FAM20A Mutation in a Patient with Enamel-Renal-Gingival Syndrome: A Case Report

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Abstract:

Objectives: Amelogenesis imperfecta with Gingival fibromatosis syndrome (AIGFS), amelogenesis imperfecta with nephrocalcinosis or Enamel-Renal syndrome (ERS), and Enamel-Renal-Gingival syndrome have been associated with mutations in the *FAM20A* gene. A number of cases in the literature have described patients with three important findings, including amelogenesis imperfecta (AI), gingival fibromatosis and nephrocalcinosis. This study was aimed to identify *FAM20A* mutations in an 11-year-old Turkish male affected with enamel-renal-gingival syndrome.

Methods: Clinical and radiographic examinations and mutational analysis of the coding exons of *FAM20A* gene were performed.

Results: The patient was the first child of non-consanguineous parents. Oral examination revealed AI and generalized gingival fibromatosis. A panoramic radiograph showed generalized absence of enamel, delayed eruption of permanent teeth, intrapulpal calcification and multiple unerupted teeth. No calcification was observed with renal ultrasound. Mutation analysis of *FAM20A* revealed a novel missense mutation in exon 10 (NM_017565.3: c.1307G>A; g.61999G>A; p.Gly436Glu). This is notable because G^{436} is highly conserved among *FAM20A* homologues.

Conclusions: Our study reports a novel *FAM20A* mutation and confirms that AIGFS and ERS actually are the same entity with different manifestations. Patients with AI, hypoplastic type with unerupted teeth and gingival fibromatosis are advocated to have renal ultrasonography to rule out nephrocalcinosis or nephrolithiasis.

Keywords: *FAM20A*, Amelogenesis imperfecta, Gingival hyperplasia, Nephrocalcinosis

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Introduction

Amelogenesis imperfecta with gingival fibromatosis syndrome (AIGFS; OMIM #614253) and amelogenesis imperfecta with nephrocalcinosis or enamel-renal syndrome (ERS; OMIM #204690) are rare autosomal recessive disorders which are caused by *FAM20A* mutations [1-7]. *FAM20A* mutations have previously been reported, by O'Sullivan et al, and Cho et al, as resulting in the AIGFS; however, none of the families in their studies were investigated by renal ultrasound [1, 2]. Kantaputra et al state that both represent the same entity as most reported patients with either syndrome sharing clinical findings, including AI, gingival fibromatosis, nephrocalcinosis, multiple unerupted teeth and heterotopic calcification in dental pulps, dental follicles and gingiva [3, 4, 6, 8-12]. In addition, a patient in one of those studies had nephrolithiasis or kidney stone [6]. Therefore, they have suggested the name "Enamel-Renal-Gingival syndrome" [6, 7]. The abnormalities found in patients with Enamel-Renal-Gingival syndrome appear to be associated with biomineralization defects [6, 7]. Here we report an 11-year-old Turkish male affected with AI, gingival fibromatosis, multiple unerupted teeth and intrapulpal calcification. Since the patient had the important features of Enamel-Renal-Gingival syndrome, we conducted a *FAM20A* analysis.

Materials and methods

Informed consent and 2 ml of peripheral EDTA blood samples from the patient and his parents were obtained under protocols approved by the Human Experimentation Committees of the Faculty of Dentistry, Chiang Mai University and Istanbul Medical Faculty, Istanbul University. All 100 unrelated normal controls were healthy Thai volunteers, in the age range of 20-35, with no evidence of *FAM20A* mutation. Genomic DNA was isolated using a Quickgene-610L DNA extracting machine (FUJIFILM, Tokyo, Japan). Eleven exons of *FAM20A* with intronic flanking sequences were amplified by polymerase chain reaction (PCR) using a GENEAMP® PCR Instrument System 9700 (Applied Biosystems, Carlsbad, California, USA) with specific primers and conditions as previously published [6]. Then, 5 μL PCR products were run on 1% agarose gel in TBE buffer using a Sub-cell® GTsystem (Bio-Rad, Hercules, California, USA) under the electrophoresis condition of 120 mA for 20 minutes and visualized by UV illumination from a Gel Doc™ XR+ System (Bio-Rad). Direct sequencing was performed to detect mutations and polymorphisms. Analyses were performed using Sequencher 4.8 Sequence analysis software (Genecodes, Ann Arbor, Michigan, USA).

Results

An 11-year-old male of Turkish origin was the first child of non-consanguineous parents. His younger sister was normal. He came to The Department of Pedodontics, Istanbul Dental Faculty, Istanbul University for dental evaluation. Oral examination revealed AI, hypolplastic type in his primary and permanent dentitions, prolonged retention and infraclusion of primary teeth, and gingival fibromatosis (Fig. 1A-B). A panoramic radiograph showed generalized absence of enamel, delayed eruption of permanent teeth, intrapulpal calcification and multiple unerupted

permanent incisors, premolars and molars surrounded by large dental follicles with sclerotic borders. The unerupted mandibular permanent second molars appeared to lie on the lower border of the mandible (Fig. 1C). No calcification was observed with renal ultrasound. His health was otherwise healthy. Mutation analysis of *FAM20A* revealed a missense mutation in exon 10 (NM_017565.3: c.1307G>A; g.61999G>A; p. Gly436Glu)(Fig. 1D). The glycine (G^{436}) that is replaced by glutamic acid is highly conserved throughout vertebrae evolution (Fig. 1E). This homozygous mutation was not present in the chromosomes of 100 normal controls nor in gene variant public databases. These evidence support the idea that this mutation in our report is likely to be disease-causing.

