

An autosomal dominant hypophosphatemic rickets phenotype in a Tunisian family caused by a new *FGF23* missense mutation

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Abstract Autosomal dominant hypophosphatemic rickets (ADHR) is a rare disease, characterized by isolated renal phosphate wasting, hypophosphatemia, and inappropriately normal 1,25-dihydroxyvitamin D₃ (calcitriol) levels. This syndrome involves rickets with bone deformities in childhood and osteomalacia, osteoporosis, articular and para-articular pain, and fatigue in adulthood. It is caused by mutations in a consensus sequence for proteolytic cleavage of the *FGF23* protein. Normally, this protein actively regulates phosphate homeostasis. Here we report a Tunisian family in which one parent and three children show clinical and biological features of ADHR. Mutation analysis of the *FGF23* gene finds a heterozygous substitution of the C at position 526 by a T (526 C → T), leading to an amino acid replacement of the *FGF23* protein (R176W) at position 176. This causative new mutation is located in the consensus sequence for the proteolytic cleavage domain. These results confirm the importance of this site in *FGF23* function and its essential role in ADHR physiopathology.

Keywords Autosomal dominant hypophosphatemic rickets · Osteomalacia · *FGF23* gene · Mutation analysis · Cleavage domain

Introduction

Maintenance of proper serum phosphate concentrations is required for normal skeletal development and for preservation of bone integrity. In addition, phosphate is essential for many cellular processes such as energy provision in the form of ATP, DNA and RNA synthesis, and kinase and phosphatase regulation of intracellular signaling [1]. Recent advances in understanding of disorders involving phosphate metabolism have shed light on the underlying mechanisms that control phosphate homeostasis in normal and in disordered states [1]. Rickets is characterized by defects in bone mineralization at the sites of growth or remodeling, leading to bone deformity and stunted growth in children. In adults, the disease is known as osteomalacia [2]. Hypophosphatemic rickets was originally termed vitamin D-resistant rickets to differentiate it from nutritional rickets, which readily responds to vitamin D supplements. Hypophosphatemic rickets is caused by the lack of renal tubular phosphate reabsorption, an important component of bone minerals. Types of hypophosphatemic rickets include X-linked hypophosphatemic rickets, autosomal dominant hypophosphatemic rickets (ADHR), hereditary hypophosphatemic rickets with hypercalciuria, tumor-induced osteomalacia, complex tubulopathies, primary hyperparathyroidism, and moderate idiopathic phosphate diabetes of adulthood [3].

Autosomal dominant hypophosphatemic rickets is a rare disease (1 in 20000) [4, 5]. The clinical manifestations depend on the age of patients and the importance of hypophosphatemia. In adult onset, it can cause osteomalacia, osteoporosis, articular and para-articular pain, tiredness, and rare cases of lithiasis. In childhood, clinical manifestations are rickets with bone deformities. The vitamin D-resistant rickets or osteomalacia should guide

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the diagnosis. Diagnosis confirmation is based on the dosage of phosphate clearance rate and the threshold of phosphate reabsorption [6].

In 2000, a genetic analysis of families with ADHR successfully identified that the fibroblast growth factor 23 gene (*FGF23*) is responsible for this disease [4]. Both human and experimental studies have convincingly demonstrated that this factor can actively regulate phosphate homeostasis. FGF23 is a circulatory hormone that can be produced by osteocytes in response to dietary phosphorus intake. Increased serum level of FGF23 induces urinary phosphate excretion to maintain the normal physiological mineral balance. This finding represents a paradigm shift from traditional thought that serum phosphate levels are passively regulated through the endocrine effects of calcitrophic hormones, such as vitamin D and parathyroid hormone (PTH).

In the FGF23 protein, amino acids from 176 to 179 consist of a consensus sequence for proteolytic cleavage: Arg176-His177-Thr178-Arg179. Mutations located in one of these two arginines such as R176Q, R179Q, and R179W lead to a cleavage-resistant protein that remains as an active intact form; this involves an exaggeration of the urinary excretion of phosphate. The discovery of FGF23 as a cause of ADHR has shed light on the humoral regulation of the reabsorption of phosphate in renal tubules and phosphate homeostasis [1].

Here we report a new mutation of *FGF23* gene in a Tunisian family with ADHR located in the consensus sequence for proteolytic cleavage domain. Some members of this family have rickets and the others osteomalacia.

