Book Chapter

Raine Syndrome and \textit{FAM20C} Variants in a Mexican Family

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Abstract

Two siblings from a Mexican family who carried lethal Raine syndrome are presented. A newborn term male (case 1) and his 21 gestational week brother (case 2), with a similar osteosclerotic pattern: generalized osteosclerosis, which is more evident in facial bones and cranial base. Prenatal findings at 21 weeks and histopathological features for case 2 are described. A novel combination of biallelic \textit{FAM20C} pathogenic variants were detected, a maternal cytosine duplication at position 456 and a paternal deletion of a cytosine in position 474 in exon 1, which change the reading frame with a premature termination at codon 207 and 185 respectively. These changes are in concordance with a negative detection of the protein in liver and kidney as shown in case 2. Necropsy showed absence of pancreatic Langerhans Islets, which are reported here for the first time. Corpus callosum absence is added to the few reported cases of brain defects in Raine syndrome. This report shows two new \textit{FAM20C} variants not described previously, and negative protein detection in the liver and the kidney. We highlight that lethal Raine syndrome is well defined as early as 21 weeks, including mineralization defects and craniofacial features. Pancreas and brain defects found here in \textit{FAM20C} deficiency extend the functional spectrum of this protein to previously unknown organs, and can lead to search for new \textit{FAM20C} targets in order to get ala better understanding of the wide pathologic and phenotypic spectrum of RS.

Keywords

Lethal Raine Syndrome; \textit{FAM20C}; New Variants; Histopathology
Introduction

Raine Syndrome (RS) (OMIM # 259775) is an autosomal recessive disease, first described in 1985 and a second case in 1989 [1-2], with a prevalence of <1 in 1,000,000 [3]. RS first cases were described as generalized osteosclerosis with periosteal bone formation, as well as characteristic craniofacial dysmorphisms, and intracerebral calcifications. Since individuals affected by RS die within the first days or weeks of life due to respiratory distress secondary to pulmonary hypoplasia, it was considered a lethal disease [4-6]. Although most cases are detected at birth, prenatal features have been detected, including craniofacial dimorphism, with hypoplastic nose and prominent eyes, in addition to cerebral alterations [7-11].

*FAM20C* gene was identified as the cause of RS in 2007 [12], due to a chromosomal rearrangement involving 7p22.3. It is a member of the family with sequence similarity 20 (FAM20), together with *FAM20A, FAM20B* [13], that encodes protein kinases acting on diverse substrates that play important roles in biomineralization [14]. The FAM20C protein is a casein kinase expressed in the Golgi apparatus and then secreted, and is considered the principal phosphokinase of the secretory pathway. FAM20C phosphorylates serine residues at S-X-E/pS motifs of phosphoproteins in serum, plasma and cerebrospinal fluid, including secreted proteins which are estimated to be over 100 genuine substrates [13,15]. In mineralized tissues, FAM20C phosphorylates members of the secretory calcium-binding phosphoprotein (SCPP) family, which include non-collagenous proteins expressed in extracellular matrices of calcified tissues known as SIBLING proteins (small integrin-binding ligand N-linked glycoproteins), such as dentin matrix proteins (DMP1), bone sialoprotein (BSP), osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE), and dentine sialophosphoprotein (DSPP) [16-19-21]. Moreover, FAM20C regulates fibroblast growth factor 23 (FGF23) secreted by osteoblasts and osteocytes, which plays a major role in the renal metabolism of phosphate, in the reabsorption of phosphate and the catabolism of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) [22-23]. Besides biomineralization, FAM20c targets participate in processes that
include wound healing, lipid homeostasis, endopeptidase inhibitory activity, adhesion and cell migration, and they also appear to be involved in cancer [15-18].

Most cases of lethal RS (LRS) are detected at birth, they present major features including craniofacial alterations like microcephaly, turribrachycephaly, wide sutures, flat facial profile, prominent eyes, hypoplastic nose and choanal stenosis/atresia, in addition to osteosclerotic bone defects, vascular and brain calcifications, as well as lung hypoplasia [7-12, 24-27]. Other features including polyhydramnios, craniofacial profile similar to Binder anomaly or Crouzon syndrome, and cerebral alterations such as large choroid plexuses, echogenic appearance of the brain, blurry ventricular walls and intracerebral calcifications have been described [7-11]. Histopathological findings derived from RS necropsies have revealed the presence of calcifications such as calcospherites within the prevascular and perivascular brain neuropile, plus pulmonary hypoplasia, and abnormal bone mineralization including trabecular bone covered by abundant osteoid, plentiful osteoblasts, bone resorption and remodeling, and osteomalacia [8,9,28-29].

