Missense Mutation in Fam83H Gene in Iranian Patients with Amelogenesis Imperfecta

S Jalal POURHASHEMI1,2, Mehdi GHANDEHARI MOTLAGH1, Ghasem MEIGHANI1, Azadeh EBRABIMI TAKALOO1, Mahsa MANSOURI1, Fatemeh MOHANDES1, Maryam MIRZAI1, Ahad KHOSHZABAN3,4, Faranak MOSHTAGHI4, Hoda ABEDKHO-JASTEH4, *Mansour HEIDARI4,5

1. Dept. of Pediatric Dentistry, Tehran University of Medical Sciences, Tehran, Iran
2. Dept. of Pediatric Dentistry, Tehran University of Medical Sciences, International Campus, Tehran, Iran
3. Dept. Bio Dental Materials, Dental Faculty of Tehran University of Medical Sciences, Tehran, Iran
4. Stem Cell Preparation Unit, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran
5. Dept. of Medical Genetics, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding Author: Email: mheidari@sina.tums.ac.ir

(Received 15 Jun 2014; accepted 10 Sep 2014)

Abstract

Background: Amelogenesis Imperfecta (AI) is a disorder of tooth development where there is an abnormal formation of enamel or the external layer of teeth. The aim of this study was to screen mutations in the four most important candidate genes, ENAM, KLK4, MMP20 and FAM83H responsible for amelogenesis imperfecta.

Methods: Geneomic DNA was isolated from five Iranian families with 22 members affected with enamel malformations. The PCR amplifications were typically carried out for amplification the coding regions for AI patients and unaffected family members. The PCR products were subjected to direct sequencing. The pedigree analysis was performed using Cyrillic software.

Results: One family had four affected members with autosomal dominant hypocalcified amelogenesis imperfecta (ADHPCAI); pedigree analysis revealed four consanguineous families with 18 patients with autosomal recessive hypoplastic amelogenesis imperfecta (ARHPIAI). One non-synonymous single-nucleotide substitution, c.1150T>A, p. Ser 342Thr was identified in the FAM83H, which resulted in ADHCAI. Furthermore, different polymorphisms or unclassified variants were detected in MMP20, ENAM and KLK4.

Conclusion: Our results are consistent with other studies and provide further evidence for pathogenic mutations of FAM83H gene. These findings suggest different loci and genes could be implicated in the pathogenesis of AI.

Keywords: Amelogenesis imperfecta, Iranian patients, FAM83H

Introduction

Amelogenesis imperfecta (AI) is a common group of inherited defects that present quantitative or qualitative tooth enamel malformation in the absence of systemic manifestations. AI has been classified into syndromic and non-syndromic forms(1). AI is sometimes associated with different syndromes such as tricho-dento-osseous (TDO) syndrome (OMIM #190320) and cone rod dystrophy. According to population-base studies, the incidence of AI varies, from 1 in 700 to 1 in 15,000 (2).
The phenotype of affected individuals is highly variable and can be divided based on whether the abnormality results in a reduced amount of enamel (hypoplasia), deficient calcification (hypocalcification), or deficient maturation of the enamel (hypomaturation) (1, 3). The mineralization level of enamel in the hypomaturation and hypocalcified AI is not normal and can be described as hypomineralized. Its genetic inheritance pattern is reported as either an X-linked or autosomal recessive (AR) or autosomal dominant (AD) (3, 4).

In order to better understand how defective enamel formation occurs through development, two fundamental questions must be addressed (1) which candidate genes are responsible for various forms of AI (2) and how do these candidate genes and their protein partners work together in normal and abnormal enamel formation? So far, changes in several ameloblast specific genes have been detected including Amelogenin (AMELX) (5-7), Ameloblastin (AMBN) (8, 9), Enamelysin (ENAM) (10-17), Kallikrein-related peptidase-4 (KLK4) (18, 19), Family with sequence similarity 83 (FAM83H) (6, 20-22) and Matrix Metalloproteinase 20 (MMP20) (14, 23-25).

In the present study, we aimed to screen the genetic alterations in the most important candidate genes, ENAM, KLK4, MMP20 and FAM83H responsible for AI in five Iranian families.