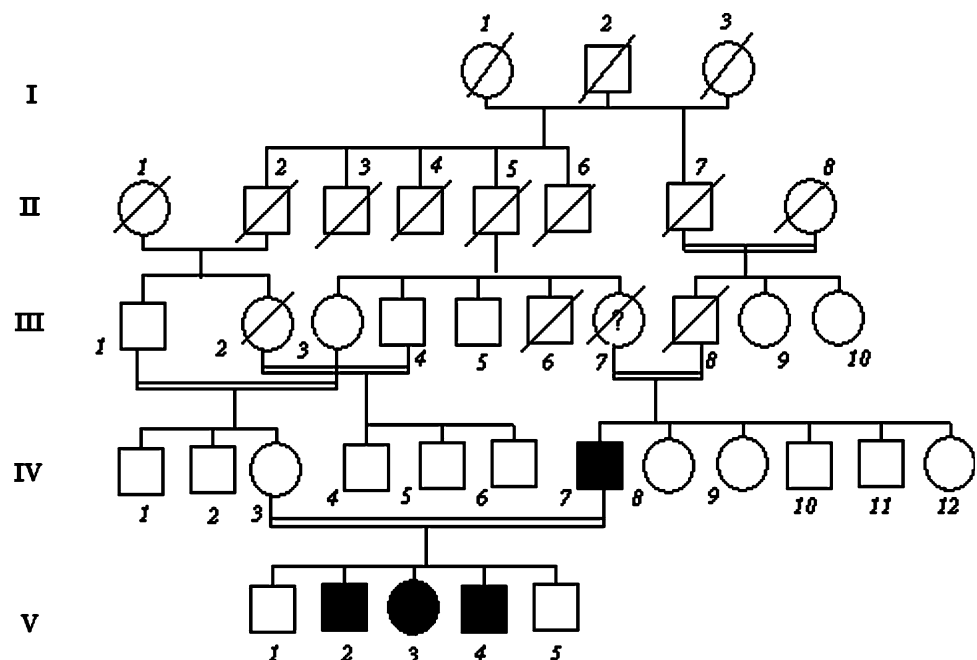
Case report

Clinical, radiologic, and biochemical findings

The reported family is composed of parents and five children. The parents are consanguineous and originate from a village in the eastern province of Tunisia. The pedigree of the family is shown in Fig. 1.

The index case is the father (IV7), aged 58 years and hospitalized in 2001, in Rheumatology Department of Monastir Teaching Hospital, for weakness of the lower limbs and a 2-year history of trouble in walking. The physical examination showed a short stature of 160 cm (average height of men in Tunisia = 170 cm), a pigeon chest deformity, difficulty with walking using two canes, and muscle wasting predominant in the pelvic muscles. The right hip joint was painful with reduced mobility. The deep tendon reflexes were normal. He had neither leg deformity nor dental abnormality. The radiograph of the pelvis showed a right femoral fracture with pelvic fissures. Biochemical investigation showed a low level of serum calcium at 2.08 mmol/l (normal 2.25–2.65 mmol/l), hypophosphatemia at 0.33 mmol/l (normal 0.8–1.4 mmol/l), a high level of total alkaline phosphatase at 1023 UI/l (normal 150–400 UI/l), increased urinary phosphate clearance at 70 ml/min (normal 5–12 ml/min), and renal threshold phosphate concentration (TmPO₄/GFR) of 0.46 mg/dl. Those biochemical abnormalities were reproducible. Serum levels of 25-hydroxyvitamin D (20.7 µg/l), PTH (40.5 ng/l), urinary pH, and protein electrophoresis were normal. There was neither aminoaciduria nor glucosuria. Bone scintigraphy showed

Fig. 1 Pedigree of the family



multiple fissures in the pelvis and ribs. Bone mineral density determined by X-ray absorptiometry method was low with a *T* score of -3.6 SD in the femur. Drug-induced and tubular causes of this phosphoric loss were excluded. Abdominal and chest scan did not show any tumoral process. This patient was treated with phosphor (4.5 g/day) and ergocalciferol (4 g/day), involving a progressive clinical and biological improvement in 2 years with regain of walking without help, and increase of serum calcium at 2.33 mmol/l and phosphate at 0.74 mmol/l, but serum level of total alkaline phosphatase still mildly high at 620 UI/l. On the other hand, there was improvement in muscle wasting and walking ability. The femoral neck fracture was treated functionally with rest.