In 2009, the first non-lethal RS (NLRS) cases were described including children, adolescents and adults, featured with a wide variable expression [23 30]. NLRS features include craniofacial dymorphism like flat profile, hypoplastic nose, ocular proptosis, additional to new features like amelogenesis imperfecta, hearing defects, ectopic calcifications, osteonecrosis, and neurodevelopmental delay [23-33,24-38].

This work is based on a previous report [39], we describe two new FAM20C gene pathogenic variants in a Mexican family with two siblings with lethal RS. Case 1 corresponds to a term newborn and case 2 corresponds to a 21 gestational week (GW) fetus. We describe prenatal findings and pathological findings for case 2, including novel pancreas features and FAM20C immunohistochemical staining in liver and kidney. Furthermore, we discuss and list FAM20C pathogenic variants in lethal Raine syndrome and its effects through different targets.
Results

Family

A Mexican family of apparently healthy parents, both of them 29 years old at the time of birth of the first case, with no history of consanguinity or inbreeding (Figure 1A). The mother’s first pregnancy (G1) (from a different father), is now a healthy nine-year old girl. Her second pregnancy (G2) corresponds to case 1, a term newborn male with clinical data compatible with RS who died on his third day of life. Case 2 corresponds to her third pregnancy (G3), a 21 gestational week (GW) fetus, with features compatible with RS, detected prenatally by ultrasound. Both pregnancies were negative to exposure to teratogens, brothers’ weight, length, and head circumference were normal according to percentiles for their gestational ages. Informed consent was obtained from both parents and this work was approved by the ethics committee of the Hospital Juárez de México on July 13th, 2018 (Ethical code: HJM 0445/18-I). Both parents gave their informed consent in writing, with alignment to the Declaration of Helsinki 1975. Sequence analysis of FAM20C exons was carried out in both parents.

Case Reports

Case 1 was a term newborn male from an uncomplicated pregnancy, delivered by cesarean section due to double loop nuchal chord. Craniofacial alterations and respiratory distress were readily detected at birth. The craniofacial phenotype was typical of RS (Figure 1B), showing turribrachycephaly, wide fontanelles, prominent frontal bone, flat facial profile, severe ocular proptosis, hypertelorism, depressed and low nasal bridge with hypoplastic nose, choanal stenosis, flaring nares, fish-like mouth, small pointed chin, rounded and low-set ears, and horizontal antitragus. Among extra-craniofacial features, he had a short, small, and slightly bell shape thorax; hands and feet presented brachydactyly, hypoplastic distal phalanges, and prominent fingerpads. Radiologic imaging showed generalized osteosclerosis (skull, vertebras, thorax and long bones) and periosteal reaction (Figure 2A). A transfontanellar ultrasound study revealed brain calcifications (Figure 2B). The patient
remained in the neonatal intensive care unit for 3 days with oxygen support, after which he developed seizures and cardio-respiratory arrest. RS diagnosis was done on the basis of osteosclerosis and craniofacial features, and parents were given genetic counseling with a 25% risk for future offspring.

Case 2 pregnancy was assessed by structural ultrasonography since week 11. Facial dymorphisms: ocular proptosis, hypertelorism, and mouth features were detected at 21 GW (Figure 1C), which lead to the parents decision to induce delivery. The patient had features like his brother, including turricephaly, high and slightly bulged frontal, ocular proptosis, depressed nasal bridge, small nose, V palate, micrognathia, and rounded ears. Among non-craniofacial features, he had prominent fingerpads, and undescended testicles. Babygram showed generalized osteosclerosis with a pattern in the base of the skull and facial bones, similar to case 1 (Figure 2C).

Figure 1: Family pedigree and phenotype of affected siblings (case 1 and 2). (A) Pedigree of the family showing reported cases (case 1 corresponds to individual II.2 and case 2 corresponds to individual II.3). (B) Clinical picture of the first proband (case 1). (C) Clinical pictures of the second patient (case 2).
Figure 2: Image findings of cases 1 and 2. (A) X-ray of cranium, hands, feet and babygram of case 1, showing generalized osteosclerosis, more prominent in cranium base and facial bones, with hypomineralization of the other skull areas; periostic reaction in humerus, distal phalangeal hypoplasia of hands, carpal ossification defects (delayed bone age), and generalized osteosclerosis. (B) Transfontanellar ultrasound of case 1, showing brain calcifications, mostly periventricular. (C) Babygram of case 2, showing cranium with osteosclerosis, also more prominent in skull base and facial bones, cranial vault hypomineralization, and increased density of all the bones. (D) Obstetric structural USG at 21GW of case 2, showing exophthalmos and oral anomalies.

