



Determination of mutation in the coding regions of FAM83H and ENAM genes in patients with imperfect enamel (Amelogenesis Imperfecta)

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ARTICLE INFO

Article Type:
Original Article

Received: 23 Feb. 2020

Revised: 28 Apr. 2020

Accepted: 25 Jun. 2020

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ABSTRACT

Introduction: Tooth enamel is a precious and highly mineralized tissue in the human body. Amelogenesis Imperfecta (AI) is a developmental, evolutionary and hereditary disease presents with the rare abnormal formation of enamel that affects the primary and secondary dentition. The molecular base of the incomplete quinizium together with clinical manifestation suggest that AI may result from mutations in the FAM83H and ENAM genes. In this study, we aimed to evaluate the association between Amelogenesis Imperfecta and mutations in the FAM83H and ENAM genes in 18 Iranian families with AI in dominant and non-syndromic form.

Materials and Methods: 18 Iranian families with at least 1 patient with Amelogenesis Imperfecta were included in this case study and were examined for related specific manifestations and also, 10CC of blood was taken from each patient followed by PCR and genome sequence for genetic alterations in FAM83H and ENAM. Genome sequences were analyzed using CLC software and CLC Sequence Viewer was used to compare them with reference sequences in the RefSeq database at the NCBI later were discussed together with clinical manifestations for each patient.

Results: All patients showed a mutation in the exon 5 of FAM83H gene in nucleotide rs56148058C/T which converted Serotonin to Aspartin. In two patients carried a mutation in the nucleotide rs546809055A/G that changed Leucine to Phenylalanine. None of patients showed significant alteration in the ENAM gene.

Conclusion: This study indicates that FAM83H gene plays an import role in incidence of Amelogenesis Imperfecta in Iran.

Keywords: Amelogenesis imperfecta; Gene mutation; FAM83H; ENAM.

Introduction

Tooth enamel is the hardest and one of the most valuable minerals in human's body. It consists of dentin, cement, and pulp, which form the tooth

structure [1]. Any disturbances in local or general factors that contribute to the formation of a normal matrix can lead to surface defects and ruptures or to stop building

up of the matrix, which together may result in a condition called enamel hypoplasia. Unlike other body tissues, enamel does not possess an amending system to reconstruct its anatomical and histological properties. Thus, any structural changes during the formation period will permanently remain for life [2]. Defective enamel (Amelogenesis Imperfecta) is a developmental and evolutionary disorder with hereditary nature that involves primary and permanent teeth. There is a wide range of defects in the enamel that is from small white points to full teeth gritting and in some cases, the teeth also turn yellow or brown [3]. The defective enamel also presents a wide range of clinical manifestations in three major categories including hypo calcify, mature, and hypoplastic forms. The prevalence of amelogenesis imperfecta (AI) varies in different populations reportedly from one in fourteen thousand to one in sixteen thousand [1], so that is found to be 1/14000, 43/10000, 1/700 and 10/10000 in the USA, Turkey, Sweden, and Argentina respectively [5]. Genetically there are four inheritance patterns assumed for AI including autosomal dominant, autosomal recessive, dominant, and reversible [6]. A study in Sweden showed that 63% of AI cases had been dominant with autosomal inheritance, while other studies from Asian countries showed the most common hereditary pattern was impermeable autosomal recessive [7].

Studies from Iran showed that the autosomal recessive pattern was the commonest type of AI. These studies also showed a higher prevalence among girls than boys with hypoplastic type being more common in both sexes than other phenotypes [6]. Advances have been made in the discovery of the molecular basis for the different clinical manifestations. It has recently been shown that mutation in the six genes including *KLK4*, *WDR72*, *FAM83*, *ENAM*, *AMLEX*, and *MMP20* are responsible for the clinical occurrence of AI [2].

Some studies have shown that mutations in *FAM83H* gene, on the long arm of chromosome number 8, were significantly associated with structural changes during the enamel calcification process [7] and two mutations including p.Arg325 and p.Gln398 in this gene result in hypo calcified defects in enamel with the dominant autosomal pattern in humans [8]. The *ENAM* gene on the long arm of chromosome 4 codes for a protein called Enamelin and is another genetic culprit causing AI. It seems that mutations in this gene lead to the production of an ineffective protein by Ameloblast cells which in turn it causes mineralization defects in enamel, which is reportedly shown to be

of both autosomal dominant and recessive types [9]. Clinical manifestations of AI such as delayed permanent teeth with an unpleasant deformity in the appearance, the presence of multiple teeth, inflammation and swelling of the gums, and abnormal dentinal pulp calcinations need advanced complex treatments including surgery, orthodontics, and the use of prosthetics [10]. So the early diagnosis of AI is an important factor for the successful outcome of treatment with other fields of medicine to be involved in order to prevent a diverse dental and systemic complications. Enamel is a barrier against the entry of pathogens into the dental structures. So it is important to produce and maintain healthy enamel. Due to lack of conclusive studies on the prevalence of genetic causations of AI in Iran, we suggested that more awareness of the causes of enamel defects might clinically help Iranian dentists to draw effective ways of preventing and treating AI. So that the purpose of this study is to investigate the presence of mutations in the *FAM83H* gene and the *ENAM* gene which are the well-known genetic causes of AI among 18 Iranian families with a non-syndromic dominant type of AI.