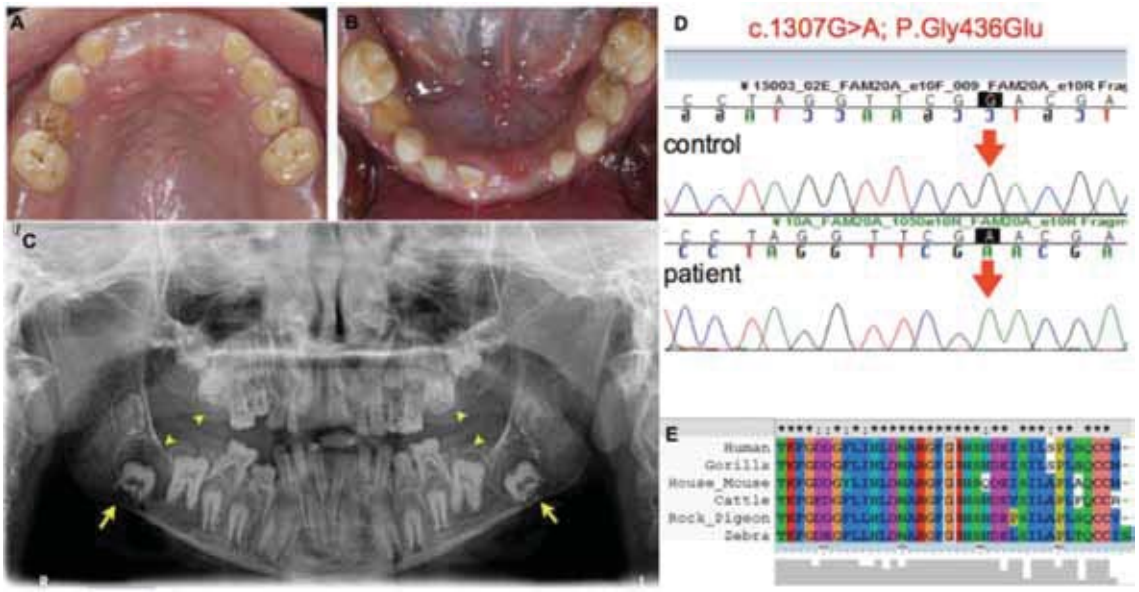

Figure 1. A-B) Oral photographs of the patient at age 11 years. Teeth affected with amelogenesis imperfecta, hypolplastic type, prolonged retention and infraclusion of primary teeth, and gingival fibromatosis. C) Panoramic radiograph shows generalized absence of enamel, delayed eruption of permanent teeth, intrapulpal calcification and multiple unerupted teeth surrounded by large dental follicles with sclerotic borders (arrow heads). The unerupted mandibular permanent second molars appear to lie on the lowerborder of the mandible (arrows). D) Exon 10 sequence revealing a homozygous missense mutation (NM_017565.3: c.1307G>A; g.61999G>A; p.Gly436Glu). E) Alignment of the FAM20A protein sequences from the human (Homo sapiens), gorilla (Gorilla gorilla), house mouse (Mus musculus), cattle (Bos taurus), rock pigeon (Columba livia) and zebra fish (Danio rerio) species using the Clustal W 2.0 multiple sequence alignment program. Stars = these positions contain identical amino acid residues in all sequences. The frame in Fig. 1E shows only the amino acid, glycine(G), in all type of vertebrates but in our patient this was changed to glutamic acid(E).

Discussion

Our study reports a novel *FAM20A* mutation in one patient characterized by AI with delayed and arrested tooth eruption, and gingival fibromatosis. Mutation analysis identified one base substitution from G to A. An amino acid sequence may have changed to glutamic acid instead of glycine at position 436 in the protein. This change may have caused the amino acid group to turn a nonpolar amino acid into a polar amino acid. Then, the change may have affected the structures and functions of *FAM20A* protein. Expression of *FAM20A* has been observed in secretory but not maturation-stage ameloblasts, odontoblasts, and selected cells within the gingival tissue [1, 5]. This might be related to the manifestations of enamel hypoplasia, pulp calcification and gingival hyperplasia in patients with *FAM20A* mutations. Our patient also had failed eruption of some of the permanent teeth and infraclusion of three first primary molars. This might be associated with *FAM20A* expression in the dental follicles just above the molar cusp tips [5]. These findings suggest that *FAM20A* plays a role in tooth eruption and has inhibitory effects on biomineralization. Although our patient shows no sign of nephrocalcinosis, this phenotype has previously been reported in individuals with *FAM20A* mutations and one of the reported patients also had nephrolithiasis [3-6]. According to Wang et al, *FAM20A* localizes in the kidneys, specifically in renal tubules [5]. Vogel et al reported that *FAM20A* null mice exhibited widespread and severe ectopic calcification throughout the body, particulary in the kidney [13]. Nephrocalcinosis is often asymptomatic and the age of onset is unknown [8, 14-16]. For these reasons, we support periodic kidney examination in all patients with *FAM20A* mutation. Further study is needed to determine whether the *FAM20A* mutations increase the patients' risk of having nephrocalcinosis or nephrolithiasis.

Conclusions

Our study reports a novel *FAM20A* mutation and confirms that AIGFS and ERS actually are the same entity with different manifestations. Patients with AI, hypoplastic type with unerupted teeth and gingival fibromatosis are advocated to have renal ultrasonography to rule out nephrocalcinosis or nephrolithiasis.

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