In 2007, his 27-year-old son (V2) was hospitalized for asthenia and low back and right foot pain. There was no suggestion of any chronic disease in his childhood. Physical examination revealed a normal height of 169 cm and anterior bowing of legs. He had no other deformities and no dental abnormalities. X-rays showed a fracture of the third right metatarsal bone. Biochemical investigations highlighted a low level of serum calcium (2.1 mmol/l), hypophosphatemia (0.44 mmol/l), elevation of alkaline phosphatase (554 UI/l), increased urinary phosphate clearance at 55 ml/min, and a low TmPO_4/GFR at 0.73 mg/dl. Bone mineral density was normal (*T* score at -1 in the lumbar site and -0.4 in the femur). Like the father, serum levels of 25-hydroxyvitamin D and PTH were normal. There were no tubular acidosis, no

proteinuria, and no glucosuria. He was treated with phosphor (1 g/day) and vitamin D (ergocalciferol 3 g/day) with immobilization of his fracture.

The repeated interrogation of the members of the family revealed that two children (one male and one female, V3 and V4) had been followed in the pediatric department for rickets since the ages of 3 and 5, respectively. They were treated by phosphate. The follow-up was ended at 9 and 12 years of age, respectively, when phosphate levels normalized and urinary loss of phosphate disappeared. They are now 23 and 26 years old. Their physical examination showed dental hypoplasia, frontal bossing, short stature, pigeon chest deformity, anterior bowing of both legs for both children, and retroversion of the pelvis for the daughter. Their serum calcium and phosphate are normal. Their bone mineral densities are normal. The mother and the brother are normal. Their paternal grandmother, who had died many years earlier, was also reported to have had short stature and leg deformities. The main clinical and biological findings of the four patients are summarized in Table 1.

Mutation analysis of the *FGF23* gene

Genomic DNA from the four patients (IV7, V2, V3, and V4) and five healthy members of this family (IV3, IV5, IV6, IV9, and V5) was isolated from peripheral blood

Table 1 Clinical and biological characteristics of patients

Patient	IV7	V2	V3	V4
Gender	M	M	F	M
Age	58	27	26	23
Age of onset	54	27	3	5
Deformities	Yes	No	Yes	Yes
Height (cm)	160	169	138	150
Clinical symptoms	Short stature Lower limb weakness Difficulty in walking Muscle wasting Pigeon chest deformity	Asthenia Low back pain Right foot pain Anterior bowing of legs	Dental hypoplasia Frontal bossing Short stature Pigeon chest deformity Anterior bowing of both legs Retroversion of the pelvis	Dental hypoplasia Frontal bossing Short stature Pigeon chest deformity Anterior bowing of both legs
Fracture	Yes	Yes	No	No
Bone mineral density (<i>T</i> score in femoral site)	-3.6 SD	-0.4 SD	1.1 SD	-0.5 SD
Serum calcium (mmol/l)	2.08	2.1	2.2	2.4
Phosphate (mmol/l)	0.33	0.44	0.9	1.1
Alkaline phosphatase (UI/l)	1023	554	190	NP
Urinary phosphate clearance (ml/min)	70	55	NP	NP
TmP/GFR (mg/dl)	0.46	0.73	NP	NP

TmP/GFR renal threshold phosphate concentration/glomerular filtration rate (TmPO_4/GFR), *NP* not performed

leukocytes by standard procedures. Mutation analysis has been carried out by sequencing the three exons and intronic flanking regions of the *FGF23* gene using these primers: exon 1 (forward: CAG GAG TGT CAG GTT TCA AT, reverse: 5'-AGG GTG CTC CCC TTC TTC); exon 2 (forward: ATT GGA TGG CAA TGA GTC T, reverse: TTA ATT GTT TGC AAA TGG TG); exon 3 (forward: AGG AGG AGC TGG GGA GTG, reverse: TGA GGG ATG GGT TAA AGA G). In all four patients, sequencing analysis revealed the presence of a heterozygous missense mutation in exon 3 with the substitution of the C at position 526 by a T (526 C → T; Fig. 2). Healthy members do not show this mutation. This mutation results in an amino acid substitution at position 176 of the FGF23 protein (R176W). The mutation is located in the consensus sequence for proteolytic cleavage (Arg176-His177-Thr178-Arg179).