Materials and Methods

This descriptive-case study was conducted on 18 Iranian families with AI who were, referred to Resalat Hospital (Department of Molecular Genetics) and the Faculty of Dentistry of Tehran University of Medical Sciences between March/2016 and March/2017. The inclusion criteria were based on clinical symptoms and radiographic findings defined by the relevant specialists and having at least 1 patient with AI in the family. In meantime declared consents were taken in writing from patient and persons who were candidates for genetic testing within families or their lawful guardians. Exclusion criteria were lack of consent at any time during the study. Then appointed specialists carried out clinical examinations and drawn pedigrees. This was followed by obtaining 10cc blood in tubes containing an anticoagulant, EDTA. DNA was extracted using the QIAamp DNA Blood Mini Kit. In this technique, PK solution and Lysis A buffer were used to extract DNA at the appropriate level of PH and iron salts while Lysis A and ethanol buffers were specifically bonded to the Biospin membrane. Denatured proteins and unwanted materials were removed by re-washing. All encoding genes from the adjacent region of *ENAM* and *FAM83H* intranets were amplified by polymerase chain reaction (PCR). Then Pouya Gostar Gene Company carried out gene sequencing for detection of genetic alterations. Designation of specific primers

was done based on the ENAM and FAM83H gene sequences using OLIGO Primer Analysis Software 7. The primers in this study covered for all encoding regions as well as the exon-Intron intersections and short regions of Introns. Chrmas software was used to analyze gene sequences and CLC sequence viewer was used to compare them with reference sequences in the RefSeq database at the NCBI. Finally, the potential nucleotide changes together with mutations and normal variants were used to draw the final report considering clinical manifestations presenting in each family.

Moral considerations

This study was approved by the Ethics Committee of Tehran University of Medical Sciences and is derived (ethics code 8823102037). Also, all participants in this study gave written consent.

Results

The present study enrolled 18 patients Mean age was (14.61±11.21) Years. male and female was 33.3% and 66.7% respectively (Table 1). All patients showed a mutation in the exon 5 of FAM83H gene in nucleotide rs56148058C/T which converted Serotonin to Aspartin. In two patients carried a mutation in the nucleotide rs546809055A/G that changed Leucine to Phenylalanine. None of patients showed significant alteration in the ENAM gene.

Molecular analysis of genetic changes in all patients showed that exon 5 of the FAM83H gene changed for nucleotide rs56148058C/T that means a serotonin was converted into aspartin, and/or the nucleotide rs546809055A/G altered causing a conversion of leucine into phenylalanine. Also in 2 patients [1,11], in addition to the above-mentioned mutations, another genetic alteration has also been observed. There was no genetic mutation in the ENAM gene in none of the patients (Fig 1).

Discussion

In this study, all patients showed mutations in the exon 5 of FAM83H gene that affected the nucleotide rs56148058C/T which changed a serotonin for Aspartatin. As previously found in other studies, in our patients the two mutations p.Arg325 and p.Gln398 in FAM83H gene also resulted in hypocalcification in enamel with an autosomal dominant inheritance pattern [25]. So far, twenty different mutations have been identified in the FAM83H gene that sometimes, like that observed in the present study, more than one mutation was discovered in a gene. In this study, all muta-

tions were only detected in the FAM83H gene in exon 5 that other studies reportedly found [13,14,15,16]. FAM83H gene sequencing in a study by Urzua et al. (2015) showed that only one member in three family families presented with a mutation in the exon 5 and one mutation was identified in the p.Gly557Cys region in a patient with a hypocalcified type of AI. In the other two families, despite the presence of phenotypic characteristics of AI hypocalcification but no mutation had been found in FAM83H gene [17]. Pourhashemi and his colleagues (2014) have shown that all five Iranian families with an autosomal dominant inheritance pattern of the disorder had the FAM83H mutation in exon 5. Also, they found a mutation in a codon 342 and nucleotides C.1150 T/A and P. ser342/T in the FAM83H gene [8]. The results from a study carried out by Haubek et al. (2011) showed that five families with autosomal dominant pattern and Hypocalcified type of AI carried a mutation in the exon 5 of FAM83H gene (8q24.3), which was in the nucleotide p. Y302X [19].

