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ORIGINAL PAPER

Hypophosphatasia may lead to bone fragility: don't miss it

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Abstract Hypophosphatasia is an inheritable disorder characterised by defective bone mineralisation due to the impaired activity of tissue-non-specific alkaline phosphatase (AP). Clinical presentation ranges from stillbirth without mineralised bone to pathological fractures in late adulthood. During childhood, the main manifestations include rickets, growth delay and dental problems. Fractures and bone pain usually characterise the adult form. A 9-year-old girl was referred for repetitive fractures after minimal trauma. She had normal growth, normal sclerae, no rickets and minimal dental abnormalities. Her sister had also presented fractures. The proband, her sister and mother had low total and bone-specific AP levels and E435K mutation in exon 12 of the liver/bone/kidney AP gene. Low AP levels must lead to genetic analysis. Bone fragility and repetitive fractures may be symptoms of hypophosphatasia in childhood, which must not be neglected. Associated factors such as vitamin D or calcium deficiency must be prevented. In conclusion, hypophosphatasia must not be forgotten as an aetiological factor of repetitive fractures or bone pain in children and AP activity should be checked accurately.

Keywords Bone pain · Fractures · Child · Osteoporosis · Hypophosphatasia

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Introduction

Hypophosphatasia (OMIM 146300, 241500, 241510) is an inborn error of the metabolism due to deficiency of the liver/bone/kidney alkaline phosphatase (AP) gene (*ALPL*: OMIM171760). Hypophosphatasia usually presents as rickets or osteomalacia. Depending on the age of clinical onset, the disorder is divided into perinatal, infantile, childhood and adult forms, and odontohypophosphatasia where only dental manifestations are observed [2, 3, 5, 8, 11, 13–15]. A mild prenatal form has recently been described, which represents a sixth clinical form [8, 11]. The disease is due to loss-of-function mutations in the

ALPL gene located on chromosome 1 in position p36.1-34. Besides the lethal perinatal presentation, paediatricians commonly encounter a severe form with impaired skeletal mineralisation, bone deformity, rickets, growth delay, dental abnormalities and respiratory and neurological complications. The adult form begins with the loss of adult teeth, followed by recurrent bone pain and fractures secondary to osteomalacia in late adulthood [3, 5, 13–15]. To the best of our knowledge, hypophosphatasia presenting as marked bone fragility is unusual during childhood. We report the case of two young sisters with repetitive fractures and low plasma AP levels, in whom an inherited mutation of the *ALPL* gene responsible for hypophosphatasia was identified. Premature tooth loss was the only associated symptom. Bone fragility as a predominant symptom of mild hypophosphatasia during childhood is discussed. We suggest that the prevalence of mild hypophosphatasia as an aetiological factor of bone fragility may be underestimated.

Case report

A girl aged 9 and a half years was admitted to our department for repetitive fractures and bone pain. She was the second child of unrelated Caucasian parents. Her weight and height were normal at birth. Her history of bone fractures was unusual. A fracture of the right tibia had been detected when she was 8 years old, after a 3-week period of pain following low-energy trauma when jumping. A second fracture of the right femoral neck was detected when she was 9 years old on X-rays performed because of hip pain. Two additional fractures had occurred without identified trauma, one of the distal metaphysis of the right femur at age 9 and a half years and a one of the right fibula 3 months later. Examination at 9 and a half years of age showed normal growth, no obvious sign of rickets, no bone deformity and normal sclerae. Dental abnormalities, mandibular in particular, with horizontal alveoloclasia and premature loss of at least five deciduous teeth, were noted. At the age of 10 years, she broke her right tibia during school physical activity and, 6 months later, her right forearm when horse-riding (again, low-energy trauma). The family history was suggestive of hereditary bone fragility. The proband's elder sister had experienced an episode of bone pain at the age of 10 years, followed 3 weeks later by the diagnosis of a femoral fracture after a benign trauma when jumping. Later, at age 15 years, X-rays performed for back pain had shown a pedicle fracture of the 4th lumbar vertebra. She had dental abnormalities similar to those of the proband. The mother and father, aged 40 and 42 years, respectively, had no history of bone or dental disease.