Materials & Methods

Patients

This study was a case study based on genetic testing of affected patients and healthy people. Inclusion criteria for our study were as follows: 1) patients were required to have an isolated form of AI; 2) Patients were included in this study with inheritance patterns. A total of 50 family members (22 affected and 28 unaffected) from five Iranian families were studied. Five Iranian families with AI were diagnosed at the Pediatric Dentistry Department of Tehran University of Medical Sciences (TUMS). The study was performed with the approval of the Institutional Review Board (IRB) and informed consent was obtained from each patient and controls before genetic testing. The pedigree analysis was carried out using Cyrillic 2.1 software.

Molecular Analysis

5 ml peripheral blood was collected in test tubes containing 0.5 M EDTA from patients, unaffected members of the family and 100 healthy controls. Then, DNA was extracted using DNGPLUS kit (Cinnagen, Tehran-Iran). The PCR amplification was typically carried out using primer pairs of exon-intron boundaries of ENAM, FAM83H, MMP20 and KLK4 genes (Table 1), 0.2U Taq DNA polymerase (Roche, Mannheim, Germany), 10pmole of each primer, 200 μM of each dNTPs, 0.67μl of 50mM MgCl2, 60ng DNA and 2.5 μl of PCR buffer in 25μl of PCR reactions. The PCR conditions included an initial denaturation step for 3 min at 95°C, 30 sec at 95°C, 45 sec at 64°C with a 1°C decrease every second cycle down to 55°C, then 55°C for 14 cycles, 1 min at 72°C for extension, and finally 10 min at 72°C. PCR products were separated on 2% agarose gels and visualized with ethidium bromide. Subsequently, to determine any mutation the PCR product was subjected to direct sequencing (Gene Fanavaran, Iran). Sequence data searches were performed in non-redundant nucleic and protein databases BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Results

Clinical descriptions and mutational analysis

After clinical examination, affected individuals from one family showed clinical features consistent with ADHCAI (Fig. 1), while 4 consanguineous families with 18 affected members were diagnosed for ARHPAI. All affected individuals were clinically and radiographically examined and showed no signs of syndromic conditions or systemic illnesses associated with enamel malformation. In addition, dental examination of the unaffected members showed no evidence of any enamel defect and any syndromic signs.