We believe that our results are consistent with the findings of above-mentioned studies in terms of inheritance pattern, the presence of mutations in the exon 5 of the gene FAM83H and also show similar c changes in a nucleotide that encode the amino acids involving etching of the teeth. The FAM83H gene produces 1,199 amino acids, and when mutations occur in this gene, the number of amino acids produced by this gene decreased to 286-693 amino acids. Many genetic studies have previously shown that if FAM83H loose less than half of its amino acids production then an autosomal dominant malformation is anticipated [20]. However this study was unable to detect any mutation in ENAM gene, a previous investigation showed that mutations in ENAM gene might result in insufficient production of a protein in the ameloblast cell that caused defects in enamel disorders with autosomal dominant pattern [9]. A study by Wang et al. (2015) indicated that mutations in the ENAM gene in the entire patient from three families presented non-systemic manifestation [19]. Additionally, Chan et al. (2012) showed that there were four mutations in the ENAM gene among eighteen families with a predominant autosomal pattern (an incidence of 67%) suggesting such gene mutations might be associated with AI in about 50% of cases in the affected families [21]. However, this study was unable to find any genetic alteration in gene ENAM from different 18 families with various inheritance pattern. One of the reasons that families from this study showed no a mutation in ENAM gene could be due to that all study samples were from families with an au-

tosomal dominant type of AI.. while disorders caused by mutation in ENAM gene more likely result in autosomal recessive inheritance pattern [21]. Comparison between the results from other studies and ours suggest that mutations in ENAM gene must be investigated in Iranian patients from a diversity of ethnic groups [18]. In general, there has been no explanation to justify why

no mutation had been found in ENAM gene in this while relevant clinical phenotypes were observed in some probands. Like other studies, we also believe that more genetic researches are required to mine this area for understanding the dynamic nature and variability of enamel formation in relation with involving genes such as FAM83H and ENAM [22].

Species group name	Sex	Age (year)	Parent family	Delayed in tooth eruption	Abnormal color	The size of the teeth is smaller than normal	Clinical manifestation	Diagnosis
1	M	9	+	+	+	-	anterior open bite-very thin tooth enamel	Hypoplastic amelogenesis imperfect
2	M	8	-	+	-	+	Mother has brownish teeth	Hypoplastic amelogenesis imperfect
3	F	11	+	+	-	+	enamel opacity is similar to dentin	Hypoplastic -hypomaturation amelogenesis imperfecta
4	M	10	-	-	+	-	The enamel thickness is half the normal state-feeling pain when using cold and hot food	Hypoplastic amelogenesis imperfect
5	M	11	+	+	-	+	There is a diastem between teeth	Hypoplastic amelogenesis imperfect
6	F	40	+	-	+	+	feeling pain when using cold and hot food-tmj with click	Hypoplastic amelogenesis imperfecta
7	F	17	-	+	+	+	The height of the clinical crown is less than normal	Hypoplastic amelogenesis imperfecta
8	F	7	+	-	+	+	Very thin enamel	Hypoplastic amelogenesis imperfecta
9	F	9	+	-	-	-	Very thin enamel-conical teeth	Hypoplastic amelogenesis imperfecta
10	F	10	+	+	+	+	Deep bite	Hypoplastic amelogenesis imperfecta
11	M	49	-	-	+	+	There is a diastem between teeth	Hypomaturation amelogenesis imperfecta
12	F	10	+	+	+	+		Hypoplastic -hypomaturation amelogenesis imperfecta
13	F	12	-	-	+	-	Very thin enamel	Hypoplastic hypomaturation amelogenesis imperfect with taurodontia
14	F	14	-	-	+	+	The height of the clinical crown is less than normal	Hypoplastic -hypomaturation amelogenesis imperfecta
15	F	12	+	-	+	-	Scalloped enamel	Hypomaturation amelogenesis imperfecta
16	M	11	+	-	-	+	Teeth with attrition	Hypoplastic amelogenesis imperfecta
17	F	13	-	+	-	-	Teeth with attrition	Hypoplastic -hypomaturation amelogenesis imperfecta
18	F	10	+	-	+	-	Very thin enamel	Hypoplastic -hypomaturation amelogenesis imperfecta

Table 1. Demographic of patients.

Species	Group Name	*****
1.	1-FAI	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
2.	9-FAI	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
3.	17-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
4.	2-FAI	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
5.	10-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
6.	18-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
7.	3-FAI	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
8.	11-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
9.	4-FAI	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
10.	12-	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
11.	5-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
12.	13-	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
13.	6-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
14.	14-	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
15.	7-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
16.	15-	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
17.	8-F	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG
18.	16-	CAAGCCCGCCCGCCAGGCCGGGGGAAAGCGGGCCCAAGGCCCGGGGGGAAACCCCGACMCCAGAGCG

Fig 1. CLC program for FAM83H gene.

Conclusion

We managed to find FAM83H gene mutations in all patients with AI condition in current study which was not in full consistency with the results of other studies which found a disease-causing role for other genes such as ENAM, so we believe further investigations are needed to decipher Iranian version of pathogenic mutations in FAM83H and other AI causing genes using different ethnicity from across Iran. No mutations have been found in ENAM gene in this study prompting us to believe that whether Iranian AI families are less carrying ENAM gene as a genetic the culprit in causation of AI manifestation or this study failed to cover sufficient samples from different ethnicities across Iran.

Conflict of Interest

There is no conflict of interest to declare.

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Please cite this paper as:

Najafi Sh, Roudgari H, Palizgir R, Naderifard M, Meighani ME; Determination of mutation in the coding regions of FAM83H and ENAM genes in patients with imperfect enamel (Amelogenesis Imperfecta). J Craniomaxillofac Res 2020; 7(3): 126-131