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Case Report

Exome sequencing reveals a mutation in *DMP1* in a family with familial sclerosing bone dysplasia

Marie-Hélène Gannagé-Yared [a,1], Periklis Makrythanasis [b,c,1], Eliane Chouery [d], Cristina Sobacchi [e,f], Cybel Mehawej [d], Federico A. Santoni [b], Michel Guipponi [c], Stylianos E. Antonarakis [b,c,g], Hanan Hamamy [b], André Mégarbané [a,h,*]

[a] Département d'Endocrinologie, Faculté de Médecine, Université Saint-Joseph, Beirut, Lebanon
[b] Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland
[c] Service of Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland
[d] Unité de Génétique Médicale, Faculté de Médecine, Université Saint-Joseph, Beirut, Lebanon
[e] CNR/IRGB, UOS Milan Unit, Milan, Italy
[f] Humanitas Clinical and Research Center, Rozzano, Milano, Italy
[g] iGE3 Institute of Genetics and Genomics of Geneva, Geneva, Switzerland
[h] Institut Jérôme Lejeune, Paris, France

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ABSTRACT

Introduction: Hypophosphatemic rickets (HR) comprises a rare group of inherited diseases. Very recently, mutations in the dentin matrix protein 1 (*DMP1*) gene were identified in patients with an extremely rare autosomal recessive form of HR (ARHR). To date, very few cases of these mutations were reported.
Materials and methods: A Lebanese consanguineous family with 2 affected sisters was studied. Patients aged 45 and 47 years old presented with short stature, severe genu varum, cranial hyperostosis and a very high bone density that led to a diagnosis of a familial sclerosing bone dysplasia. Molecular analysis of known genes involved in osteopetrosis showed normal results. A combination of genotyping and exome sequencing was performed in order to elucidate the genetic basis of this pathology.
Results: Biochemical analysis was consistent with normal serum calcium and 1-25$(OH)_2$D levels, low to normal serum phosphorus and elevated PTH values. Serum c-terminal FGF-23 was elevated in one of the two patients. A homozygous mutation disrupting the initiation codon of the *DMP1* gene (OMIM 600980), NM_001079911.2: c.1A>G, p.Met1Val, was identified by exome sequencing and confirmed by Sanger sequencing.
Conclusion: We report here a family of ARHR secondary to a *DMP1* mutation located in the first coding exon of the gene. Our cases show that some ARHR cases may develop with age an unaccountable increase in bone density and bone overgrowth.

Introduction

Hypophosphatemic rickets (HR) comprises a group of rare inherited diseases with an incidence of 4 per 100,000 live births [1]. The most common form, the X-linked HR is associated with mutations in the phosphate-regulating endopeptidase homologue X-linked (*PHEX*) gene identified in 1995 [2]. Subsequently, mutations in several other genes have been reported. In 2000, the principal regulator of phosphate metabolism, the fibroblast growth factor 23 (*FGF23*) was isolated and mutations of this gene were associated with the autosomal dominant form of the disease [3]. Additionally, pathogenic variants in two other genes the dentin matrix protein 1 (*DMP1*) [4–10] and the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) [11] were identified in patients with an extremely rare autosomal recessive HR (ARHR) type. The clinical, biochemical, and histomorphometric parameters found in ARHR are very similar to those observed in both X-linked and autosomal dominant HR and includes bowing of legs and growth failure during childhood, elevated FGF-23 and inappropriately normal 1-25-dihydroxyvitamin D (1-25$(OH)_2$D) concentrations.