Available at:  http://ijph.tums.ac.ir
| Primer | Sequence | PCR Product Size(bp) |
|--------|----------|---------------------|
| **ENAM** | | |
| EN1F | 5'-CTGTGCCAAGCTTTCTGACA-3' | 923 |
| EN1R | 5'-TGTTGGCCCTCTCAAGTGTT-3' | |
| EN45F | 5'-CCCCATCCATTTCCATACTC-3' | 523 |
| EN45R | 5'-TGGATGCGCTGGGAATATTACT-3' | |
| EN6F | 5'-TCAGAAATATTCTACTGGGAAG-3' | 323 |
| EN6R | 5'-TGTGAGAAGATGGGCAAT-3' | |
| EN7F | 5'-GAGGATGAGACACGCTGAA-3' | 302 |
| EN7R | 5'-CGGGCTGAGGTTGATTATGT-3' | |
| EN8F | 5'-GGGAGATGTAGACTCCCAAGTTT-3' | 307 |
| EN8R | 5'-TGATGCACTGGTTTGTTTCA-3' | |
| EN9F | 5'-GATCCAGCTGAAGGCTTGT-3' | 324 |
| **MMP20** | | |
| M1F | 5'-CAGGACCTGGAGGAACAATC-3' | 201 |
| M1R | 5'-CCAGACCAACCATCTAGGTTGA-3' | |
| M2F | 5'-CCCTGCCTTACCTGAGCAT-3' | 470 |
| M2R | 5'-GCTTGAAGCAGAGATGGTAA-3' | |
| M3F | 5'-CCGGATTATCCCAACTGTCT-3' | 472 |
| M3R | 5'-ACTGTGCGAAGGAGATGTC-3' | |
| M4F | 5'-TGTCATGCTACTCAAAATGTCC-3' | 312 |
| M5F | 5'-AGTTAGGAGAAGGGAGATGTA-3' | 193 |
| M5R | 5'-CTGATGGAAGGATATTTTG-3' | |
| M6F | 5'-CATGTCAGCTGAAGGATGT-3' | 300 |
| M6R | 5'-GCTGAGCAGATGGAGATGAGG-3' | |
| M7F | 5'-AGTAAACGATGCGCCCTTC-3' | 266 |
| M7R | 5'-AAACAGGCAAGGGCAAGG-3' | |
| **KLK4** | | |
| K1F | 5'-GAGTTAGGAGCAGGCTGAGAG-3' | 1000 |
| K1R | 5'-ACAAGGAGTGGCAGGAGAC-3' | |
| K2F | 5'-CTGCTCTGAACCTCTGACC-3' | 615 |
| K2R | 5'-ATTCCTCATCCCCATCTCCTA-3' | |
| K3F | 5'-TGACTGCTCTGAACTCTG-3' | 149 |
| K3R | 5'-CTCTGCCGCGTTTATGATTG-3' | |
| K4F | 5'-GCGGTTGAGATGAGTAGTG-3' | 615 |
| K4R | 5'-GGCCCTGTTGCTTCTGCTT-3' | |
| K7F | 5'-AAACTGACCTGGCCCTCGT-3' | 195 |
| K7R | 5'-TGTCAGACTCGGAGCAAGG-3' | |
| **FAM83H** | | |
| FAM83PF1 | 5'-CTCGCCAGGAGCCCTTGCTTCTG-3' | 486 |
| FAM83PR1 | 5'-GGAAGGCGACAGGAAGT-3' | |
| FAM83PF2 | 5'-CCCTCTCCCTCCCTCTAAG-3' | 523 |
| FAM83PR2 | 5'-CGCCCAGGTTGGAAGTCA-3' | |
| FAM83PF3 | 5'-CTACACAGACCTGACAGTG-3' | 652 |
| FAM83PR3 | 5'-CGAAGCAGGAAATGAGTCTC-3' | |
| FAM83PF4 | 5'-GCTTCCACCAAGACTGC-3' | 497 |
| FAM83PR4 | 5'-CTCGCTGTGAAAGGAGTGC-3' | |
| FAM83PF5 | 5'-GTGCGCCAGCAGCAAGA-3' | 566 |
| FAM83PR5 | 5'-GACTCCCCGAGATGGTAAG-3' | |
| FAM83PF6 | 5'-CAGGATTTCATGAGCAGAAGG-3' | 593 |
| FAM83PR6 | 5'-GCTGAAACCTTGTGCTTCTG-3' | |
| FAM83PF7 | 5'-AAGGCCATTTCTGGAGCAGAT-3' | 689 |
| FAM83PR7 | 5'-GACGGTGCAAGGATGAGT-3' | |
Pourhashemi et al.: Missense Mutation in Fam83h Gene in Iranian Patients

Fig. 1: Pedigree analysis, Clinical Characterizations and Molecular Study of Family 1 Affected with ADHCAI. (A) The pedigree of the family represents an autosomal dominant pattern of inheritance with four affected patients. (B) Phenotype demonstrating hypocalcified amelogenesis imperfecta. (C) Radiographic examination shows lack of tooth enamel in proband. (D) Chromatogram from wild type FAM83H gene. (E) DNA sequencing revealed heterozygous in codon 342 for amino acid serine to threonine (c.1150T>A, p. Ser 342Thr). Arrows indicate the proband and base substitution.

The ADHCAI individuals were typically characterized by soft enamel, which wears off the tooth soon after eruption and following exposure to mastication forces. As it is shown in Fig. 1C enamel cannot be distinguished from dentin by its opacity on radiographs; however, ARHPAI patients presented reduced enamel and spacing between teeth.

Mutation screening in ADHCAI-affected members revealed one novel non-synonymous single-nucleotide substitution in the FAM83H gene. This mutation was detected in codon 342 for amino acid serine to threonine (c.1150T>A, p. Ser 342Thr, Fig. 1E).

