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Novel *FAM83H* mutations in Turkish families with autosomal dominant hypocalcified amelogenesis imperfecta

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To the Editor: Amelogenesis imperfecta (AI) are a heterogeneous group of disorders that perturb enamel development. Historically, 14 subtypes have been recognized based on clinical phenotype and mode of inheritance (1). To date, mutations in *AMELX*, *ENAM*, *KLK4*, and *MMP20* have been documented in hypoplastic and hypomaturation AI (2–6). However, the genetic basis for most cases of autosomal dominant AI in the Turkish population remained unknown. Recently, mutations underlying a hypocalcified form of AI were reported (7, 8). In the present study, we evaluated eight Turkish kindreds with autosomal dominant hypocalcified AI (ADHCAI). Genetic linkage studies were consistent with linkage to chromosome 8q24.3 in seven families. Sequence analysis identified *FAM83H* nonsense mutations in all eight families.

Eight probands were identified from Dental School Clinics at Ege University, the University of Istanbul and Yeditepe University, Istanbul, Turkey, in accordance with Institutional Review Board approval from the corresponding University and the National Institutes of Health. Available family members received an oral examination and dental radiographs to determine affection status. The presence of AI was established by generalized yellow–brown discoloration of the teeth, hypoplastic appearing enamel and pathological loss of enamel. Affected individuals were diagnosed with ADHCAI based on previously proposed criteria (1) (Fig. 1). Seven kindreds were consistent for autosomal dominant transmission of AI. In an eighth family, only the proband was affected, but the phenotype was consistent with hypocalcified AI and the known AI genes *AMELX*, *ENAM*, *KLK4*, and *MMP20* had been excluded based on sequence analysis. A total of 50 individuals were clinically examined,

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including 26 affected individuals. DNA extraction from peripheral venous blood was performed as previously described (6). DNA extraction from saliva was performed according to the Oragene protocol (DNA Genotek Inc., Ottawa, Ontario, Canada).

Primers and conditions used to amplify the *FAM83H* gene are shown in Table 1. The large approximately 4.8 kb exon 5 was split into eight overlapping amplicons. Polymerase chain reaction (PCR) products were purified using Exo-Sap and sequenced in both directions to minimize sequencing artifacts using ABI Big Dye Terminator chemistry, version 3.1, and an ABI 3730 (Applied Biosystems, Foster City, CA).

Six short tandem repeat polymorphism (STRP) markers (D8S1836, D8S15018mg, D8S373, D8S2334, D8S1925 and D8S1926) spanning a 2.35-Mb interval that includes the *FAM83H* locus were genotyped. Markers were amplified from genomic DNA using fluorescently labeled primers and AmpliTaq Gold PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol. PCR products were electrophoresed using an ABI 3130 DNA analyzer with size standard GeneScan 400HD Rox. Data were analyzed with GeneMapper ID software, v3.7. Haplotypes were generated based on allele transmission, incorporating sequence variants identified by sequencing.

Genotyping of STRP loci surrounding the *FAM83H* locus in families 1–7 were consistent with genetic linkage to this interval of chromosome eight. As we were unable to amplify the large exon 5 using the published primers (7), primers were redesigned as shown in Table 1 and required a variety of conditions to successfully amplify this region. Three mutations were identified (Fig. 1, Table 2). Affected individuals from family 1 were found to have a C to T substitution at nucleotide position 1192 of the complementary DNA reference sequence (Gen-Bank accession number: NM_198488.3), a mutation previously identified in a Korean family (7). A novel c.1366C>T mutation was identified in family 2. This substitution is predicted to be a nonsense mutation, p.Q444X (Fig. 1b). Affected individuals from the remaining six families all had a novel nonsense mutation, p.Q456X, due to a c.1366C>T mutation (Fig. 1c). The finding of a common c.1366C>T mutation in six families (families 3–8) raised the possibility of a mutational hot spot or a founder effect. Based on haplotype analysis, the c.1366C>T mutation appears to have been inherited 'identical by descent' from a common ancestor in five of these families with a shared haplotype extending over an interval of 1.8 Mb spanning the *FAM83H* locus (Fig. 1d). In family 8, the c.1366C>T mutation appears on a different haplotype background. Additionally, the proband was the only affected family member. Mutational analysis confirmed neither parent has a *FAM83H* mutation. Thus, consistent with the haplotype analysis, this mutation appears to have arisen *de novo*, but germline mosaicism in one of the parents cannot be excluded.

FAM83H mutations were identified in affected individuals from all eight Turkish families studied. With these results, eight *FAM83H* mutations are now reported (Table 2). Only two mutations, c.1192C>T and c.1366C>T, have been found in more than one kindred. The c. 1192C>T mutation reported previously in a Korean family was identified in one Turkish family (family 1) in the current study (7). Based on haplotype analysis, the c.1366C>T mutation identified in six Turkish families reported here appears to have occurred as a founder mutation in five Turkish families (families 3–7) and as an apparent *de novo*

mutation in another Turkish family (family 8, Fig. 1d). Lee et al. (8) also reported a *de novo* p.E415X *FAM83H* mutation. Identification of two *de novo* mutations from a total of eight mutations suggests that the *FAM83H* gene may have a high mutation rate, which is surprising given that the gene is relatively small (98,126 bp).