DMP1 was isolated originally from a rat incisor library and codes for a noncollagenous extracellular matrix protein. It is highly expressed in the bone and teeth [12] and promotes mineralization [13] and

* Corresponding author at: Unité de Génétique Médicale. Faculté de Médecine., Université Saint-Joseph, 42, rue de Grenelle, 75007 Paris, France. Fax: +961 1 421 021.
E-mail addresses: mhcyared@terra.net.lb (M.-H. Gannagé-Yared), Periklis.Makrythanasis@unige.ch (P. Makrythanasis), eliane.chouery@usj.edu.lb (E. Chouery), Cristina.Sobacchi@humanitasresearch.it (C. Sobacchi), Cybel.mehawej@net.usj.edu.lb (C. Mehawej), Federico.Santoni@unige.ch (F.A. Santoni), Michel.Guipponi@unige.ch (M. Guipponi), Stylianos.Antonarakis@unige.ch (S.E. Antonarakis), hananhamamy@yahoo.com (H. Hamamy), megarbane@usj.edu.lb (A. Mégarbané).
[1] Both first authors collaborated equally to this work.

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odontogenic differentiation [14,15]. *DMP1* acts as a transcriptional activator of osteoblast-specific genes such as alkaline phosphatase and osteocalcin, it moves out to the extracellular matrix during the osteoblast–osteocyte transition phase to promote mineralization and phosphate homeostasis [16]. To date only 7 mutations have been reported in the 6 exons of the *DMP1* gene [4–10]; most of them are located in exon 6, suggesting that this area could be highly susceptible to genetic changes [17].

We report here a *DMP1* mutation in 2 sisters born to consanguineous Lebanese parents. Both patients were previously reported as presenting a new atypical form of sclerosing bone dysplasia [18].

Materials and methods

This study was granted approval from the Saint Joseph University of Beirut Committee on Clinical Investigation and conformed to the tenets of the Declaration of Helsinki.

A Lebanese family with two sisters previously reported by Chouery et al. [18] was studied. In both patients, fasting serum calcium, phosphorus, albumin, creatinine and alkaline phosphatase were measured using an automated Cobas Integra by Roche Diagnostics. 25-hydroxyvitamin D (25(OH)D) and third generation parathormone (PTH) were measured using the Diasorin automate Liaison. 1-25-dihydroxyvitamin D ($1\text{-}25(OH)_2D$) was measured using the Diasorin radioimmunoassay. Human FGF-23 was measured using an ELISA kit that detected epitopes in the C-terminal end of the protein (Labor Limbach laboratory).

After informed consent was obtained from patients, EDTA blood samples were collected and DNA was extracted, then amplified using standard methods. In order to elucidate the genetic basis of the pathology, a combination of homozygosity mapping and exome sequencing was adopted. DNA from the patients and their unaffected siblings were first genotyped using the HumanOmniExpress Bead Chip by IlluminaInc® to define Runs of Homozygosity (ROH). The SNP array tests 730K SNPs with a mean distance of 4 kb between the SNPs. ROH for every individual were identified using PLINK. We defined as ROH the regions with 50 homozygous consecutive SNPs irrespective of the total size of the genomic region, allowing for one mismatch. The ROH region was the one demarcated by the first heterozygous SNPs flanking each established homozygous region. Subsequently, exome sequencing was performed in one of the patients. Exome was captured using the SureSelect Human All Exons v3 reagents (Agilent Inc®). Sequencing was performed in an IlluminaHiSeq 2000. The raw results were analyzed using a custom pipeline which utilizes published algorithms in a sequential manner (BWA for mapping the reads, SAMtools for detection of variants, Pindel for the detection of indels, ANNOVAR for the annotation). All experiments were performed using the manufacturer's recommended protocols without modifications. After calling the variants, homozygous exonic and splicing variants (± 2 bp from the intron–exon junction) were first selected. ROH coordinates, genotypes and exome results were then processed using CATCH 1.1, an in-house algorithm that takes into account the family information and assigns every variant to a different class according to how well it respects the segregation of the ROH. The next parametrisable filtering step selects variants with allele frequencies lower than 0.01 in public databases (dbSNP 135, 1000 genomes, Exome Variant Server).

