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Title: Enamel renal syndrome: a novel homozygous *FAM20A* founder mutation in 5 new Brazilian families

Running title: *FAM20A* founder mutation in enamel renal syndrome

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Abstract

Enamel renal syndrome (ERS) is a rare autosomal recessive disorder that still not fully characterized. Here we investigated ERS characteristics in 11 patients from 5 Brazilian families through clinical examination, imaging, renal ultrasonography, laboratory tests and DNA sequencing. The patients' age ranged from 6 to 25 years old, and the presence of hypoplastic amelogenesis imperfecta, microdontia, intra-pulpal calcification, impacted posterior teeth with hyperplastic pericoronal follicles, gingival fibromatosis, ectopic calcifications on gingival and pericoronal tissues, and nephrocalcinosis were common findings to all patients. Only 4 patients showed abnormal laboratory tests (vitamin D, parathyroid hormone, phosphate, calcium). Intellectual disability and renal cysts were present in 2 patients each. Biallelic loss of function mutations in *FAM20A* gene, characterized by one base pair deletion in exon 11, resulting in a frameshift replacing a glutamine at codon 483 for a lysine and terminating at position 24 [NG_029809.1: c.1447delG; p.(Glu483Lysfs*24)], were detected in all patients, strongly suggesting a founder effect. Our results reinforce the distinct orofacial features of ERS, which are the clue for kidney examination and genetic testing. Early diagnosis is essential to minimize the deleterious effects related to ERS. Here we report the largest series of patients with ERS in the same population, and describe, for the first time, a founder mutation for *FAM20A*.

Keywords: amelogenesis imperfecta; nephrocalcinosis; gingival fibromatosis; syndrome; *FAM20A*

Introduction

Firstly described in 1972 by McGibbon as "generalized enamel hypoplasia and renal dysfunction"[1], enamel renal syndrome (ERS, OMIM #204690) is a rare autosomal recessive disorder that remains not fully characterized. Similar phenotypes have been described under different names, including amelogenesis imperfecta and nephrocalcinosis syndrome[2,3], amelogenesis imperfecta and gingival hyperplasia syndrome[4] and enamel-renal-gingival syndrome[5,6]. It is believed that these conditions represent in fact the same disease, caused by underlying *FAM20A* gene mutations[4,7,8,9].

Clinically, the common oral characteristics include hypoplastic amelogenesis imperfecta (AI), delayed tooth eruption, pulp calcifications, hyperplastic dental follicles, and gingival hyperplasia with variable severity and presence of calcified nodules[9]. In addition, nephrocalcinosis (NC) and other kidney disorders have been included as frequent findings, especially in the early adulthood[10,11]. In that sense, it is speculated that even those individuals with the oral characteristics showing no renal defects, but with biallelic *FAM20A* mutations, will eventually develop NC[7] . The protein encoded by *FAM20A* is expressed in the ameloblasts during secretory and maturation stages of enamel development, in suprabasal cells of the gingiva, odontoblasts, and dental pulp cells, indicating its fundamental role in enamel development and gingival homeostasis[4]. Several *FAM20A* mutations have been described in individuals with the ERS phenotype, including stopgain, frameshift, missense, and splice-site mutations[7,8,12-16].

Here we describe 11 unreported patients with ERS from 5 different Brazilian families harboring a homozygous founder loss of function mutation in *FAM20A*. The early diagnosis can have an impact on the overall morbidity caused by ERS, hence it is important that child caregivers are aware of the main features beginning during the childhood.

Patients and Methods

The patients included in this study were evaluated at the Stomatology Clinic of the Dental School at the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM, Brazil). This study was approved by the Research Ethical Committee of UFVJM (number 074/12), and a written informed consent was obtained from patients, parents or guardians, as appropriate. In general, the oral aesthetics and functional impairment caused by the ERS were the main complaints of the patients seeking professional care. The probands and the relatives up to three generations were evaluated and the families' pedigrees were built to verify the inheritance pattern of the syndrome.

