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CASE REPORT

A novel *FAM20C* mutation causing hypophosphatemic osteomalacia with osteosclerosis (mild Raine syndrome) in an elderly man with spontaneous osteonecrosis of the knee

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Abstract

Raine syndrome is characterized by FGF23-mediated hypophosphatemic osteomalacia with osteosclerosis caused by mutations in the *FAM20C* gene. We report a case of a 72-year-old man who presented with rapid progressive spontaneous osteonecrosis of the knee (SONK). A full osteologic assessment including dual energy X-ray absorptiometry (DXA), high-resolution peripheral quantitative computed tomography (HR-pQCT), and serum analyses revealed a high bone mass in the lumbar spine and hip (DXA T-score + 7.5 and + 4.7/+4.2) with increased bone microstructural parameters in the distal radius and tibia (BV/TV 127%, 140% of the age-matched mean, respectively), as well as a low bone turnover state. Phosphate levels were low due to renal phosphate wasting and high FGF23 levels (126.5 pg/ml, reference range 23.2–95.4 pg/ml). Using gene panel sequencing, we identified a novel *FAM20C* heterozygous missense mutation in combination with a homozygous duplication that potentially alters splicing. Taken together, this is the first case of mild Raine syndrome with spontaneous osteonecrosis of the knee, phosphate wasting, and a pronounced trabecular high bone mass phenotype.

Keywords *FAM20C* · High bone mass · Osteomalacia · Raine syndrome

Introduction

Raine syndrome is a very rare genetic skeletal disorder that was first described in 1989 and that is defined by osteomalacia with osteosclerosis expressed by increased bone mass and hypophosphatemic osteomalacia [1]. At the molecular level, the disease is caused by homozygous or compound heterozygous mutations of *FAM20C*, encoding a protein kinase located in the Golgi apparatus facilitating the phosphorylation of various secreted proteins [2]. These include several proteins with relevance to biomineralization, such as the small integrin-binding ligand, N-linked glycoproteins (SIBLINGs) and also FGF23, an osteocyte-derived key hormone of mineral homeostasis [3]. More specifically, it was shown that FAM20C-mediated phosphorylation on Ser180 inhibits O-glycosylation of FGF23, thereby promoting FGF23 inactivation [4]. Alternatively, it was reported that FAM20C affects *FGF23* expression in a DMP1-dependent manner [5].

Although the full complexities underlying the action of FAM20C in mineral homeostasis remain to be established, increased levels of bioactive FGF23 in the serum are most

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likely the major explanation for the hypophosphatemia observed in patients with Raine syndrome [6, 7]. Importantly, whereas most reported patients with Raine syndrome are severely affected, presenting with additional cerebral calcification and high neonatal lethality, [8, 9], also mild forms of non-lethal Raine syndrome were recently reported [10, 11]. This implies that FAM20C-dependent disease states are more frequent than previously anticipated and that *FAM20C* mutations may cause late-onset conditions, as described in the following case report.

Patient and methods

Here, we report our findings in a 72-year-old man, who experienced rapid progressive spontaneous osteonecrosis of the right knee (SONK, Ahlbäck disease) accompanied by a patchy bone marrow edema of the right distal femur. In order to further analyze his bone status, the areal bone mineral density (aBMD) was evaluated using dual energy X-ray absorptiometry (DXA, Lunar iDXA, GE Healthcare; Madison, WI, USA).

Serum and urinary biochemical bone turnover markers including calcium, 25- and 1,25-hydroxyvitamin D, parathyroid hormone (PTH), osteocalcin, bone-specific alkaline phosphatase (BAP), and deoxypyridinoline (DPD), were assessed. Phosphate metabolism was determined by multiple fasting serum phosphate measurements as well as tubular maximal rate of phosphate reabsorption in relation to the glomerular filtration rate (TmP/GFR, mmol/l). Intact FGF23 (iFGF23) was measured by an immuno-chemiluminescent sandwich assay (DiaSorin Liaison XL), while c-terminal FGF23 (cFGF23) was additionally measured by ELISA. For bone microstructure analysis, high-resolution peripheral quantitative computed tomography (HR-pQCT; XtremeCT, Scanco Medical, Switzerland) was performed at the distal radius and tibia in a standardized procedure using the in vivo protocol. HR-pQCT results were compared to age- and sex-matched reference values from the literature (50th centile for men aged 70–80 years) [12].

