0(2008).

References

Hart TC, Hart PS, Gorry MC *et al.* (2003) Novel ENAM mutation responsible for autosomal recessive amelogenesis imperfecta and localised enamel defects. *Journal of Medical Genetics* **40**, 900–6.

Hart PS, Hart TC, Michalec MD *et al.* (2004) Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. *Journal of Medical Genetics* **41**, 545–9.

Hart PS, Becerik S, Cogulu D *et al.* (2009) Novel FAM83H mutations in Turkish families with autosomal dominant hypocalcified amelogenesis imperfecta. *Clinical Genetics* **75**, 401–4.

Hu JC, Chun YH, Al Hazzazzi T, Simmer JP (2007) Enamel formation and amelogenesis imperfecta. *Cells, Tissues, Organs* **186**, 78–85.

Kim JW, Simmer JP, Hu YY *et al.* (2004) Amelogenin p.M1T and p.W4S mutations underlying hypoplastic X-linked amelogenesis imperfecta. *Journal of Dental Research* **83**, 378–83.

Kim JW, Simmer JP, Hart TC *et al.* (2005) MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. *Journal of Medical Genetics* **42**, 271–5.

Kim JW, Simmer JP, Lin BP, Seymen F, Bartlett JD, Hu JC (2006) Mutational analysis of candidate genes in 24 amelogenesis imperfecta families. *European Journal of Oral Science* **114**(Suppl. 1), 3–12. discussion 39–41, 379.

Kim JW, Lee SK, Lee ZH *et al.* (2008) FAM83H mutations in families with autosomal-dominant hypocalcified amelogenesis imperfecta. *American Journal of Human Genetics* **82**, 489–94.

Lee SK, Hu JC, Bartlett JD *et al.* (2008) Mutational spectrum of FAM83H: the C-terminal portion is required for tooth enamel calcification. *Human Mutation* **29**, E95–9.

Tan JT, Kremer F, Freddi S *et al.* (2008) Competency for nonsense-mediated reduction in collagen X mRNA is specified by the 3′ UTR and corresponds to the position of mutations in Schmid metaphyseal chondrodysplasia. *American Journal of Human Genetics* **82**, 786–93.

Witkop CJ Jr (1988) Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited: problems in classification. *Journal of Oral Pathology* **17**, 547–53.

Wright JT, Daly B, Simmons D *et al.* (2006) Human enamel phenotype associated with amelogenesis imperfecta and a kallikrein-4 (g.2142G>A) proteinase mutation. *European Journal of Oral Science* **114**(Suppl. 1), 13–7. discussion 39–41, 379.