Pathological Features

Necropsy carried out for case 2, described a 22 GW male, weight 450 g, length 28.7 cm, head circumference 19 cm, thoracic perimeter 14.6 cm, abdominal perimeter 16 cm, and feet 4.2 cm. The brain weighed 71 g and measured 7 × 6 × 4cm, with lissencephaly and agenesis of corpus callosum (Figure 3A). Histological sections of the bone showed hyaline cartilage and immature trabecular bone. Brain sections showed disorganization of the cortical layers and multiple zones of microcalcifications (Figure 3A–C). In the pancreas, islets of Langerhans were absent (Figure 3D). No other tissue alterations were detected.
Figure 3: Anatomopathological findings of case 2. (A) Brain section showing corpus callosum agenesia. (B) Histological analysis of brain sections revealed brain parenchyma with disorganized cortex layers, and zones with laminated microcalcifications (arrows). (C) Neuropile with concentric calcospherites. (D) Pancreas with absence of Langerhans’ islets. (H&E stain).

FAM20C Variants Analysis

In order to identify FAM20C variants (NM_020223.3), we performed sequence analysis by polymerase chain reaction (PCR) and bidirectional Sanger sequencing using BigDye Terminator v3.1 kit (Applied Biosystems, Foster city, CA, USA) of exons 1–10 on peripheral blood DNA from both parents. Primers used were those reported by Acevedo et al. [31]. Detected variants were searched in public databases (i.e., gnomAD, ExAC, 1000 Genomes, and ESP) [40-41].

We detected a duplication at position 456 (c.456dupC) in exon 1 in the mother and a deletion of a cytosine in position 704 also in exon 1 (c.474delC) in the father. Both variants are novel in RS (reference sequence NM_020223), as shown in Figure 4. The
first one alters the reading frame, resulting in a premature termination codon at position 207 (p.Gly153ArgfsTer56), and the second also changes the reading frame due to a premature stop codon at codon 185 (p.Ser159ProfsTer28). The two RS cases would have inherited these variants and therefore they appear to be compound heterozygous. The fact that they are different variants, confirms the history of non-consanguinity. The variants found in both parents, have not been described in patients or families affected with RS, nor have they been reported as variants in ExAC, ESP, and 1000 Genomes databases [44-46]. The paternal variant (p.Ser159ProfsTer28) is reported in gnomAD, which includes exome samples from Africans, Ashkenazi Jews, East Asian, European, South Asian, Latino, and other populations, and represents one from 134998 alleles (allelic frequency of 0.000007408). Since this allele was detected in Latino population, it represents one from 23776 alleles, and an allelic frequency of 0.00004206.
Figure 4: FAM20C gene sequence analysis from Family. (A) Electropherogram showing wild-type FAM20C sequence and localization sites of maternal and paternal variants. (B) Electropherogram showing identified maternal variant sequence c.456dup corresponding to p.Gly153Argfs*56 and effect on size protein. (C) Electropherogram showing paternal variant sequence c.474delC corresponding to p.Ser159Profs*28 and size protein effect.

Immunohistochemical Detection of FAM20C

FAM20C immunodetection was done in formalin fixed paraffin embedded tissue sections (5 µm thickness) from kidney and liver, derived from case 2, as well as in necropsy tissues of an unrelated 31 GW newborn without RS (control) (deceased due to
non-syndromic congenital heart malformation). Liver and kidney sections were used because FAM20C expression is higher in these tissues (at least in adults), because of FAM20C activity in protein targets synthesized and secreted from hepatocytes, and because of its participation in kidney on regulating excretion of phosphate through binding to FGFRs/α-K.

Anti-FAM20C antibody ab154740 was used, diluted 1:50 (rabbit polyclonal, Concentration 100 µL at 0.72 mg/mL, Abcam, Cambridge, UK). This antibody identifies the fragment corresponding to a region within amino acids 233-512 of Human FAM20C (UniProt ID: Q8IXL6). It was used by Cozza G and Knab VM (16, 71). For the negative control immunostaining, the primary antibody was replaced by PBS. Antigen-antibody complexes were detected by avidin-biotin-peroxidase with a Starr Trek Universal HRP Detection System KIT (Catalogue: STUHRP700 H, L10, Biocare Medical). Assays were performed in triplicate and photographed under a light microscope (Zeiss model 473028, Carl Zeiss, Oberkochen, Germany). Assays were done assuming a qualitative analysis.