Mutational analysis was carried out by enrichment using a custom designed SureSelect XT gene panel (Agilent, Santa Clara, CA, USA) containing all coding exons of 409 genes in which mutations have been associated with changes in bone mass, skeletal dysplasias, dysostoses, or connective tissue diseases (skeletal disorder associated genome (sDAG)). The sequencing was done on a MiSeq sequencing machine (Illumina, San Diego, CA, USA). Data were analyzed by the software tools GeneTalk and PhenIX. The pathogenicity of the prioritized variants was judged using MutationTaster (http://www.mutationtaster.org). The mutations were verified by Sanger sequencing.

Results

The patient presented with normal stature and appearance (183 cm, BMI 28.2 kg/m^2, no dysmorphic face, no clinical signs of dysplasia). Besides the pronounced osteonecrosis of the medial femur condyle and patchy T2-hyperintense bone marrow lesions detected by magnetic resonance imaging (MRI, Fig. 1a), no other diseases or symptoms had been detected. Intracerebral calcifications were not investigated. The time between the onset of knee symptoms and the well-advanced osteonecrosis was around 6 months (Fig. 1a). The patient had not suffered from any fractures. The osteonecrosis was readily visible in radiography (Fig. 1b) and unicondylar knee replacement was performed.

The initial osteologic assessment by DXA revealed a high bone mass in the spine (DXA T-score + 7.5) and hip (DXA T-score + 4.7/+ 4.2 left/right) (Fig. 1c, Table 1). Neither compression fractures nor sandwich vertebrae were detected by vertebral fracture assessment (VFA) (Fig. 1d). High-resolution peripheral quantitative computed tomography (HR-pQCT) at the distal radius and tibia indicated increased trabecular parameters (mostly trabecular number) at both skeletal sites compared to reference values from the literature [12], while cortical thickness was not increased (Fig. 1e, Table 1).

Biochemical analyses showed low phosphate levels, low tubular phosphate reabsorption (TmP/GFR), and high normal c-terminal FGF23 levels as well as low markers of bone formation (i.e., osteocalcin and BAP) and moderately increased bone resorption (DPD cross links) (Table 2). Kidney function, alkaline phosphatase, calcium, 25-hydoxyvitamin D3, 1,25-dihydoxyvitamin D3, and parathyroid hormone were within the normal range. Other causes for hypophosphatemia and osteonecrosis were excluded by appropriate laboratory tests. In fact, levels of hemoglobin, gamma-GT, thyroid stimulating hormone, testosterone, and gastrin were normal, while protein electrophoresis and immunofixation were also inconspicuous. At 6-month follow-up, laboratory analyses showed a persistent phosphate wasting with high iFGF23 and c-terminal FGF23 values (126.5 pg/ml, reference range 23.2–95.4 pg/ml; 117 kRU/l, reference range 26–110 kRU/l, respectively).

Mutational analysis revealed two variants in the *FAM20C* gene (Fig. 1f). The first variant c.906C>A results in a heterozygous exchange p.(Phe302Leu) on the protein level and was ranked to be pathogenic due to complete evolutionary conservation of the residue and absence of the variant in ExAC and gnomAD databases (Fig. 1g). The second variant c.952_956+30dup is a duplication of a gene section comprising parts of exon 4 and of intron 4, which we found in a homozygous state (Fig. 1f). This duplication is a common polymorphism, which alone is not disease causing. However, we speculate that it