The clinical examination was focused on oral aspects of the syndrome (teeth and gingiva conditions, alveolar ridge shape, and tooth absence). The patients were evaluated by periapical and panoramic x-ray, in addition to renal ultrasonography (USS), which was analyzed by an experienced nephrologist. Furthermore, the patients were tested for alterations in their blood (calcium, ionized calcium, phosphate, parathyroid hormone, vitamin D (25OH and 1,25(OH)2), alkaline phosphatase and creatinine) and urine collected during 24 hours (calcium, phosphate, creatinine, osmolarity, specific gravity and glomerular filtration rate). Gingival tissues, teeth and pericoronary tissues removed for the purpose of oral rehabilitation were evaluated by classic H&E staining, and by screening electron microscopy (SEM) as published previously[17].

For sequencing analysis, genomic DNA was extracted from oral mucosa cells through saliva collection as previously described[18]. Exons and flanking splice junctions of *FAM20A* were amplified with specific primers by polymerase chain reaction[7], followed by bidirectional sequencing in an ABI Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing data were analyzed and compared with the published reference sequence for *FAM20A* (NG_029809, February 2018).

Results

The age of patients (7 males and 4 females) ranged from 6 to 25 years-old. Pedigrees from the 5 families revealed an autosomal recessive transmission pattern. In 7 patients from 3 different families, consanguineous marriage of parents and/or grandparents were observed (Fig. 1). The patients' previous medical history did not reveal any significant information, especially regarding renal or urinary disorders. Two patients from Family 1 (1.III-5 and 1.III-11) presented intellectual disability (ID) concomitant to other ERS features (Table 1).

Table 1 summarizes the most common clinical and imaging findings in the diagnosed patients. Clinical examination revealed that all individuals presented microdontia, spaced teeth with yellow-brownish discoloration, occlusal and incisal wear with molars showing flat cusps characterizing hypoplastic AI, and gingival overgrowth in different levels of severity (Fig. 2). Besides, other findings were also observed although not always present, such as tooth translucency caused by reduced enamel thickness, rough tooth surface, prolonged retention of deciduous teeth, malocclusion (loss of the vertical occlusion dimension, anterior open bite, crossbite) semi-lunar shape of central incisors edges, in addition to loss of periodontal support (Fig. 2). Those later findings were related to the time of tooth eruption. According to patients' reports and direct observation of remaining deciduous teeth, the dental defects are present since the first dentition and, usually, the patients did not report any tooth pain or sensitivity.

The radiographic analysis confirms the delayed eruption of permanent dentition, revealing impacted teeth with crown resorption and incomplete rhizogenesis in some cases (Fig. 3 A-D). Those impacted teeth were localized in aberrant areas, crossing the cortical bone and inducing an irregular shape of alveolar ridge, with agenesis of permanent teeth being sporadically observed (Table 1; Fig. 3 A-D). A common finding on the unerupted teeth was the presence of pericoronal

radiolucencies with sclerotic margins (hyperplasia of dental follicle), sometimes even covering the root (Fig. 3A-D). Intra-pulpal calcifications were frequently found, assuming a needle shape in the incisors and a round shape in the molars (Fig. 3A-D). There was a lack of regular contrast between enamel and dentin, representing tissue hypomineralization (Fig. 3A-D).

In the USS analysis, the renal parenchyma was hyperechoic in 5 cases, and corticomedullar dedifferentiation was a common finding. Eight patients showed bilateral NC with mineralization foci of different sizes and 1 patient (1.III.5) developed NC only in the right kidney (Fig 3E-F). Two patients showed cystic areas adjacent to the lower pole of left kidney (2.III.1) and in the cortical portion (1.III.11) (Fig. 3G). Two patients could not be investigated for renal calcifications (4.III.1 and 4.III.2).

The blood and urine tests revealed values within the normality parameters for most of the patients (Table 1). In 4 patients the values were altered for D vitamin, calcium, parathyroid hormone, urine phosphate and alkaline phosphatase. Patient 1.III.11 showed low levels of 25OH vitamin D (27.9 ng/mL) and urine phosphate (395 mg/24 h), patient 2.III.3 presented low levels of 25OH D vitamin (23.3 ng/mL) and ionized calcium (1,15 mmol/L), patient 3.III.4 showed slightly higher levels of parathyroid hormone (62 pg/mL), and patient 5.III.4 had higher levels of parathyroid hormone (70 pg/mL), alkaline phosphatase (493 U/L) and 1,25(OH)2 D vitamin (80 pg/mL).