The mutation was identified in the affected members of the families with ADHCAI, but not in the unaffected individuals as well as in 100 healthy controls, indicating that this genetic change is not common variant in the Iranian populations. Figure 2 indicates the positions of mutations identified in the FAM83H gene.

Moreover, although no significant mutations in ENAM, KLK4 and MMP20 genes were detected in any probands, different polymorphisms were identified within non-coding or/coding region sequences of these genes (Table 2).
**Fig. 2:** Diagram of the FAM83H based on the human sequence. The human FAM83H gene is composed of five exons (orange boxes) and four introns (blue line). The 5'-UTR (untranslated regions) and 3'-UTR regions are indicated with black boxes. Arrows show the location of identified missense mutations in the C-terminal sequences.

| Gene   | Site of Variation | gDNA         | cDNA           | Protein                       | AI Phenotype |
|--------|------------------|--------------|----------------|-------------------------------|--------------|
| FAM83H | Exon5            | g.10061G>A   | c.846G>A       | GCG>GCA(Ala>Ala)             | RHPAI        |
| FAM83H | Intron4          | g.9945G>C    | -              | -                             | ADHCAI       |
| KLK4   | Exon2            | g.6329G>T    | c.66G>T        | TCG>TCT(Ser>Ser)             | ARHPAI       |
| KLK4   | Intron1          | g.5193C>G    | -              | -                             | ARHPAI       |
| ENAM   | Intron7          | g.8232T>G    | -              | -                             | ARHPAI       |
| ENAM   | Intron7          | g.8562A>T    | -              | -                             | ARHPAI       |
| MMP20  | Intron5          | g.18514T>C   | -              | -                             | ARHPAI       |
| MMP20  | Intron5          | g.18505A>G   | -              | -                             | ARHPAI       |

**Table 2:** Identified single Nucleotide Polymorphisms (SNPs) in this study

**Discussion**

In this study, we performed direct PCR sequencing for 5 families having at least two affected individuals with AI. ARHPAI was diagnosed in 4 out of 5 families. The clinical features of hypoplastic AI in our patients were typically yellow-brown discoloration of the teeth and evidence of pathological enamel loss during wear as well as fracturing. One family was diagnosed for ADHCAI. The clinical characteristics of ADHPCAII usually presented with yellow-brown teeth discoloration and poorly mineralized enamel that have increased enamel proteins.

Our mutation screening within coding sequences of three AI candidate genes including KLK4, ENAM and MMP20 failed to detect any mutation in affected patients. However, several single nucleotide polymorphisms were found in the studied samples. In agreement with our results, several studies could not detect any mutation in the aforementioned genes involved in enamel defects in different populations (14, 15, 26). Ghandehari et al. studied the mutations in MMP20, ENAM and KLK4 genes in Iranian families with generalized hypoplastic phenotypes; however, no mutations were detected in their samples (27). In addition, another study by Kim et al. in 24 families with non-syndromic enamel defects found only a few disease-causing mutations (14). These results strongly suggested that several genes and loci might be associated with AI.

Although we were not able to identify any relevant mutation in the hypoplastic AI patients, one novel missense mutation in the FAM83H gene, c.1150T>A, p. Ser342Thr was detected in the ADHPCAI patients. The mutation was cosegregated among affected members of families with the disease phenotype but not in those that were unaffected. Mutations in the candidate genes, FAM83H, KLK4, MMP20 and ENAM have been suggested to be important in the etiology of AI (19, 21, 28). Even though our results are the first report that demonstrates the genetic alterations of FAM83H in Iranian patients, several mutations within this gene have been identified in AI patients in different ethnic groups (20-22, 29-33). Kim et al. (21) studied two families with autosomal-dominant hypocalcified AI and identified nonsense mutations (R325X and Q398X) in the FAM83H gene. In addition, a
study previously described two nonsense transition mutations in a single allele of FAM83H (c.1379G>A; g.5663G>A in the C-terminal exon 5) in the Chinese population (34). More recently, Wang et al. identified disease-causing mutations (g.6930delG; c.245delG; p.Gly82Alafs*87) in their samples (19). Up to now, the majority of genetic alterations (~14 mutations) have been reported in the last exon of FAM83H, which encodes the C-terminal region. According to our findings as well as the literature published so far, two main questions needed to be addressed namely: 1) Which parts of this gene induces more pathogenic effects and 2) How can we define the molecular characterization of the FAM83H gene? To address the first question, as early pointed out, all disease-causing mutations in FAM83H are located within exon 5. Therefore, it seems that this part of FAM83H gene could be the hotspot for genetic alterations. In order to address the second question, several molecular studies such as sub-cellular localization, using knockout mice experiments and bioinformatics strategies are required. It is believed that investigating biological functions of the genetic variations provide a great opportunity for understanding molecular bases of human diseases. Even though some features and their predicted functions of human FAM83H have been previously reported the C-terminal region function(s) was not adequately analyzed (34). It has been suggested the implication of FAM83H protein in normal and abnormal functions through either direct or indirect interactions with several protein partners.