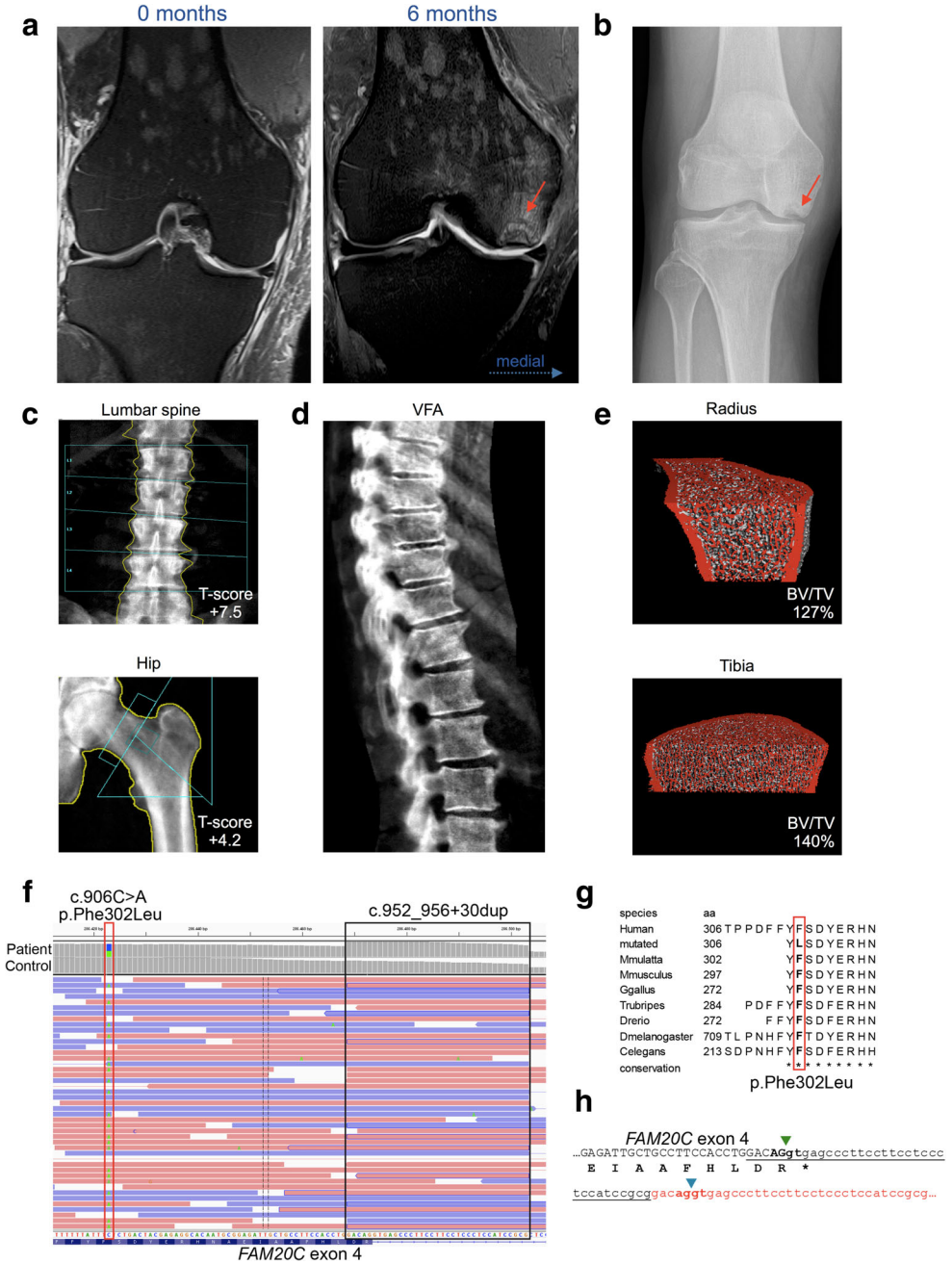

Fig. 1 Spontaneous osteonecrosis of the knee in a patient with mild Raine syndrome. **a** MRI findings at initial presentation and after 6 months. Note the patchy bone marrow edema and the osteonecrosis in the medial condyle. Proton density (PD) weighted sequences at 6 months. **b** Anteroposterior radiography with the osteonecrosis area (red arrow) and unicondylar knee replacement. **c** Increased bone mass in detected by dual energy X-ray absorptiometry (DXA) in the spine and the hip. **d** No fractures or sandwich vertebrae (vertebral fracture assessment, VFA). **e** Bone microstructure analysis using HR-pQCT in the distal radius and tibia pointed to increased trabecular bone mass characterized by predominant increase in trabecular number. **f**–**h** Results of molecular genetic testing. **f** IGV browser image showing the heterozygous variant c.906G>A; p.Phe302Leu and the duplication c.952_956+30dup at the end of exon 4 of the *FAM20C* gene. **g** Multispecies alignment of the amino acid residues surrounding p.Phe302 showing high conservation. **h** Possible effects of the duplication on the splice donor site of exon 4. Exonic bases are given in capital letters, intronic bases in minuscule. The first copy of the duplicated sequence stretch is underlined and the second copy is given in red letters. Arrowheads indicate splice sites. Green: normal splice site, blue: novel potential splice site. If splicing occurs in the novel splice site (blue), the exon 4 sequence would be extended leading to a premature stop codon (*)

results in a mild alteration of splicing by usage of the second downstream splice donor site predicted by Human Splice Finder (Fig. 1h) (Supp. Fig.). The resulting transcripts would harbor a premature stop codon. In combination with the missense variant, this possibly causes the observed mild phenotype. We found no variants in *FGF23*, *PHEX*, *DMP1*, or

Table 1 Bone mineral density by DXA and bone microstructural measurements by HR-pQCT