FAM20C indirect immunoreactivity was positive in the cytoplasm of tubular epithelial cells of the kidney in a 31 GW newborn control, whereas in our RS patient (case 2) there was no detection (Figure 5). In the liver, FAM20C indirect immunoreactivity was detected in control hepatocytes, but it was not detectable in RS patient.
Figure 5: Representative images of FAM20C detection by immunohistochemistry (40×) in kidney and liver of patient with lethal Raine Syndrome (RS) (case 2) and necropsy tissue from a newborn without RS (gestational age 31 weeks). (A) Kidney from case 2, (a) negative control. (B) Kidney from newborn without RS (31GW), (b) negative control. (C) Liver from case 2, (c) negative control. (D) Liver from newborn without RS (31GW), (d) negative control.

Discussion

Since the first report by Raine 30 years ago, 32 lethal RS cases have been described [1,7-10,12, 24-27, 30-39], including our two cases. All individuals affected with lethal RS survive hours, days or weeks, with death mainly resulting from respiratory failure, but some survive up to two years. More than 20 years later (in 2009), non-lethal cases of Raine syndrome were reported in two unrelated individuals of 8 and 11 years, and to date, 25 cases have been described [4-6, 11, 24-35, 53] Thus, two types of RS are recognized: lethal and non-lethal RS. The cases reported herein have the classical features of LRS, mainly defined by mineralization defects, facial phenotype, and perinatal respiratory mortality.
The craniofacial phenotype of case 1 corresponds to the classical features, such as wide fontanelles, prominent frontal bone, flat facial profile, severe ocular proptosis, hypertelorism, depressed and low nasal bridge with hypoplastic nose, and choanal stenosis. The second patient (case 2), had similar craniofacial features as his older brother but slightly less pronounced, probably due to a shorter gestational age (21 weeks). Among extracraniofacial features both had a short and narrow thorax, slightly bell shaped; brachydactyly, hypoplastic distal phalanges in hands, feet, and prominent fingerpads, but were less pronounced in case 2. Radiologically, both present abnormalities compatible with RS such as generalized osteosclerosis, which was more prominent at the cranial base and facial bones, plus long bone periostic reaction and hypoplastic distal phalanges in the hands. They also had carpal ossification defects (delayed bone age), not a common feature of RS. Babygram of case 2 also showed prominent osteosclerosis at the cranial base and facial bones. To date, the earliest RS osteosclerosis reported was in a prenatal ultrasound at 23 GW, but we detected it in our case 2 at 21 GW despite of structural ultrasound assessment since 11 GW, making it the earliest known case of mineralization defects that could represent a striking and early LRS feature. Other described RS features like metaphyseal flaring, shortening and bowing of long bones, irregular contour of ribs, fractures, pseudofractures, flattened vertebral bodies, were not present in the cases described here. In summary, there were three major clinical and radiological components in our LRS cases: craniofacial phenotype, bone mineralization defects, and respiratory disturbances.

It is important to point the brain defects present in case 2, as necropsy shows absence of the corpus callosum, an apparently uncommon RS feature. To date, neurological defects have been reported in three lethal cases, including corpus callosum hypoplasia, encephalocele, cortical atrophy, cerebellar hypoplasia, pachygyria and optic nerve hypoplasia [37,44,53], as well as two non-lethal cases including corpus callosum dysgenesis and cortical atrophy, apparent pituitary gland absence, posterior cerebellum hypoplasia and postnatal
hydrocephaly. Additionally, some patients present developmental delay [6,25].

Since its original description, RS was considered an autosomal recessive disorder and the responsible gene was detected in 2007 [11 12]. Table 1 summarizes FAM20C pathogenic variants for LRS reported to date (according to RefSeq NM_020223.3) [12,39,47,48,49,51,54,55].