Discussion

Osteomalacia or rickets by phosphaturic diabetes poses an etiological problem: it can be inherited or acquired, and needs different kinds of treatment. In ADHR, the urinary phosphate clearance is increased more than 15 ml/min, the rate of tubular reabsorption of phosphate is decreased under 85%, and the renal threshold phosphate concentration is decreased under 0.8 [7]. In adult patients, this disease causes pain in the spinal bones and in the joints and peri-articular areas, osteomalacia, osteoporosis, and fracture without leg deformities. This definition corresponds to our findings since the father had femoral fracture. In childhood, ADHR involves rickets with growth deficiency, small stature, bone deformities, and low serum levels of 1,25-OH₂D₃ [4, 8]. Curiously, as in our two cases, sometimes the renal loss of phosphate disappears in adulthood [9].

In cases of phosphaturic diabetes of genetic cause, the familial context can often indicate the diagnosis. The differential diagnosis of hereditary phosphate diabetes includes X-linked hypophosphatemic rickets, Fanconi's

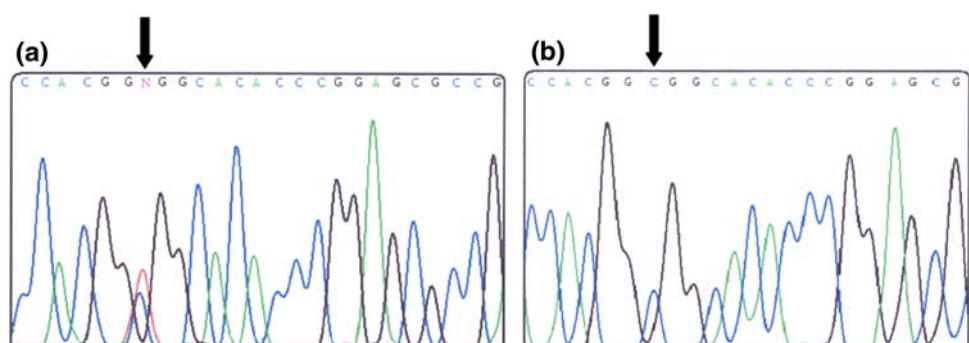
syndromes, distal tubular hereditary acidosis, and primary hyperparathyroidism [10–18]. However, the absence of bowing or knock-knee deformity in the lower limbs, dental abscess, glucosuria, renal wasting of amino acids, calcium, and bicarbonates, muscular hypotonia, and PTH perturbation can eliminate all these diagnoses. Otherwise, the diagnosis can be difficult if the clinical presentation is different among members of the same family. In this case, genetic analysis is essential for the diagnosis and also to screen patients before the onset of symptoms.

In our case, the family history could suggest autosomal recessive or dominant inheritance. The first is corroborated by the multiple loops of consanguinity and would be caused by *DMP1* gene mutations. However, clinical and biochemical findings do not support this hypothesis. The findings are more suggestive of an autosomal dominant form, which is supported by the presence of affected members in two successive generations. The mutation found in the *FGF23* gene confirms this hypothesis.

FGF23 mutations are responsible for ADHR [4]. Up to now, three different mutations in four unrelated families have been described (R176Q, R179Q, R179W) [4]. Interestingly, they were all located in the cleavage motif (RHTR) responsible for the processing of the protein [19–21]. The new mutation that we found (R176W) is located in this conserved proteolytic cleavage site. The arginine residue at position 176 is conserved among species from fish to mammals [22]. These mutations make protein resistant to cleavage and are associated with gain-of-function. Consequently, the full-length FGF23 persists at its active form and continue to inhibit renal phosphate reabsorption, leading to hypophosphatemia and often severe ectopic calcifications [22, 23].

The main targets of FGF23 are kidney and bone tissues through 1C receptors in the distal tubule and cartilage and 2C receptors of the osteoblasts [24, 25]. FGF23 contributes to the regulation of the tubular reabsorption of phosphate independently from PTH and decreases the 1,25-OH₂D₃ production in the proximal tubules, contributing to osteomalacia in ADHR. Consequently, patients should have

Fig. 2 Sequence analysis of exon 3 of the *FGF23* gene in four affected individuals (IV7, V2, V3, and V4) (a) and the healthy mother (IV3) (b). *Black arrow* in a corresponds to the heterozygous missense mutation with the substitution of a C by a T at position 526 (526 C > T); in b, it corresponds to the wild-type homozygous C nucleotide



calcitriol supplementation. Antibodies against FGF23 are being studied for treatment of chronic hypophosphatemia [26].

In conclusion, this report provides additional evidence that FGF23 is a physiological regulator of phosphate homeostasis and proves the importance of the stability of the consensus site in the processing procedure. Further studies will be necessary to understand the role of FGF23 in the regulation of phosphate levels.

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