Decalcified teeth showed intra-pulpal calcifications in both crown and root, sometimes even obliterating the area (Fig. 4A-B). Microscopic analysis of the gingival tissue revealed epithelial hyperplasia and a dense and fibrous connective tissue (Fig. 4). Deep in the connective tissue, large areas of dystrophic calcifications arranged in lobes and surrounded by fibrous tissues with mild chronic inflammatory infiltrate were commonly found (Fig. 4C-D). The hyperplastic

dental follicles also presented lobular dystrophic calcifications, apparently originated from small islands of odontogenic epithelium scattered in a collagenous stroma (Fig. 4E). These odontogenic epithelium islands showed vacuolated cells, probably representing a degenerative process (Fig. 4F). SEM analysis of extracted teeth revealed surface wear and oblique enamel cracks, areas of uneven mineral deposition, rough, porous, and void spaces (Fig. 4G-I).

For the genetic analysis, saliva from affected patients and their relatives was collected. Sanger sequencing revealed that all evaluated patients presenting ERS phenotypes showed a novel *FAM20A* homozygous one base pair deletion in exon 11, causing a frameshift and a premature stop codon [NG_029809.1: c.1447delG; p.(Glu483Lysfs*24)] (Fig. 5). Fitting the expected recessive segregation, the relatives investigated showed a heterozygous state for the deletion (Fig. 5B).

After considering the findings above mentioned, the 11 individuals were diagnosed with autosomal recessive ERS caused by a founder *FAM20A* mutation. The patients are under oral rehabilitation and are monitored by a nephrologist. They also received genetic counseling.

Discussion

This study detailed the clinical and imaging characteristics of multiple patients with ERS from 5 different Brazilian families, all originated from the same geographic region, and possibly with the same ethical background. In Brazil, three main population groups, Europeans, Africans and native American Indians (Amerindians), substantially contribute to the variable ancestry within Brazilian population, and each Brazilian contains different proportions of genomic DNA from these 3 main groups[19]. The families in this study are from a geographic area of Brazil with great African immigration.

Indeed, clinical, imaging, laboratory and genetical analysis were combined for diagnosis process to cover the syndrome spectrum. De la Dure-Molla et al.[9] suggested the following pathognomonic features in ERS: hypoplastic or absent enamel, primary and permanent teeth affected, flat cusps on posterior teeth, microdontia and spaced teeth, intra-pulpal calcifications, delayed tooth eruption, impacted posterior teeth with hyperplastic follicle, root dilacerations of impacted teeth, gingival fibromatosis, and gingival and dental follicle ectopic calcifications on biopsies. All these characteristics were presented in the patients evaluated in this study. Besides, other less common characteristics presented in this current series, such as malocclusion, periodontal disease, supra-incisive diastema, the semilunar shape of central incisors and dental and bone resorption, have been reported before[6,8,15,16,20].

ERS was associated with ID in 2 patients from Family 1, and was also reported in 2 previous studies[21,22]. Martelli-Junior et al.[21] considered ID as a characteristic superimposed on ERS phenotype in their case since other 6 relatives presented this feature as an isolated entity. Interestingly, in our study ID was presented only in association to ERS and its interpretation as an uncommon ERS finding cannot be excluded. On the other hand, the Family 1 is highly consanguineous, and ID could represent an unrelated condition to ERS. The genetic profile associated to ID was not addressed in this study and should be further confirmed in ERS patients.

AI represents a complex group of inherited conditions causing dental enamel malformations in quantity or quality, either as an isolated finding or as a characteristic of a syndrome, such as ERS[23]. In this study, AI was subclassified as hypoplastic type, represented by defects in the primary organic matrix of the enamel that may be thin and smooth, rough and with craters, or even presented as enamel agenesis[24]. The presence of abnormalities in tooth shape and intra-pulpal calcifications suggests that morphogenesis and dentinogenesis are also affected by

ERS[20]. Previous reports have identified reparative and amorphous dentin inside the pulpal chambers of erupted and non-erupted teeth in ERS[21]. Similarly, irregular dentine with dilated tubules and pulpal calcifications showing osteodentine tissue were also found in the present study. In addition, the histopathological analysis of the hyperplastic gingiva and dental follicle revealed dystrophic calcification bodies related to islands of odontogenic epithelial cells, similar to previous reports [5,13]. The hypothesis is that the odontogenic epithelial cells might have roles in the formation of these calcified bodies, but subsequently degenerate and remain as epithelial rests[11]. Future studies should be performed to define the nature of these calcifications.