DXA	L1-L4	Left Hip
BMD (g/cm^2)	2.12	1.63
T-score	+ 7.5	+ 4.2
HR-pQCT	Radius	Tibia
BV/TV, % (% mean)	19.9 (127%)	21.3 (140%)
Tb.N, n/mm (% mean)	2.61 (127%)	2.8 (142%)
Tb.Th, mm (% mean)	0.076 (104%)	0.076 (95%)
Ct.Th, mm (% mean)	1.0 (103%)	1.27 (94%)

HR-pQCT data were compared to sex- and age-matched reference values [12]. *BMD* bone mineral density, *BV/TV* bone volume/tissue volume, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Ct.Th* cortical thickness

ENPP1 that could explain the hypophosphatemia, especially not in combination with the considerably elevated bone mineral density. Furthermore, there were no variants in the known gene mutations for osteosclerotic bone disorders (e.g., *CLCN7*, *SOST*, *LRP5*).

Discussion

In this report, we outline a new phenotype of hypophosphatemic osteomalacia with osteosclerosis (or Raine syndrome) associated with osteonecrosis of the knee caused by two FAM20C variants in an elderly man. The elevated BMD T-scores in the hip and spine were accompanied by increased trabecular parameters in HR-pQCT, which has not been performed yet in patients with *FAM20C* mutations. Similar to our data, a homozygous *FAM20C* mutation was found in a 61-year-old man with hypophosphatemic osteomalacia and increased FGF23 levels [11]. Furthermore, there are other studies reporting non-lethal Raine syndrome [10, 13–15]. However, the common forms of Raine syndrome have been defined by characteristic face, brain abnormalities including intracerebral calcifications, and neonatal lethality [1, 8, 16]. In general, *FAM20C* was found to be a crucial molecule for bone development [13].

Bone marrow edema syndrome and osteonecrosis of the knee have not been reported previously in patients with Raine syndrome. The concept that mineralization defects (i.e., osteomalacia) can cause bone marrow edema with subsequent risk of osteonecrosis has previously been suggested [17, 18]. Consistently, bone marrow edema or osteonecrosis was observed during pregnancy or vitamin D deficiency [18, 19], both situations associated with mineralization defects. Therefore, it is likely that in our case the osteonecrosis (SONK) was at least partly caused by the mineralization defect due to hypophosphatemia. Interestingly, we also detected low bone formation markers with moderately increased bone resorption indicating an overall low bone turnover, which does not necessarily match the phenotype of osteomalacia typically being associated with increased levels of (bone-specific) alkaline phosphatase.

In our patient, the first *FAM20C* variant was ranked pathogenic, while the second variant was reported to be present in 11% of the general population. The combination of both variants was related to an altered gene function causing the respective mild but characteristic phenotype in terms of high bone mass and phosphate wasting. It is not uncommon that polymorphisms can influence expression of genetic disorders. For example, the severity of spinal muscular atrophy (SMA) depends on the copy number of the SMN2 gene, which is variable in the population [20].

In conclusion, we reported a novel FAM20C heterozygous missense mutation in combination with a homozygous duplication in a 72-year-old patient, which extends the genetic and clinical spectrum of patients with mild Raine syndrome. Here, the clinical phenotype is in line with laboratory and skeletal features that have been reported in (mild) Raine syndrome, while we additionally describe bone marrow edema and rapid progressive spontaneous osteonecrosis of the knee.

Table 2 Laboratory results

Parameter	Patient	Reference
Creatinine (mg/dl)	0.98	0.6–1.3
AP (U/l)	49	40–129
Ca (mmol/l)	2.39	2.13–2.63
25-OH-D_3 (μg/l)	50.5	> 30
1,25-$(OH)_2$-D_3 (ng/l)	56	20–79
P (mmol/l)	*0.61*	0.77–1.50
TmP/GFR (mmol/l)	*0.41*	0.8–1.35
PTH (ng/l)	51.9	17–84
FGF23 (kRU/L)	97 (*117*)	26–110
Osteocalcin (μg/l)	*6.6*	12–52.1
BAP (μg/l)	8.1	5.5–22.9
DPD cross-links (nmol/mmol)	*6*	2–5

AP alkaline phosphatase, *Ca* calcium, *25-OH-D_3* 25-hydroxyvitamin D, *P* phosphate, *TmP/GFR* tubular maximal rate of phosphate reabsorption in relation to the glomerular filtration rate, *PTH* parathyroid hormone, *FGF23* fibroblast growth factor 23, *BAP* bone-specific alkaline phosphatase, *DPD* deoxypyridinoline. Italics indicate abnormal values. C-terminal FGF23 levels were increased at 6-months of follow-up (in parentheses)

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Compliance with ethical standards

Conflicts of interest None.

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