Laboratory tests showed normal values of serum calcium, phosphorus, parathyroid hormone (PTH), 25 OH D and 1-25 $(OH)_2$ D in all members of the family. The total and bone-specific AP levels were low for age in the proband, her sister and her mother (Table 1). Serum and urine phosphoethanolamine (measured by ion exchange chromatography at Necker Hospital, Paris, France) were within the normal ranges in the proband and her mother. The sister's serum and urinary phosphoethanolamine levels were at the upper limit of the normal range. In the proband, the biopsy of a hypertrophic tibial lesion in the tibia near the fracture zone revealed excessive amounts of undermineralised osteoid matrix. The proband,

Table 1 Biological parameters

	Proband				Sister	Mother	Father
Age (years)	9	10	13	14	19	40	42
Calcium: (2.15–2.70 mmol/L)	2.37	2.35	2.47	2.36	2.28	2.33	2.23
Phosphorus: (1.30–1.85 mmol/L)	1.49	1.59	1.45	1.5	1.26	1.23	1.18
Total AP: (335–935 IU/L) paediatric normal range, (50–126 IU/L) adult normal range	162*	234*	113*	72*	32*	44*	72
BSAP: (38–64 µg/L) paediatric normal range, (8–15 µg/L) adult normal range	6*		9*	7.6*	2.5*	2.7*	
PTH: (15–85 pg/mL)	17	25	27	30	16	27	
25 OH D: (9–45 ng/mL)	38	33	25	31	38	50	
1-25 OH_2 D: (18–60 pg/mL)	45	58	73	42	45	54	
Phosphoethanolamine: (<4 µmol/L)				ND	5**	ND	
Calcium/creatinine ratio: (0.4–0.7 mmol/mmol)	0.1*	0.7	1.9				
Phosphoethanolamine/creatinine ratio: (<25 mmol/mmol)				19	24	19	

*Low level for age (normal values in parentheses); **high level for age
Bone-specific alkaline phosphatase (BSAP) was determined by immunoassay (Tandem R Ostase, Hybritech Inc., CA). Phosphoethanolamine was measured by ion exchange chromatography (Necker Hospital, Paris, France)
ND = not detectable

her sister and her mother showed X-ray abnormalities similar to those found in hypophosphatasia: irregular osteoporosis, distorted bone trabeculation, projection of non-ossified tissue into the medial metaphysis, pseudo-fracture in the proband and copper-beaten skull in the mother, without craniosynostosis (Fig. 1). There were no other abnormalities, such as bone deformity, long bone spurs, abnormal shape of the terminal phalanges, nephrocalcinosis or dysmorphic facial appearance. In particular, the clinical appearance of the lower limbs was normal in both sisters and not suggestive of a history of rickets. The curvature of the tibia also appeared to be normal (Fig. 1). *ALPL* gene sequencing showed the presence of a heterozygous G>A substitution at position 1345, responsible for E435K mutation in exon 12 in the proband, the sister and the mother. No additional mutation was detected after the complete sequencing of coding sequences and intron–exon borders of the *ALPL* gene. Despite the low AP levels found in some other members of the pedigree (Fig. 2), none had a history of bone fragility. The premature loss of teeth had occurred in three individuals (II-2, II-4, II-6 and III-1). Dual-energy X-ray absorptiometry (DEXA) of the lumbar spine and the total body bone mineral density found low values in all three patients (Table 2). Interestingly, the bone mineral content very markedly decreased during the immobilisation in the right leg of the proband, as compared with the left, and became normal after complete healing and the resumption of physical activity. This suggests that repeated immobilisation may have promoted the recurrence of fractures between the ages of 8 and 10 years.