Another classic finding of ERS is NC, though kidney phenotypes are not always present[1,10,25]. All patients in this study investigated by renal USS showed renal calcifications, but none developed renal complications. Renal complications in ERS range from renal failure[1,10,26] to recurrent infections[1,10], pyelonephritis[3], polycystic kidney and distal renal tubular acidosis[22,25,27], occurring between the second and third decades of life. Some studies have explained NC as an epithelial and paracellular disorder in calcium transport, predominantly caused by mutations in calcium channel proteins[28, 29]. These systems either reabsorb calcium filtered from the urine through the tubular renal cell or release calcium from the tubular cell into the interstitial compartments. When dysfunction is present, increased urinary calcium precipitates in the interstitium, resulting in NC, which is invariably accompanied by hypercalciuria[7]. However, this does not appear to be the mechanism by which patients develop NC in this syndrome since 5 reports have shown hypocalciuria in their patients[2,10,30-32]. Another hypothesis is that NC is associated with an increase of urate or oxalate, or even a decrease in inhibitors of crystallization, such as citrate[33]. NC was not associated with changes in calcium levels in our patients, which suggests its genetic etiology. The formation of renal calcifications is probably the result of

synergistic effects of altered function in many predisposing genes (including *FAM20A*) to increase individual susceptibility above the threshold of stone formation[6]. Despite the typical absence of alterations in the laboratory tests, previous cases reported hypocalciuria and hypophosphaturia[30], elevated alkaline phosphatase[20,32], low levels of vitamin D 25-OH[32], and high parathyroid hormone[5,10,27]. Changes in levels of alkaline phosphatase, vitamin D, parathormone and phosphate in urine were also found in 4 patients of this study.

FAM20A is considered a pseudokinase due to a mutation within its catalytic site, but it can form a functional complex with FAM20C and can enhance the capacity of the latter to phosphorylate extracellular proteins in their secretory pathways [17,34]. The role of FAM20A in amelogenesis may be indirect, and it can be hypothesized that FAM20A loss of function would result in reduced phosphorylation of enamel matrix proteins, thus disrupting amelogenesis beyond the first stages of inner enamel deposition, and leading to a poorly mineralized matrix [17]. Several *FAM20A* mutations including missense, nonsense, splice site, and insertion/deletion, have been previously reported in ERS patients[8]. Combining homozygosity mapping and whole exome sequencing, O'Sullivan et al.[4] identified the first homozygous mutation in *FAM20A* in a consanguineous family with ERS. Using genome-wide linkage analysis, exome capture, next-generation sequencing and Sanger sequencing, Jaureguiberry et al.[7] described 20 different biallelic *FAM20A* mutations segregating with the disease in 25 ERS patients from 16 families.

Previous reports have shown homozygous mutations in all 11 exons, and some introns, of *FAM20A* in ERS, whereas heterozygous carriers appear to be phenotypically healthy[9]. Most of the previous studies reported ERS-associated mutations inducing protein truncation (premature stop codons) and only 4 missense mutations have been reported[7,13,14,23]. In the current report, all ERS patients were identified with a novel homozygous nonsense mutation in exon 11 [NG_029809.1:

c.1447delG; p.(Glu483Lysfs*24)]. This led us to speculate that this mutation is probably have arisen from a common ancestor with a founder effect.

In closing, we reported a large cohort of patients with ERS, illustrating the clinical and imaging features and revealing one novel and founder mutation in *FAM20A*. It is suggested that patients presenting hypoplastic AI in association with delayed teeth eruption, intra-pulpal calcifications, gingival hyperplasia, and hyperplastic pericoronal radiolucences should be referred for renal investigation of NC. Genetic counseling is important given the inheritance pattern of the disease. Finally, early diagnosis and treatment of this condition will decrease the renal effects of ERS, and oral rehabilitation is a must to provide better function, aesthetics and improve the patients' quality of life.

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Figure Legends

Figure 1. Pedigrees representing the five studied families.

Figure 2. Different clinical aspects of the orodental ERS findings in three different patients (4.III.2, 1.III.11, and 2.III.1, according to the pedigrees codes). In A, B, and C, it is possible to visualize the presence of a thin and smooth enamel layer, widely spaced and small teeth, flat molar cusp, the absence of molars, and gingival enlargement. In D, E and F, the enamel translucence, yellowish teeth color, teeth absence (upper and lower incisors), gingival enlargement, loss of the typical teeth shape and of the vertical occlusion dimension, are evident. Figures G, H, and I, show the absence of frontal and posterior teeth, gingival enlargement, loss of the periodontal support in an inferior molar, and anterior open bite.

