Iranian J Publ Health, Vol. 43, No.12, Dec 2014, pp.1680-1687

Original Article

Missense Mutation in *Fam83H* Gene in Iranian Patients with Amelogenesis Imperfecta

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(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 imperfect

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 (ARHPAI). 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 presentquantitative 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*, *FAM38H*, *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 95ºC, 30 sec at 95ºC, 45 sec at 64ºC with a 1ºC decrease every second cycle down to 55ºC, then 55ºC for 14 cycles, 1 min at 72ºC for extension, and finally 10 min at 72ºC. 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.

Table1: Primer sequences used in this study

Primer	Sequence	PCR Product Size(bp)
	ENAM	
EN1F	5'-CTGTGCCAAGCTTTCTGACA-3'	923
EN1R	5'-TGTTTGGCCCTCTCAAGTGT-3'	
EN45F	5'-CCCCATCCATTTCCATACTC-3'	523
EN45R	5'-TGATGGCTGGGGAAATTACT-3'	
EN6F	5'-TCAGAAATTTTTACACTGGGAAG-3'	323
EN6R	5'-TGTGAGAGGATAGGGGCAAT-3'	
EN7F	5'-GAGGATGGAGACAGCCTGAA-3'	302
EN7R	5'-CGGGCTGAGGTTGATTATGT-3'	
EN8F	5'-GGGAGATGTAGACTCCCAAGTTT-3'	307
EN8R	5'-TGATGCACTGGTTTTGTTTCA-3'	
EN9F	5'-GATCCAGCTGAAGCCTTTGT-3'	324
	MMP20	
M1F	5'-CAGGACCTGGAGGAACAACT-3'	201
M1R	5'-CCAGACACCAATCTAGGTGGA-3'	
M2F	5'-CCCTGCCTTACCTGAGCAT-3'	470
M2R	5'-GCCTGACGGATGGATGTAAA-3'	
M3F	5'-CCGGATTATCCCAACTGTCT-3'	472
M3R	5'-ACTGTGCGAAGGAGGAGTGT-3'	
M4F	5'-TGTCAATGCTACTCAAAATGTCC-3'	312
M5F	5'-AGTTAGGAGAAGGAGATGGG-3'	193
M5R	5'-CTGATGGGTCTGTGGAATG-3'	
M6F	5'-CATGTCCAGCGTGAAAGTGT-3'	300
M6R	5'-GTCTGGGAGTGGAGATGAGG-3'	
M7F	5'-AGTAAGCAGTGCCCCTCTC-3'	266
M7R	5'-AAACAAGGCAAGGCAAGG-3'	
	KLK4	
K1F	5'-GAGTTGAGGCAGCCTGAGAG-3'	1000
K1R	5'-ACAAGGAGTTGCAGGGACAC-3'	
K2F	5'-CTGCTCCTGAACCTCTGACC-3'	615
K2R	5'-ATTCCCATCCCCATCTCCTA-3'	
K3F	5'-TGACTGCTCCTGAACCTCTG -3'	149
K3R	5'-CCTCGCCGTTTATGATTTG-3'	
K4F	5'-GGGGTTGAAGATGAGAATGG-3'	615
K4R	5'GGCCCTGTGTGTCTCTGTCT-3'	
K7F	5'-AAACTGACCTGCCCTCCGT-3'	195
K7R	5'-TGTCAGACTCGGACACGGA-3'	
	FAM83H	
FAM83PF1	5'-CTCGCCAGGAGCCCTTGTCCTGTAGA-3'	486
FAM83PR1	5'-GGAAGGCCGACAGGAAGT-3'	
FAM83PF2	5'-CCCTTCTCCTTCCCTAAACG-3'	523
FAM83PR2	5'-CGCCCAGGGTGAAGTCAT-3'	
FAM83PF3	5'- CTACCAGCAGCAGTACCAGTG-3'	652
FAM83PR3	5'- CGAGCGGAATGAGTCCTG-3'	
FAM83PF4	5'- GCCTTCCCCACCAAGGTC-3'	497
FAM83PR4	5'- CTGCTGTGCAAAGGAGTCG-3'	
FAM83PF5	5'- GTTGCCAGCCACAGCAAG-3'	566
FAM83PR5	5'- GACTCCCCGGAGATGGTAAG-3'	
FAM83PF6	5'- CAGGATTTATCGAGCAGAAGG-3'	593
FAM83PR6	5'- GGCTGAACACTTGTCCTTGTC-3'	
FAM83PF7	5'- AAGGCCATTCTGGAGCAGAT-3'	689
FAM83PR7	5'- GACGGTGCAGAGATGAAGGT-3'	