**Conclusion**

Our findings indicated an association between AI and FAM83H mutations. However, the exact mechanism(s) by which this gene acts in the pathogenesis of AI remains to be clarified. To define direct or indirect influences of FAM83H on normal and abnormal enamel formation different techniques such as chromatin immunoprecipitation assay (ChIP Assay) and protein-protein interaction using specific antibodies may be useful strategies for identification of its down-stream target signaling pathways.

**Ethical considerations**

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

**Acknowledgements**

This work was supported by a grant from the Tehran University of Medical Sciences. The authors declare that there is no conflict of interest.

**References**

1. Witkop CJ (1988). Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited: problems in classification. J Oral Pathol17:547-553.
2. Chaudhary M, Dixit S, Singh A, Kunte S (2009). Amelogenesis imperfecta: Report of a case and review of literature. J Oral Maxillofac Pathol13:70-77.
3. Aldred MJ SR, Crawford PJ (2003). Amelogenesis imperfecta: a classification and catalogue for the 21st century. Oral Disease9:19-23.
4. Gadhia K, Mcdonald S, Arkutu N, Malik K (2012). Amelogenesis imperfecta: an introduction. Br Dent J212:377-379.
5. Hu JCC, Chan H-C, Simmer SG, Seymen F, Richardson AS, Hu Ymilkovich RN, Estrella NM, Yildirim M, Bayram M, Chen CF, Simmer JP (2012). Amelogenesis imperfecta in two families with defined AMELX deletions in ARHGAP6. PLoS One, 7:e52052.
6. Lee K-E, Lee S-K, Jung S-E, Song SJ, Cho SH, Lee Zhkim JW (2011). A novel mutation in the AMELX gene and multiple crown resorptions. Eur J Oral Sci, 119:324-328.
7. Wright JT, Torain M, Long K, Seow K, Crawford P, Aldred MJ, Hart PS, Hart TC (2011). Amelogenesis imperfecta: genotype-phenotype

Available at:  http://ijph.tums.ac.ir
16. Mardh CK, Backman B, Holmgren G, Hu JCC, Simmer JP, FORSMAN-SEMB K. (2002). A nonsense mutation in the enamelin gene causes local hypoplastic autosomal dominant amelogenesis imperfecta (AIH2). *Hum Mol Genet*, 11:1069-1074.

17. Hart TC, Hart PS, Gorry MC, Michalec MD, Ryu OH, Uygun C, Ozdemir D, Firatli S, Aren G, Firatli E (2003). Novel ENAM mutation responsible for autosomal recessive amelogenesis imperfecta and localised enamel defects. *J Med Genet*, 40:900-906.

18. Hart PS, Hart TC, Michalec MD, Ryu OH, Simmons D, Hong S, Wright JT. (2004). Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. *J Med Genet*, 41:545-549.

19. Wang SK, Hu Y, Simmer JP, Seymen F, Estrella N, Pal S, Reid BM, Yildirim M, Bayram M, Bartlett JD, Hu JC. (2013). Novel KLK4 and MMP20 mutations discovered by whole-exome sequencing. *J Dent Res*, 92:266-271.