Figure 3. Imaging findings on panoramic radiography of four different ERS patients: 1.II.4 (A), 1.III.11 (B), 2.III.1 (C), and 5.III.4 (D), according to the pedigrees codes. In general, the pattern is unique and the images reveal delayed eruption and several impacted permanent teeth, intra-pulpal calcifications (arrow), pericoronal radiolucences (asterisks), crown reabsorption of impacted molars (§) as in A and B, and teeth in aberrant location like the impacted molars invading the cortical mandibular bone in Figure C. Figures E and F represent the findings on renal ultrasonography of both right and left kidneys, respectively. Hyperechoic areas of different sizes represent the calcified bodies and were interpreted as a sign of nephrocalcinosis. Figure G shows the renal cyst in the patient 1.III.6, which is an uncommon finding in ERS patients.

Figure 4. Microscopic findings common in ERS. Figure A shows incisive in a longitudinal section, and the squared area is presented in a higher magnification of the pulp region in B, revealing the presence of multiple calcified bodies. Figure C represent the histologic findings of the gingival tissue, where the specimen shows epithelial hyperplasia and the presence of dystrophic calcified lobular tissue deep in the connective tissue in the absence of inflammatory infiltrate. The squared area in C is in higher magnification on D, showing basophilic, strongly stained lobular osteodentine tissue. E shows the histologic findings in the pericoronal tissue of impacted teeth, also presenting dystrophic calcification bodies in a fibrous stoma, and the higher magnification in F highlights the presence of odontogenic epithelial cells surrounding calcified tissue. G, H, and I are SEM images showing the rough, irregular enamel surface (G), the presence of cracks and intra-pulpal calcified tissue (H), and irregular dentin deposition around the pup chamber (I). Figures A, B, C, D, E, and F are regular H&E stained sections (A and B were previously decalcified). (Original magnification: A, C:50x; B, E:100x; D: 200x; F:400x). e= enamel, d= dentin, ct= calcified tissue.

Figure 5. Representative images from the sequencing chromatograms of *FAM20A* exon 11 analyzed in the patients of this study. This homozygous loss of function mutation, characterized by one base pair deletion, as shown in the proband 2.III.1 (A), resulted in a premature stop codon [NG_029809.1: c.1447delG; p.(Glu483Lysfs*24)]. Fitting the expected recessive segregation, his mother (2.II.10) revealed heterozygosity for this deletion (B). The analysis of a healthy non-affected control showed a normal *FAM20A* sequence (C).

Table 1. General clinical, imaging and laboratory findings of the patients diagnosed with Enamel Renal Syndrome.

		1.III.1 (M/18)	1.III.4 (M/13)	1.III.5 (F/11)	1.III.6 (M/25)[§]	1.III.11 (M/14)	2.III.1 (M/15)	2.III.3 (F/13)	3.III.4 (M/12)	4.III.1 (F/06)	4.III.2 (F/13)	5.III.4 (M/13)	Total
CLINICAL FEATURES	Microdontia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Yellow-brownish teeth	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Enamel translucency	No	No	No	No	Yes	Yes	No	No	No	No	Yes	03
	Rough tooth surface	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	06
	Occlusal/incisal wear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Prolonged retention of deciduous teeth	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	10
	Malocclusion	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	08
	Spaced teeth	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Gingival hyperplasia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Semilunar shape of central incisors	Yes	Yes	Yes	No	No	No	No	No	No	No	No	03
	Periodontal involvement	Yes	No	No	Yes	No	Yes	No	Yes	No	No	No	04
X-RAY/ USS	Impacted permanent teeth	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Teeth in ectopic areas	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No	07
	Dental Agenesis	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No	04
	Pericoronal radiolucences	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Lack of the contrast enamel/dentin	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	Intra-pulpal calcifications	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
	External dental resorption	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No	04
	Localized bone resorption	Yes	No	No	Yes	No	No	No	No	No	No	No	02
	Renal calcifications	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-	Yes	09
	Blood and urine tests alterations	No	No	No	No	Yes	No	Yes	Yes	No	No	Yes	04
	Other alterations	No	No	No	ID/RC[¶]	MR	RC	No	No	No	No	No	-

[§]Case report presenting the oral rehabilitation of this patient previously published[36].