In the present cases, sequencing analyses revealed that both parents were heterozygous carriers. The effect of the nucleotide duplication and deletion in exon 1, yield a frameshift in the reading frame with a premature termination codon at codon 207 (maternal variant) and 185 (paternal variant), that predict 207 and 185 amino acid proteins [42-43]. On the basis of this, both variants can be clearly classified as pathogenic, and represent variants producing a very short protein without kinase domain. The paternal variant has been detected in only one allele (allelic frequency of 0.000007408) at gnomAD, from an apparently healthy latino individual, but it is not included in the ExAc, ESP and 1000 Genomes databases [39-42]. The maternal variant is not described in any of the main databases (gnomAD, ExAc, ESP and 1000 Genomes). We could not verify the presence of these variants in both cases due to DNA degradation of tissues in case 2 and unavailable tissue samples for case 1, neither from the nine year old daughter. Nevertheless, as RS has an autosomic recessive inheritance, we can assume that both variants were inherited together in cases 1 and 2 (compound heterozygous). If this was the case, these two variants represent a novel combination of biallelic FAM20C variants in Raine osteosclerotic dysplasia and therefore, they are new variants associated to lethal RS, although one of them had been previously reported in only one heterozygous healthy individual.
Table 1: FAM20C variants related to lethal Raine syndrome.

<table>
<thead>
<tr>
<th>RV</th>
<th>Localization</th>
<th>PKDA</th>
<th>Coding Seq</th>
<th>Protein Seq</th>
<th>M</th>
<th>Sp</th>
<th>I/NS</th>
<th>GR</th>
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<td>7p22</td>
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<td>45, XY pseud (7,7) (p22;p22)</td>
<td>—</td>
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<td>2007</td>
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<td>2</td>
<td>7p22 (48 Kb)</td>
<td>X</td>
<td>46,XX,ar[hg18] 7p22.3 (36480-53371)+0</td>
<td>—</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>2013</td>
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<tr>
<td>3</td>
<td>F1</td>
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<td>c.556dupC</td>
<td>p.Glu186Cys</td>
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<td>2020</td>
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</tr>
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<td>X</td>
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<td>p.Ser158PhefsTer26</td>
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<td></td>
<td></td>
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<td>2020</td>
<td>Hernández-Zevala et al. [39]</td>
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<td>5</td>
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<td>X</td>
<td>c.813+5G&gt;C</td>
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<td>X</td>
<td>c.956 + 5G &gt; C</td>
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<td>2007</td>
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<td>8</td>
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<td>c.1007T &gt; G</td>
<td>p.Met336Trp</td>
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<td></td>
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<td>c.1104G &gt; A</td>
<td>Gly368Asp</td>
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<td>p.Leu379Arg</td>
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<td>p.Arg408Cys</td>
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<td>p.Arg548Ter</td>
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<td></td>
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</table>

The premature stop codon of both alleles is in concordance with a disease-causing loss of function variant. Moreover, because the gene would produce a short transcript, it could undergo nonsense-mediated mRNA decay (NMD) [56,57,60], but it was not corroborated because RNA samples were not available. On the other hand, the FAM20C protein was not detectable by immunohistochemistry in kidney and liver tissues, which normally have a high expression according to The Human Protein Atlas database [58]. As the antibody used recognizes the region spanning aa 233–512, and both mutant proteins lack this region, these truncated proteins are not detectable, in addition to being non-functional as predicted by the lack of the kinase domain located between aa 354–565 of wild type FAM20C. Therefore, the absence of any functional form of this protein could cause the lethal phenotype.

As RS is a congenital syndrome, FAM20C is important for proper embryonic development. The FAM20C protein importance in early development is supported by the 21 GW fetus phenotype (case 2), as well as its detection in a 31GW control fetus. To our knowledge, FAM20C expression in these tissues was previously unknown. The phenotype of RS patients appears to be related to changes in the distribution of FAM20C and/or its kinase activity [58-61], which ranges from absent to varying degrees of impairment of S-x-E/pS motif phosphorylation of over 100 target proteins, besides the possible role of NMD. This would also explain the phenotypic spectra and the severity of lethal or non-lethal RS [16,60-61].

FAM20C participates in biomineralization through phosphorylation of different SCPP proteins, including the SIBLING and other proteins including FGF23. SIBLING proteins include OPN, DMP1, BSP, extracellular MEPE, and DSPP. Other proteins include specific extracellular matrix and transport proteins, proteases and protease inhibitors, plus biologically active peptide hormones, which together with SIBLING proteins regulate calcium phosphate precipitation as hydroxyapatite. On the other hand, some of these proteins, as well as other FAM20C targets like BMP4 (Bone morphogenetic
protein 4), have roles in cell bone differentiation and maturation [20, 21, 64-67].

