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Exclusion of Candidate Genes in Seven Turkish Families With Autosomal Recessive Amelogenesis Imperfecta

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Abstract

Amelogenesis imperfectas (AI) are a group of inherited defects of dental enamel formation that show both clinical and genetic heterogeneity. Seven Turkish families segregating autosomal recessive AI (ARAI) were evaluated for evidence of a genetic etiology of AI for the seven major candidate gene loci (*AMBN*, *AMELX*, *ENAM*, *FAM83H*, *KLK4*, *MMP20*, and *TUFT1*). Dental and periodontal characteristics of the affected members of these families were also described. The mean scores of DMFS and dfs indices were 9.7 and 9.6, respectively. The mean PPD was 2.2mm and the percentage of the sites with plaque and BOP were 87.8% and 72.4%, respectively. The exons and intron/exon junctions of the candidate genes were sequenced and no gene mutations were identified in any individuals. These findings support the existence of an additional gene(s) that are etiologic for ARAI in these families.

Keywords

autosomal recessive amelogenesis imperfecta; *AMBN*; *AMELX*; *ENAM*; *FAM83H*; *KLK4*; *MMP20*; *TUFT1*

INTRODUCTION

The Amelogenesis imperfectas (AIs) are a group of clinically and genetically heterogeneous disorders that affect enamel development resulting in abnormalities of amount, composition, and/or structure of enamel in the absence of any generalized or systemic diseases [Bailleul-Forestier et al., 2008]. Although the ideal method of classifying AI is not yet established, four main clinical types of AI have been described: hypoplastic, hypocalcified, hypomaturational, and hypomaturational–hypoplastic with taurodontism. AI is classified into 14 distinct subtypes based on the clinical phenotype and mode of inheritance [Witkop, 1988].

Familial segregation patterns for AI include autosomal dominant, autosomal recessive, X-linked modes of transmission.

Amelogenin, enamelin, ameloblastin, and tuftelin are the main structural proteins involved in enamel formation. Mutations in the genes coding for the amelogenin and enamelin proteins are now known to be associated with different types of AI. Most cases of X-linked AI result from mutations in the *AMELX* gene which codes for amelogenin [Lagerström et al., 1991; Hart et al., 2002a,b; Kim et al., 2004]. Enamelin (*ENAM*) gene mutations have been identified in both autosomal dominant [Rajpar et al., 2001; Kida et al., 2002; Hart et al., 2003a] and recessive [Hart et al., 2003b] forms of AI. Transgenic mice overexpressing the ameloblastin (*AMBN*) gene develop AI [Paine et al., 2003].

Mutations in genes encoding the predominant enamel proteinases; kallikrein 4 (*KLK4*) and matrix metalloproteinase 20 (*MMP20*) are also etiologic for AI. The first mutation in the *KLK4* gene has been identified as being associated with autosomal recessive hypomaturation AI [Hart et al., 2004]. Mutations in the *MMP-20* gene have also been described as being associated with autosomal recessive pigmented hypomaturation AI [Kim et al., 2005; Ozdemir et al., 2005a; Papagerakis et al., 2008]. Recently mutations in the *FAM83H* gene have been identified with autosomal dominant hypoplastic forms of AI in Korea and in Turkey [Hart et al., 2009; Kim et al., 2008; Lee et al., 2008].

The prevalence of AI is reported as 1/700 in northern Sweden [Bäckman and Holm, 1986], 1/14,000 in the USA [Witkop and Sauk, 1976], and 1/8,000 in Israel [Chosack et al., 1979]. Autosomal recessive forms of AI are the most prevalent in the Middle East and some parts of Asia, while the autosomal dominant forms of AI are the most prevalent in the United States and Europe [Witkop and Sauk, 1976; Chosack et al., 1979].

Dental anomalies associated with AI include; quantitative and qualitative enamel deficiencies, pulpal calcification, tooth sensitivity, poor dental esthetics, decreased occlusal vertical dimension, multiple impacted teeth, delayed eruption, congenitally missing teeth, hypercementosis, root malformation, taurodontism, gingivitis, and periodontitis. Patients often have anterior open bites or vertical deep bites which further complicate the restorative treatment considerations [Hart et al., 2003b; Ravassipour et al., 2005; Poulsen et al., 2008].

The aim of this study was to evaluate evidence for a genetic etiology for the seven major candidate gene loci (*AMBN*, *AMELX*, *ENAM*, *FAM83H*, *KLK4*, *MMP20*, and *TUFT1*) in seven Turkish families segregating autosomal recessive AI (ARAI) and describe the dental and periodontal characteristics of these patients.