Results

Clinical findings

The patients, two sisters aged 45 and 47, were born to healthy first cousins parents. At a very young age, the parents' attention was drawn by the appearance of a progressive genu varum in both sisters followed a few years later by a progressive enlargement of the jaw. In addition, the oldest sister was operated 3 times from her genu varum during her childhood. At the age 35, she presented neurologic signs such as cervical pain radiating to shoulders, cubital paresthesia and weakness of the lower limbs. A cervical laminectomy was not possible because the bones were too hard. In 2005, both sisters were first seen by us at the age of 37 and 39. At that time, they presented with short stature (155 and 151 cm, 10th and 3rd percentiles respectively) and dysmorphic features comprising a square face appearance, high forehead, mild proptosis, symmetrical enlargement of the jaw and protruding chin [16]. Bone abnormalities included cranial base and vault hyperostosis with increased density of the orbits, severe genu varum, cortical bone thickening mainly at the diaphysis of the lower limb long bones, diaphyseal modeling defects, increased width of the ribs, and short femoral necks [16]. A marked increase in bone mineral density (values 200–300% above values for age) was also observed [18]. When seen again in 2012, no changes in the clinical phenotype were noted. Recent X-rays show diffuse osteocondensation (sacroiliac articulations, long bones, calcifications of the anterior and posterior spine ligaments) dysmorphic, enlarged and convex aspect of the femoral diaphysis (Fig. 1).

Biochemical analysis findings

Laboratory results in 2005 in both affected patients were unremarkable except for slightly low serum phosphorus and high serum C-telopeptide values. Results in 2012, when patients were reevaluated at the age of 45 and 47, are shown in Table 1. Normal serum calcium and $1\text{-}25(OH)_2D$ levels with low to normal serum phosphorus and elevated PTH values were observed in both sisters while serum c-terminal FGF-23 was elevated in one of them.

Molecular findings

By genotyping data analysis, a total of 90, 94, 95 and 94 ROH were detected in the four genotyped individuals for a total size of 362, 298, 360 and 123 Mbp, respectively. Pairwise IBD comparisons between the individuals using PLINK showed that they shared 50.4% to 63.09% of their genome confirming the sibship. After exome sequencing 69,323,813 reads were on RefSeq protein coding exons, resulting in a coverage of at least 8× in 95.04% of them. A total of 21,232 exonic and splicing variants were detected of which, 10,489 were synonymous and 9169 were missense. After using CATCH only 48 were found respecting the selection criteria and the ROH segregation. 25 were excluded after being found with high frequency in our local database of people with the same ethnic origin, 8 variants were also filtered out for their low quality, 8 variants for affecting the polypyrimidine tract of the acceptor splice site and 1 for wrong attribution of homozygous status. For the 6 remaining variants in *RIF1*, *DMP1*, *DNAH12*, *FRAS1*, *PLK5* and *RAPSN* a voting system among SIFT, PolyPhen2 and MutationTaster was used demanding that at least 2 of them would estimate the variant

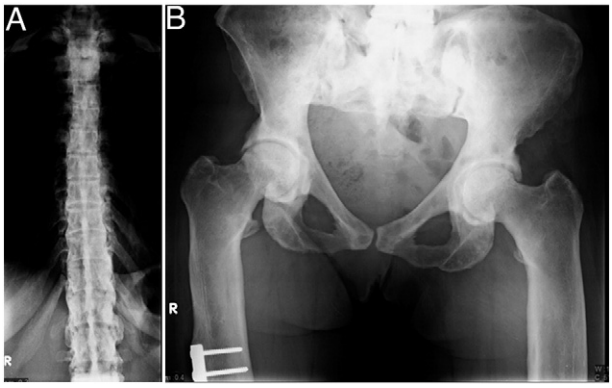

Fig. 1. X-rays of the eldest patient at the age of 47 showing (A) diffuse osteocondensation and (B) Thick and undermodeled femur with short femoral neck.