Some RS syndrome patients present elevation of FGF23 and PTH, altered levels of 1,25(OH)2D3, as well as neonatal hypocalcemia, hypophosphatemia which develops postnatally, occasional hyperphosphaturia, serum alkaline phosphatase urinary and deoxypyridinoline elevation, among others [22, 27-29]. Case 1 presented hypocalcemia the first day, but was corrected with no other registered alterations.

O-glycosylation of FGF23, inhibits its proteolysis and secretion, therefore it is present as an intact protein with functional activity. Meanwhile, phosphorylation of FGF23 at Ser180 by FAM20C prevents O-glycosylation and undergoes proteolytic cleavage by furin protease, resulting in inactive FGF23 [23,67]. Its activity is in the kidney through binding and activating its receptor complex composed of α-klotho and FGFR1, leading to the repression of the expression of the NPT2a and NPT2c type II co-receptors of the proximal tubules, to lower phosphate reabsorption. Additionally, FGF23 reduces synthesis of the 1,25(OH)2D3 in the kidney, in order to lower Ca2+ and phosphate absorption in the intestine. Therefore, inactivation of FGF23 is mediated by its cleavage in serum, secondary to FAM20C activity, in order to increase phosphate reabsorption and to decreases waste urinary phosphate. Also, FGF23 deficiency can result in vitamin D3 elevation, with secondary hypercalcemia, hyperphosphatemia, ectopic calcifications and bone mineralization defects [64-68].

Hence, the absence of FAM20C results in FGF23 hypophosphorylation favoring O-glycosylation, which in turn decreases its degradation and increases its circulant levels and activity, leading to FGF23 elevation. More active FGF23 results in greater phosphate wasting and secondary hypophosphatemia, additionally to a low circulating 1,25(OH)2D3 [64-68]. This translates into hypophosphatemia and hypocalcemia or hypophosphatemic rickets in some RS patients [6,22, 33, 35,55]. Moreover, FGF23 probably induces parathyroid hormone (PTH) secretion by the parathyroid glands through its interaction with
Klotho protein (KL), which is also induced by the decrease of serum calcium. PTH increases the reabsorption of calcium and phosphates and stimulates the production of 1,25(OH)2D3 by proximal tubular cells [66,68]. As a result of this, we suggest that all patients with unexplained bone sclerosis and RS-like features should have a metabolic bone screening.

In addition to bone tissue, FAM20C is expressed in teeth, causing severe dentin and enamel defects in non-lethal RS patients [70-73], including Amelogenesis imperfecta (AI) and abnormal dentinogenesis, which are considered NLRS common features [31-32]. As most patients with lethal RS die within the first days of life, it is not possible to determine them in affected individuals as in cases 1 and 2, and to date only one lethal case that was two years old with teeth defects has been reported.

In regards to the absence of Langerhans islets in case 2, which was not previously reported, there are no data indicating a role of FAM20C in pancreatic development. However, FAM20C has a potential role in regulating β-cell secretory pathway function through multiple protein targets in mice [74]. This points to a FAM20C role in pancreas activity and supports potential FAM20C targets in pancreatic development. Finally, neurological involvement in NLRS, as well as in two lethal cases (including our case 2), suggests that FAM20C also plays a role in brain development and function. This is another tissue to look into for new target involvement. Although RS is presently defined by bone and mineralization disorders, the recent identification of several targets in different tissues gives new clues to the understanding of its pathogenesis.

In summary, these two FAM20C variants in the parents of two siblings with LRS represents two new pathogenic variants. These variants were apparently inherited as a biallelic combination in compound heterozygous, both of them causing a frameshift out of frame producing truncated proteins (185 and 207 aa). FAM20C protein was not detected with anti-FAM20C in the liver and the kidney of the 21GW fetus, supporting a possible lethal phenotype caused by truncated protein or kinase domain absence. Remarkable features are pancreas defects (lack of
Langerhans islets), reported here for the first time, as well as agenesis of the corpus callosum, which is an uncommon feature. The craniofacial phenotype and radiological features of the present cases are highly representative of LRS, which include osteosclerosis (highly striking in the skull base and facial bones) and periostic reaction in long bones; the facial phenotype, plus defects in the brain and pancreas. FAM20C activity is evident during early embryonic development, since the phenotype in FAM20C deficiency was evident at 21GW. The addition of brain and pancreas development defects in RS, can lead to search for new FAM20C targets in order to get a better understanding of the wide pathologic and phenotype spectrum of RS.

References


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