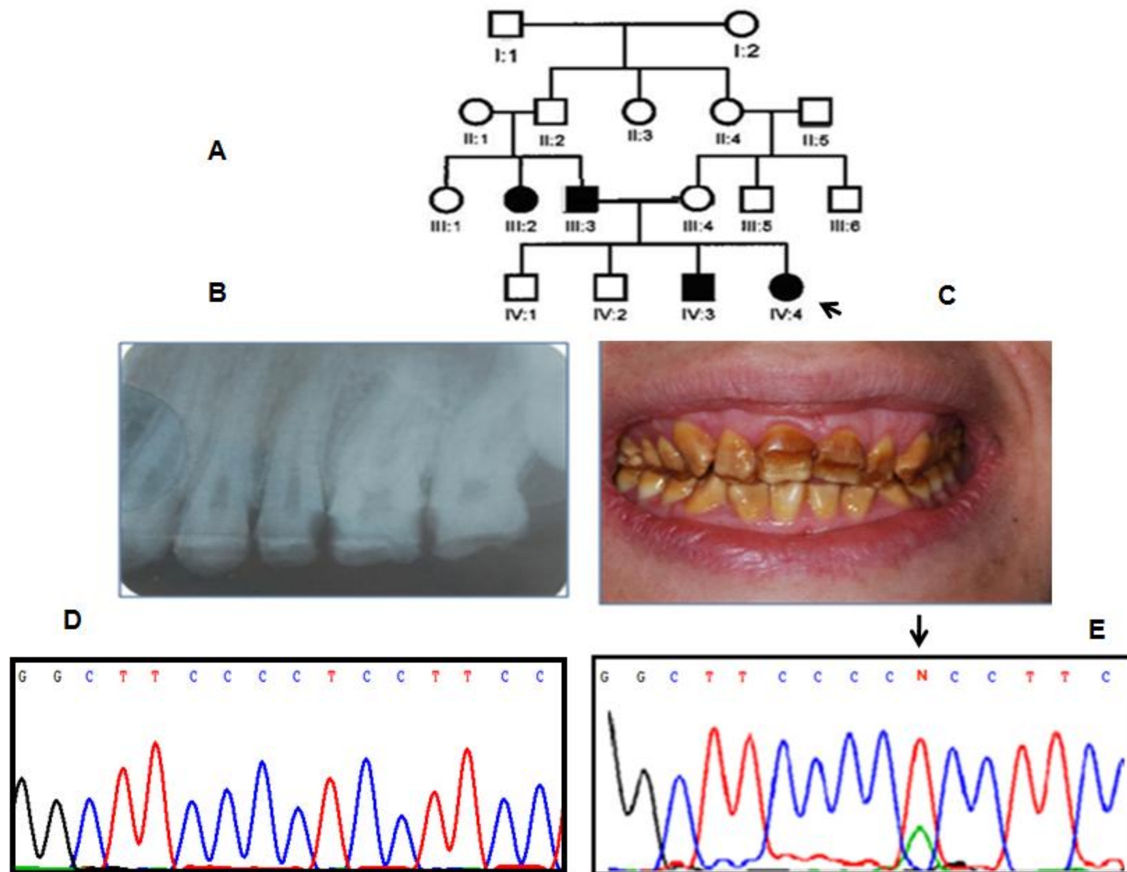

Fig. 1: Pedigree analysis, Clinical Characterizations and Molecular Study of Family 1 Affected with ADHCAI.A. (A) The pedigree of the family represents an autosomal dominant pattern of inheritance with four affected patients. (B) Phenotype demonstrating hypocalcified amoelogenesis imperfecta. (C) Radiographic 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 subsituation

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/and 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

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

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

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 ADHPCAI 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. Ser 342Thr was detected in the ADHPCAI patients. The mutation was co-segregated 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.

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