[¶]ID=intellectual disability; RC=renal cyst. The subjects are identified by their code on the pedigrees, with information regarding sex (M=male, F=female) and age at the diagnosis (in years) inside the parenthesis.

References

1. MacGibbon D. Generalized enamel hypoplasia and renal dysfunction. Aust Dent J. 1972;17(1):61-3.
2. Normand de la Tranchade I, Bonarek H, Marteau JM, Boileau MJ, Nancy J. Amelogenesis imperfecta and nephrocalcinosis: a new case of this rare syndrome. J Clin Pediatr Dent. 2003 Winter;27(2):171-5.
3. Hall RK, Phakey P, Palamara J, McCredie DA. Amelogenesis imperfecta and nephrocalcinosis syndrome. Case studies of clinical features and ultrastructure of tooth enamel in two siblings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;79(5):583-92.
4. O'Sullivan J, Bitu CC, Daly SB, Urquhart JE, Barron MJ, Bhaskar SS, et al. Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. Am J Hum Genet. 2011;88(5):616-20.
5. Kantaputra PN, Kaewgahya M, Khemaleelakul U, Dejkhamron P, Sutthimethakorn S, Thongboonkerd V, et al. Enamel-renal-gingival syndrome and FAM20A mutations. Am J Med Genet A. 2014;164A(1):1-9.
6. Kantaputra PN, Bongkochwilawan C, Kaewgahya M, Ohazama A, Kayserili H, Erdem AP, et al. Enamel-Renal-Gingival syndrome, hypodontia, and a novel FAM20A mutation. Am J Med Genet A. 2014;164A(8):2124-8.
7. Jaureguiberry G, De la Dure-Molla M, Parry D, Quentric M, Himmerkus N, Koike T, et al. Nephrocalcinosis (enamel renal syndrome) caused by autosomal recessive FAM20A mutations. Nephron Physiol. 2012;122(1-2):1-6.
8. Pêgo SPB, Coletta RD, Dumitriu S, Iancu D, Albanyan S, Kleta R, et al. Enamel-renal syndrome in 2 patients with a mutation in FAM20 A and atypical hypertrichosis and hearing loss phenotypes. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123(2):229-234.e2.
9. De la Dure-Molla M, Quentric M, Yamaguti PM, Acevedo AC, Mighell AJ, Vikkula M, et al. Pathognomonic oral profile of Enamel Renal Syndrome (ERS) caused by recessive FAM20A mutations. Orphanet J Rare Dis. 2014;9:84.
10. Dellow EL, Harley KE, Unwin RJ, Wrong O, Winter GB, Parkins BJ. Amelogenesis imperfecta, nephrocalcinosis, and hypocalciuria syndrome in two siblings from a large family with consanguineous parents. Nephrol Dial Transplant. 1998;13(12):3193-6.