Consistent with previous findings, all mutations found in these Turkish families were nonsense mutations (7, 8). The finding of only nonsense mutations raises the question of whether the underlying molecular mechanism is haploinsufficiency or a dominant negative effect. Although all reported mutations are in the terminal exon and the corresponding messenger RNAs might be expected to escape nonsense-mediated decay (NMD), there are examples in humans of terminal exon nonsense mutations triggering NMD, notably in *COL10A1* (9). If mutant transcripts undergo NMD then haploinsufficiency of *FAM83H* leads to ADHCAI. If the transcripts escape NMD then the truncated products may interfere with the normal product and produce a phenotype through a dominant negative effect. The finding that all mutations are nonsense mutations that cluster within a 380 amino acid region is more supportive of a dominant negative effect. As mutations are identified in more families, the underlying molecular mechanism may become clearer.

FAM83H represents the first gene widely expressed outside of the developing tooth to be associated with AI. Kim et al. (7) demonstrated expression in mouse eye, liver and kidney. We verified expression in human kidney, liver and gingiva (data not shown). The phenotype observed in association with *FAM83H* mutations indicates that FAM83H functions in mineralization, but its expression in non-mineralized tissues suggests that either it does not directly function in mineralization or it has another function in non-mineralized tissues. Enamel matrix proteins are almost completely removed as enamel crystallites grow and mineralization progresses (10). In both hypomaturation and hypocalcified forms of AI, significant amounts of protein are detected in fully developed enamel (11). It is not surprising, therefore, that mutation in the known enamel proteases, kallikrein 4 and matrix metalloproteinase 20, result in hypomaturation phenotypes (4–6). *MMP20* and *KLK4* mutations result in autosomal recessive phenotypes, suggesting that haploinsufficiency of these proteinases is sufficient for normal enamel development. Analysis of the predicted protein sequence of FAM83H does not identify obvious homology outside of the FAM83 family. Given that *FAM83H* mutations cluster within a 380 amino acid region, this region appears to be critical for normal enamel development and calcification. *FAM83H* mutations were identified in all eight of the Turkish families segregating ADHCAI, suggesting that *FAM83H* mutations may be responsible for the majority of ADHCAI in Turkey. The generality of the founder effect in the Turkish population remains to be seen.

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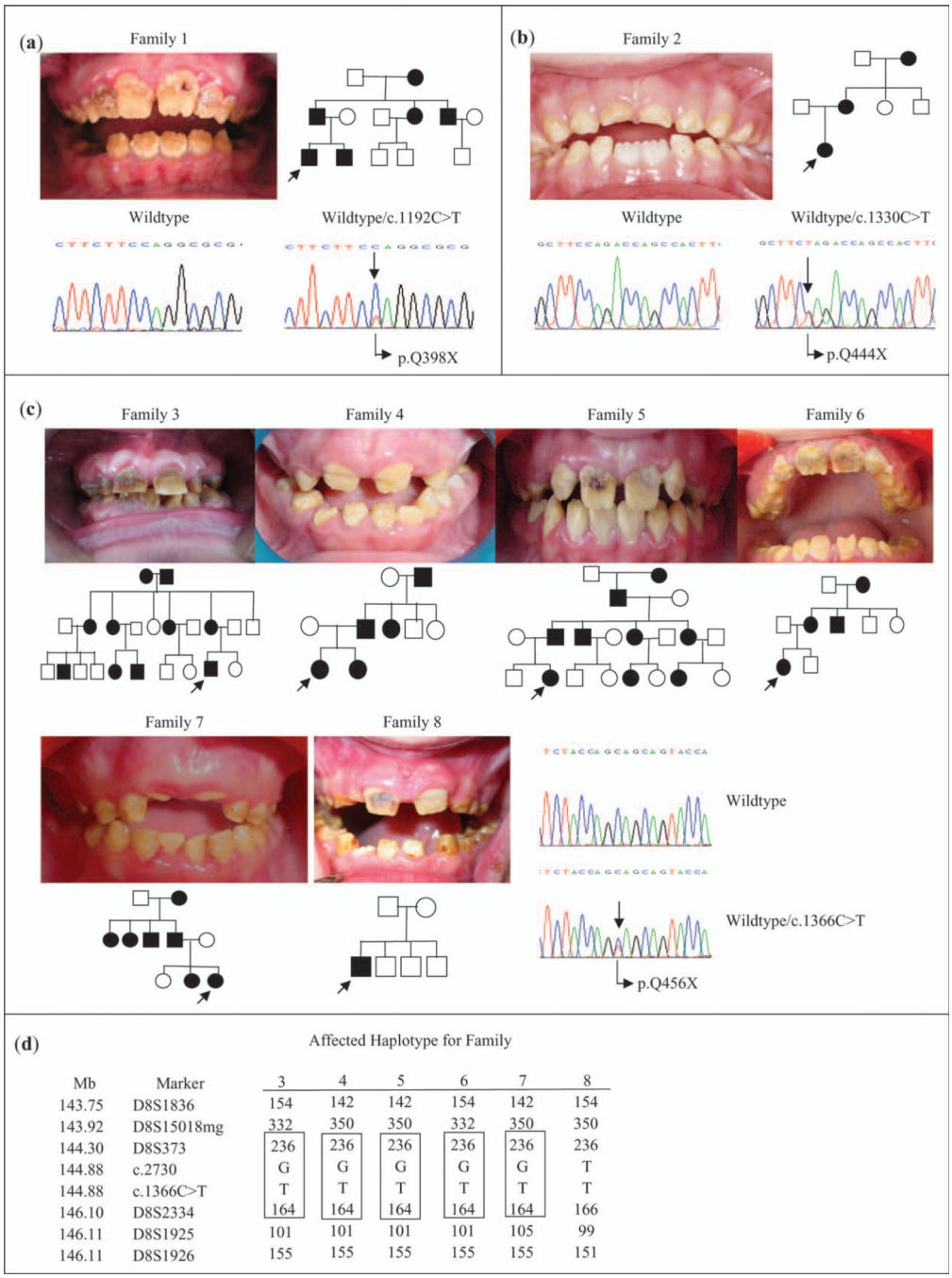