20. Hyun HK, Lee SK, Lee KE, Kang HY, Kim EJ, Choung PH, Kim JW (2009). Identification of a novel FAM83H mutation and microhardness of an affected molar in autosomal dominant hypocalcified amelogenesis imperfecta. *Int Endod J*, 42:1039-1043.

21. Kim J-W, Lee S-K, Lee ZH, PARK J-C, Lee K-E, Lee M-H, Park JT, Seo BM, Hu JC, Simmer JP (2008). FAM83H mutations in families with autosomal-dominant hypocalcified amelogenesis imperfecta. *Am J Hum Genet*, 82:489-494.

22. Lee S-K, Hu JCC, Bartlett JD, Lee K-E, Lin BPJ, Simmer JP (2008). Mutational spectrum of FAM83H: the C-terminal portion is required for tooth enamel calcification. *Hum Mutat*, 29:95-99.

23. Papagerakis P, Lin HK, Lee KY, Hu Y, Simmer JP, Bartlett JD, Hu JC (2008). Premature stop codon in MMP20 causing amelogenesis imperfecta. *J Dent Res*, 87:56-59.

24. Ozdemir D, Hart PS, Ryu OH, Choi SJ, ozdemir-karatas M, Firatli E ET AL, Piesco N, Hart TC (2005). MMP20 active-site mutation in hypomaturation amelogenesis imperfecta. *J Dent Res*, 84:1031-1035.

25. Lu Y, Papagerakis P, Yamakoshi Y, Hu JCC, Bartlett JD, Simmer JP (2008). Functions of KLK4 and MMP-20 in dental enamel formation. *Biol Chem*, 389:695-700.

26. Santos MCLG, Hart PS, Ramaswami M, Kanno CM, Hart TC, Line SRP (2007). Exclusion of known gene for enamel development in two Brazilian families with amelogenesis imperfecta. *Head Face Med*, 3:8-8.
27. Ghandehari motlagh M, Bahmanipour M, Aref P, Pourhashemi SJ, Shahrabi M, Nazarian AR, Raoofian R, Mahlubinejad F, Heidari M (2009). No Evidence for Association between Amelogenesis Imperfecta and Candidate Genes. Iran J Public Health, 38:4-9.

28. Becerik S, Cogulu D, Emingil G, Han T, Hart PS, Hart TC (2009). Exclusion of candidate genes in seven Turkish families with autosomal recessive amelogenesis imperfecta. Am J Med Genet A, 149:1392-1398.

29. Chan H-C, Estrella NMRP, Milkovich RN, Kim J-W, Simmer JP, Hu JCC (2011). Target gene analyses of 39 amelogenesis imperfecta kindreds. Eur J Oral Sci, 119:311-323.

30. Kweon Y-S, Lee K-E, Ko J, Hu JCC, Simmer JP, Kim J-W (2013). Effects of Fam83h overexpression on enamel and dentine formation. Arch Oral Biol, 58:1148-1154.

31. Wright JT, Frazier-Bowers S, Simmons D, Alexander K, Crawford P, Han ST, Hart PS, Hart TC (2009). Phenotypic variation in FAM83H-associated amelogenesis imperfecta. J Dent Res, 88:356-360.

32. Haubek D, Gjorup H, Jensen LG, Juncker I, Nyegaard M, Borglum AD, Poulsen S, Hertz JM (2011). Limited phenotypic variation of hypocalcified amelogenesis imperfecta in a Danish five-generation family with a novel FAM83H nonsense mutation. Int J Paediatr Dent, 21:407-412.

33. El-Sayed W, Shore RC, Parry DA, Inglehearn CF, Mighell AJ (2010). Ultrastructural analyses of deciduous teeth affected by hypocalcified amelogenesis imperfecta from a family with a novel Y458X FAM83H nonsense mutation. Cells Tissues Organs, 191:235-239.

34. Song YL, Wang CN, Zhang CZ, Yang K, Bian Z (2002). Molecular characterization of amelogenesis imperfecta in Chinese patients. Cells Tissues Organs, 196(3):271-9.

Available at: http://ijph.tums.ac.ir