11. Martelli-Júnior H, dos Santos Neto PE, de Aquino SN, de Oliveira Santos CC, Borges SP, Oliveira EA, et al. Amelogenesis imperfecta and nephrocalcinosis syndrome: a case report and review of the literature. Nephron Physiol. 2011;118(3):p62-5.
12. Cho SH, Seymen F, Lee KE, Lee SK, Kweon YS, Kim KJ, et al. Novel FAM20A mutations in hypoplastic amelogenesis imperfecta. Hum Mutat. 2012 Jan;33(1):91-4.
13. Kantaputra PN, Bongkochwilawan C, Lubinsky M, Pata S, Kaewgahya M, Tong HJ, et al. Periodontal disease and FAM20A mutations. J Hum Genet. 2017;62(7):679-686.
14. Wang SK, Reid BM, Dugan SL, Roggenbuck JA, Read L, Aref P, et al. FAM20A mutations associated with enamel renal syndrome. J Dent Res. 2014;93(1):42-8.
15. Cherkaoui Jaouad I, El Alloussi M, Chafai El Alaoui S, Laarabi FZ, Lyahyai J, Sefiani A. Further evidence for causal FAM20A mutations and first case of amelogenesis imperfecta and gingival hyperplasia syndrome in Morocco: a case report. BMC Oral Health. 2015;15:14.
16. Volodarsky M, Zilberman U, Birk OS. Novel FAM20A mutation causes autosomal recessive amelogenesis imperfecta. Arch Oral Biol. 2015;60(6):919-22.
17. Lignon G, Beres F, Quentric M, Rouzière S, Weil R, De La Dure-Molla M, et al. FAM20A Gene Mutation: Amelogenesis or Ectopic Mineralization? Front Physiol. 2017;8:267.
18. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J. 2007;18:148-52.
19. Moura RR, Coelho AV, Balbino Vde Q, Crovella S, Brandão LA. Meta-analysis of Brazilian genetic admixture and comparison with other Latin America countries. Am J Hum Biol. 2015;27(5):674-80.
20. Paula LM, Melo NS, Silva Guerra EN, Mestrinho DH, Acevedo AC. Case report of a rare syndrome associating amelogenesis imperfecta and nephrocalcinosis in a consanguineous family. Arch Oral Biol. 2005;50(2):237-42.
21. Martelli-Júnior H, Bonan PR, Dos Santos LA, Santos SM, Cavalcanti MG, Coletta RD. Case reports of a new syndrome associating gingival fibromatosis and dental abnormalities in a consanguineous family. J Periodontol. 2008;79(7):1287-96.
22. Suda N, Kitahara Y, Ohyama K. A case of amelogenesis imperfecta, cleft lip and palate and polycystic kidney disease. Orthod Craniofac Res. 2006;9(1):52-6.
23. Wang SK, Aref P, Hu Y, Milkovich RN, Simmer JP, El-Khateeb M, et al. FAM20A mutations can cause enamel-renal syndrome (ERS). PLoS Genet. 2013;9(2):e1003302.

24. Deutsch D, Leiser Y, Shay B, Fermon E, Taylor A, Rosenfeld E, et al. The human tuftelin gene and the expression of tuftelin in mineralizing and nonmineralizing tissues. Connect Tissue Res. 2002;43(2-3):425-34.
25. Elizabeth J, Lakshmi Priya E, Umadevi KM, Ranganathan K. Amelogenesis imperfecta with renal disease--a report of two cases. J Oral Pathol Med. 2007;36(10):625-8.
26. Lubinsky M, Angle C, Marsh PW, Witkop CJ Jr. Syndrome of amelogenesis imperfecta, nephrocalcinosis, impaired renal concentration, and possible abnormality of calcium metabolism. Am J Med Genet. 1985;20(2):233-43.
27. Hunter L, Addy LD, Knox J, Drage N. Is amelogenesis imperfecta an indication for renal examination? Int J Paediatr Dent. 2007 Jan;17(1):62-5.
28. Shan Q, Himmerkus N, Hou J, Goodenough DA, Bleich M. Insights into driving forces and paracellular permeability from claudin-16 knockdown mouse. Ann N Y Acad Sci. 2009;1165:148-51.
29. Dimke H, Hoenderop JG, Bindels RJ. Molecular basis of epithelial Ca2+ and Mg2+ transport: insights from the TRP channel family. J Physiol. 2011;589(Pt7):1535-42.
30. Kala Vani SV, Varsha M, Sankar YU. Enamel renal syndrome: a rare case report. J Indian Soc Pedod Prev Dent. 2012;30(2):169-72.
31. Kirzioglu Z, Ulu KG, Sezer MT, Yüksel S. The relationship of amelogenesis imperfecta and nephrocalcinosis syndrome. Med Oral Patol Oral Cir Bucal. 2009;14(11):e579-82.
32. Poornima P, Katkade S, Mohamed RN, Mallikarjuna R. Amelogenesis imperfecta with bilateral nephrocalcinosis. BMJ Case Rep. 2013;2013. pii:bcr2013009370.
33. Sayer JA, Carr G, Simmons NL. Nephrocalcinosis: molecular insights into calcium precipitation within the kidney. Clin Sci (Lond). 2004;106(6):549-61.
34. Li LL, Liu PH, Xie XH, Ma S, Liu C, Chen L, et al. Loss of epithelial FAM20A in mice causes amelogenesis imperfecta, tooth eruption delay and gingival overgrowth. Int J Oral Sci. 2016;8(2):98-109.
35. Costa DC, Dourado MR, Figueiredo de Carvalho MF, Santos CR, da Cruz Batista MA, Mesquita AT. Enamel Renal Syndrome: A Case History Report. Int J Prosthodont. 2017;30(1):22-24.