MATERIALS AND METHODS

Study Population and Clinical Recordings

The study protocol was approved by the Ethics Committee of the Ege University School of Medicine. All consecutive subjects were recruited from the Ege University, School of Dentistry. Prior to participation, the purpose and procedures were fully explained to all

patients and all participants and/or parents gave written informed consent in accordance with Helsinki declaration.

Seven families segregating ARAI were identified and clinical and radiographical examinations were performed for all available family members. The presence of AI was established by generalized yellow-brown discoloration of the teeth, radiographic evidence of decreased enamel mineralization, and pathological loss of enamel. Using modifications of clinical criteria previously proposed by Witkop [1988], affected individuals were classified into one of four groups: hypoplastic, hypomaturational, hypocalcified, and hypomaturational/hypoplasia with taurodontism. In addition to oral examinations, complete medical histories were taken to identify any additional clinical findings that would be consistent with syndromic conditions. Individuals with syndromic presentations of AI were excluded from the analyses. The presence of nephrocalcinosis was evaluated with renal ultrasound for all probands. Cephalometric and panoramic radiographs and photographic records were obtained to evaluate the presence of skeletal open-bite malocclusion in all affected individuals.

In the intraoral examination, decay index (DMFS/dfs) (WHO) was performed and clinical symptoms including; congenitally missing teeth, anterior and posterior open-bite and cross-bite, pulpal calcification, dentin dysplasia, root anomalies, taurodontism, and dental malocclusion were recorded for all affected individuals. The patients were asked if they experience dentinal hypersensitivity and their answers were recorded. The following periodontal clinical parameters were also recorded at six sites on each tooth: probing pocket depth (PPD; mm), dichotomous plaque index (PI; %), bleeding on probing (BOP), and width of the keratinized tissue (KT; mm). All measurements were performed by a single blinded and calibrated examiner using a manual William's periodontal probe.

Molecular Genetic Analysis

Genomic DNA was extracted from either peripheral venous blood samples or saliva using the QIAamp blood kit (Qiagen, Santa Clara, CA) or Oragene DNA self-collection kit (DNA Genotek, Ottawa, Canada), respectively. The oligonucleotide primers and the conditions used to amplify and sequence all exons, including intron/exon splice sites for *AMBN*, *AMELX*, *ENAM*, *FAM83H*, *KLK4*, *MMP20*, and *TUFT1* were described previously [Hart et al., 2003a; Hart et al., 2004, 2009; Kim et al., 2005, 2006; Santos et al., 2007]. The PCR products were electrophoresed through 1% agarose gels and the amplicons extracted using GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Piscataway, NJ). Extracted amplicons were sequenced using the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3130 DNA Sequencer™. PCR products were sequenced in both directions to minimize sequencing artifacts. Sequence data were compared with NCBI reference sequences using the Sequencer program (Gene Codes Corporation, Ann Arbor, MI).

RESULTS

Examinations of available members from all families studied indicated 10 of the 27 family members evaluated were affected with ARAI. Affected individuals showed no signs of

syndromic conditions or systemic illnesses associated with defective enamel development. None of the unaffected family members had generalized enamel defects clinically and they showed no evidence of radiographic enamel defects, taurodontism or other dental abnormalities.

The mean age (years) of the ARAI patients were 10.8 ± 4.48 (mean $\pm$ standard deviation). The age and gender distribution of the patients are shown in Table I. Six of the ARAI patients diagnosed as type I AI, three as type II AI, and one as type III AI. Parents of six of the seven families were consanguineous, and parents in only one family (family 5) were not consanguineous. Because of the association of AI with nephrocalcinosis [Lubinsky et al., 1985; Hall et al., 1995], affected individuals were evaluated with renal ultrasound to indicate the presence of nephrocalcinosis. Nephrocalcinosis was identified by renal ultrasound in only one patient (1a). All primary and permanent teeth had generalized yellow-brown discoloration and evidence of decreased enamel mineralization and pathological loss of enamel were seen radiographically in patients who were in the mixed dentition. Seven of the ARAI patients had delayed dental eruption (1a, 1d, 3a, 4a, 5a, 6a, and 7a), and one patient (3a) had congenitally missing teeth (maxillary and mandibular second premolars). Taurodontism was present in one patient (7a). Seven of the ARAI patients (1a, 2a, 2c, 3a, 4a, 5a, and 7a) reported dental hypersensitivity with thermal or chemical stimulation. Anterior open-bite was present in five patients (2a, 2c, 3a, 5a, and 7a) while none of the patients showed posterior open-bite. Three of the patients (1a, 3a, and 7a) had anterior cross bite and five of them (1a, 2c, 3a, 5a, and 7a) had posterior cross bite. Malocclusion was seen in six patients (1a, 2a, 2c, 3a, 4a, and 5e) (Table I). Pulpal calcification, dentinal dysplasia, and root anomalies were not detected in any of the AI patients (Figs. 1–8).