Mb	Marker	3	4	5	6	7	8
143.75	D8S1836	154	142	142	154	142	154
143.92	D8S15018mg	332	350	350	332	350	350
144.30	D8S373	236	236	236	236	236	236
144.88	c.2730	G	G	G	G	G	T
144.88	c.1366C>T	T	T	T	T	T	T
146.10	D8S2334	164	164	164	164	164	166
146.11	D8S1925	101	101	101	101	105	99
146.11	D8S1926	155	155	155	155	155	151

Fig. 1.

Pedigrees, clinical photographs, mutations and haplotype analysis for Turkish families segregating autosomal dominant hypocalcified amelogenesis imperfecta. (**a**) Family 1 segregating a previously identified mutation, c.1192C>T (p.Q398X). (**b**) Family 2 segregating a novel mutation, c.1330C>T (p.Q444X). (**c**) Families 3–8 segregating a novel mutation, c.1366C>T (p.Q456X). (**d**) Haplotype analysis of families 3–8.

Table 1

Primers and conditions to amplify *FAM83H*

Region	Sequence 5′–3′	Size (bp)	Annealing temperature	Conditions
Promoter	F:CGTCTGGTATGAGGCTTGGTGTG; R: TCCGGCTCAGTCGCTAGGAAAC	696	60	*a*
Exon 1	From Kim et al. (1F/1R)	240	60	*a*
Exon 2	From Kim et al. (2F/2R)	617	60	*a*
Exons 3 and 4	From Kim et al. (3F/3R)	527	60	*a*
Exon 5, set 1	F: CGGGTCCCACTGGTACTGCT; R: ACCTCACATCCCTGCGTCCTC	748	57	*b*
Exon 5, set 2	F: GTGTCGCGGCAGACGTTCCTC; R: GAGCGGAATGAGTCCTGCTTGG	725	57	*b*
Exon 5, set 3	From Kim et al. (7F/7R)	610	60	*a*
Exon 5, set 4	F: GCCTGCCTTCCCGCTTCC; R: CTAGCTCGGGGCTGTTGTGGG	726	64	*b*
Exon 5, set 5	F: GCCAACGCCTTGTACAGCAGC; R:TGCTGAGGGGAGAAAGGCACTG	709	64	*b*
Exon 5, set 6	F: GAAGGGCCCAGAGAATGAGG; R: TCGCTGCCCCGGTCATC	634	62	*b*
Exon 5, set 7	F:ATGAATTTCACGAGGCTATCATCT; R: TTGGATTCTCCTGCTCGCT	714	57	*a*
Exon 5, set 8	F: GCCCCATTCTCAGGTCCTTTCC; R: TCACAGGGGAAATCTGGGAGG	734	64	*b*

a, accuprime + enhancer;

b, sigma.

Table 2

Mutations in *FAM83H*

Complementary DNA	Protein	Exon	Ethnicity	Reference
c.891T>A	p.Y297X	5	Korean	(8)
c.973C>T	p.R325X	5	Korean	(7)
c.1192C>T	p.Q398X	5	Korean, Turkish	(7, this study)
c.1243G>T	p.E415X	5	Hispanic	(8)
c.1330C>T	p.Q444X	5	Turkish	This study
c.1366C>T	p.Q456X	5	Turkish	This study
c.1380G>A	p.W460X	5	Caucasian	(8)
c.2029C>T	p.Q677X	5	Caucasian	(8)