The mean DMFS/dfs indices were 9.7/9.6. The mean PPD and width of keratinized tissue were measured as 2.2 and 6 mm, respectively. The percentage of sites with plaque was 87.8%, while 72.4% of the sites had BOP (Table II).

The exons and intron/exon junctions of the *AMBN*, *AMELX*, *ENAM*, *FAM83H*, *KLK4*, *MMP20*, and *TUFT1* genes were sequenced and no genemutations were identified in any individuals. Autozygosity analysis for SNP and STRP genotyping of the candidate genes did not indicate homozygosity at any of the gene loci tested that would support an etiologic AI gene.

DISCUSSION

The AI are a clinically heterogeneous group of inherited defects in enamel formation. The malformed enamel can be abnormally thin, soft, rough, and discolored. The condition is genetically heterogeneous, resulting in difficulty classifying specific forms of the condition, and making diagnosis of individual cases problematic. Studying rare genetic diseases is important, as it can contribute important information with significant implications for dentistry and medicine. Identification of the genetic basis of a disorder often provides a first step for genetic testing, understanding the disease pathology and the development of etiology based treatments.

Mutations in five genes (*AMELX*, *ENAM*, *FAM83H*, *KLK4*, and *MMP20*) have been found to be etiologic for AI in humans. Of these, three (*ENAM*, *KLK4*, and *MMP20*) can cause ARAI [Hart et al., 2003b; Hu and Yamakoshi, 2003; Hart et al., 2004; Kim et al., 2005; Ozdemir et al., 2005a; Papagerakis et al., 2008]. Of these three genes etiologic for ARAI, mutations of *ENAM* are the most frequent, although the genetic basis for most forms of ARAI in the Turkish population remains unknown. Mutations of the *ENAM* and *FAM83H* genes are also etiologic for autosomal dominant AI, with the majority due to mutations of the *FAM83H* gene, whose function in enamel development is unknown [Kim et al., 2008; Lee et al., 2008].

In contrast to Europe and North America, where autosomal dominant forms of AI are the most common, ARAI are the most prevalent forms in Turkey, probably due to the higher rates of parental consanguinity [Chosack et al., 1979; Sundell and Koch, 1985; Bsoul et al., 2004]. In the present study, among seven families with ARAI, consanguinity was present in six families. While autosomal dominant forms of AI are present in Turkey, they are not as frequent as ARAI forms. Additionally, autosomal dominant AI in Turkey appears primarily due to *FAM83H* mutations [Hart et al., 2009], with a far smaller number of cases due to *ENAM* mutations [Hart et al., 2003a; Kim et al., 2005b; Ozdemir et al., 2005b]. To date only four mutations for *KLK4* and *MMP20* have been identified in humans and none of these has occurred in the Turkish population [Hart et al., 2004; Kim et al., 2005; Ozdemir et al., 2005a; Papagerakis et al., 2008]. *ENAM* mutations have been identified in a relatively small number of autosomal recessive hypoplastic AI patients; with all reports to date from the Turkish population [Hart et al., 2003b; Ozdemir et al., 2005b]. Findings in the present study for seven Turkish families segregating ARAI, indicate that no gene mutations were identified in coding regions and intron/exon boundaries in genes coding for the enamel matrix proteins (*AMBN*, *AMELX*, *ENAM*, and *TUFT1*), in genes coding for enamel proteinases (*KLK4* and *MMP20*) or in the *FAM83H* gene. Although exonic and splice site mutations were not identified, it is possible that other types of mutations such as intronic or regulatory region mutations may be etiologic. However, data from available SNP polymorphisms and genotyped short tandem repeat polymorphic (STRP) markers spanning the candidate genes do not indicate that the genetic interval spanning these candidate genes were homozygous in AI affected individuals in the six consanguineous families. These findings suggest that the candidate genes evaluated are not etiologic for ARAI in these families, and support the existence of an additional gene(s) that are etiologic for ARAI in these families. These findings support a greater genetic heterogeneity for ARAI.

A rare syndrome associating AI with nephrocalcinosis precipitation of calcium salts in the kidney has been reported [Lubinsky et al., 1985; Hall et al., 1995]. In the present study nephrocalcinosis was identified in one patient. Interestingly, an AI affected sibling from the same family did not show evidence of nephrocalcinosis by renal ultrasound, possibly due to the young age of the child (3 years). Whether this family segregates a form of AI that is etiologically distinct from the other cases studied remains to be seen.

Dental hypersensitivity is frequently reported by AI patients as a symptom that severely influences the patients' daily lives [Poulsen et al., 2008]. In the present study most of the patients reported complaints about dental hypersensitivity with thermal or chemical

stimulation. Dental hypersensitivity severely affects the oral health related quality of life of the AI patients and also makes applying oral hygiene regimens difficult for the patients. We therefore evaluated the periodontal status of these AI patients. The plaque indices and dental hypersensitivity were considerably higher in the AI affected patients, possibly reflecting the presence of morphological abnormalities of the enamel which may complicate the removal of dental plaque. As a result of poor oral hygiene the percentage of sites with BOP was also high in the present study (PI: $87.8 \pm 20.9\%$; BOP: $72.4 \pm 22.7\%$). Periodontal problems ranging from gingival enlargement and gingivitis to periodontitis, often combined with poor oral hygiene have been reported in AI patients [Bouvier et al., 1999; Toksavul et al., 2004; Türkün, 2005; Poulsen et al., 2008]. In spite of the apparently poor oral hygiene observed in the AI patients, we did not see evidence of increased periodontitis.

Recently, Poulsen et al. [2008] reported that taurodontism was the most frequent dental anomaly reported in AI patients. In the current study taurodontism was seen in only one ARAI patient. An anterior open-bite malocclusion, generally considered to be of skeletal origin, is a frequent associated finding in AI [Rowley et al., 1982; Hoppenreijns et al., 1998; Cartwright et al., 1999; Ravassipour et al., 2005]. Open-bite malocclusion has been reported in the Turkish population associated with ARAI due to *ENAM* mutations [Hart et al., 2003b; Ozdemir et al., 2005b]. In the present study anterior open-bite was seen in the five of the ten ARAI patients. Alterations in the eruption process of the permanent teeth reported in AI include delayed eruption, retention, or impaction of teeth and the presence of follicular cysts [Poulsen et al., 2008].

In summary, results of the current study indicate that an additional gene(s) is/are responsible for a significant number of cases of ARAI in the Turkish population. While the PI and BOP scores indicate that oral hygiene appears to be suboptimal in these AI affected individuals, we did not see evidence of destructive periodontitis. In this population, anterior open-bite is frequently present and dentinal sensitivity is a significant problem.

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FIG. 1.
Pedigree of the 6th family.

FIG. 2.
Intraoral appearance of 6a demonstrating generalized yellow-brown discoloration of the dentition.

FIG. 3.
Panaromic radiograph of 6a, clinically evident anterior open-bite is seen.

FIG. 4.
Lateral cephalograph of 6a.

FIG. 6.
Intraoral appearance of 7a demonstrating anterior open-bite.

FIG. 7.
Panaromic radiograph of 7a.

FIG. 8.
Lateral cephalograph of 7a.

TABLE I

The Demographic Characteristics and the Intraoral Findings of the ARAI Patients

Patient no.	Gender	Age	Type of AAI	Consanguinity	Tooth sensitivity	Delayed eruption	Anterior open-bite	Anterior cross-bite	Posterior cross-bite	Malocclusion	Congenital missing teeth	Taurodontism	Nephrocalcinosis
1a	Male	7	Type I	+	+	+		+	+	+			+
1d	Male	3	Type I	+		+							-
2a	Female	10	Type I	+	+		+			+			-
2c	Female	9	Type II	+	+		+		+	+			-
3a	Male	18	Type I	+	+	+	+	+	+	+	^a		-
4a	Male	12	Type II	+	+	+				+			-
5a	Male	7	Type I		+	+	+		+				-
5e	Male	18	Type I							+			-
6a	Female	12	Type III	+		+							-
7a	Male	12	Type II	+	+	+	+	+	+			+	-

^aMaxillary and mandibular second premolars.

TABLE II

The Periodontal Parameters and the DMFS/dfs Index of the ARAI Patients

Patient no.	PPD (mm)	PI (%)	BOP (%)	KT (mm)	DMFS	dfs
1a	2	100	100	5.3	8	12
1d					0	0
2a	2.10	100	100	7.4	10	7
2c	1.76	87	100	6.2	12	10
3a	2.51	66	40	5.66	18	0
4a	2.29	37	45	6.63	4	0
5a	2	100	55	4.97	16	0
5e	2.3	100	65	5.9	16	0
6a	2.55	100	83	5.2	5	0
7a	2.34	100	64	6.3	8	0
Mean $\pm$ SD	2.2 $\pm$ 0.2	87.8 $\pm$ 20.9	72.4 $\pm$ 22.7	6 $\pm$ 0.8	9.7	9.6