Discussion

Hypophosphatasia is highly variable in its clinical expression, which ranges from stillbirth to pathological fractures and bone pain occurring during late adulthood [14]. We describe the case of two young sisters presenting with symptoms of the adult form (bone pain and fragility), without bone deformity, obvious rickets or growth delay, which are the classic signs of the paediatric forms. Both girls had premature loss of teeth, a hallmark of hypophosphatasia in childhood. Hypophosphatasia predominantly presenting with repetitive fractures during childhood appears unusual.

We suggest that the prevalence of hypophosphatasia as a cause of bone fragility may be underestimated. First, the diagnosis of hypophosphatasia is based on the determination of AP activity. AP levels and normal ranges vary depending on age and development stage. Such variations are often neglected by physicians. Some laboratories do not even specify the lower limit of AP in children and some physicians may pay attention only to elevated levels and

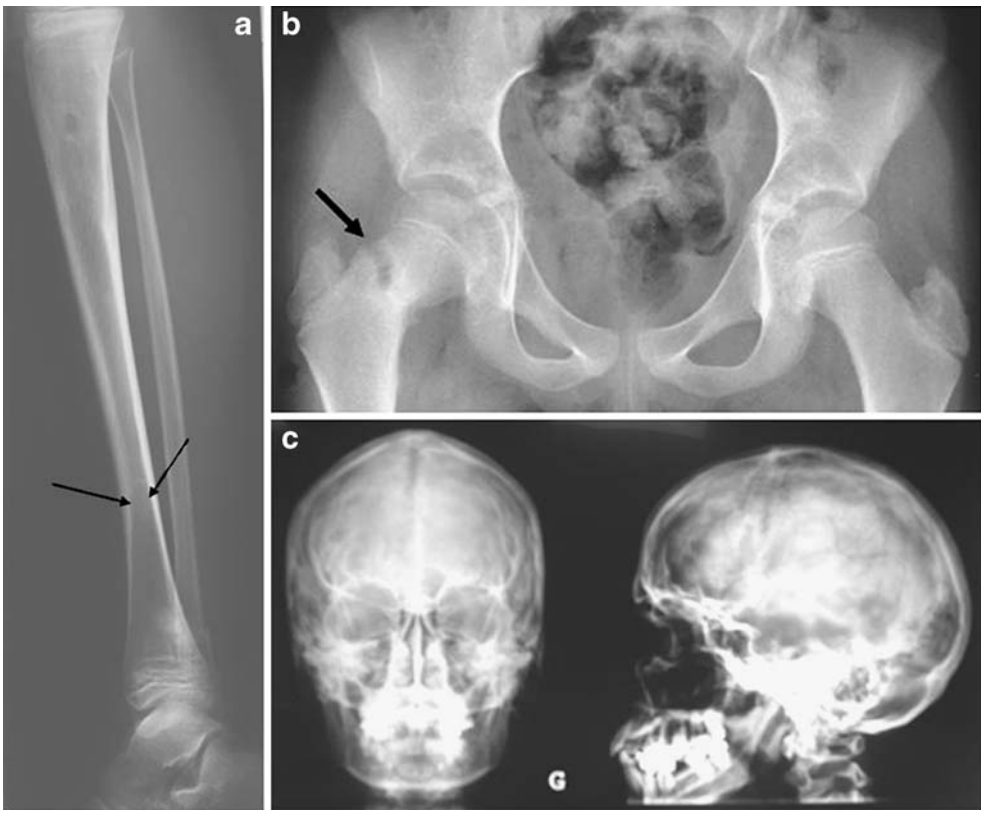

Fig. 1 X-ray abnormalities of the proband (aged 9 years) and her mother (aged 39 years). **a** Distorted trabeculae and irregular osteoporosis with projection of non-ossified tissue ("tongue" of radiolucency, the upper limit shown by the *arrows*) into the medial metaphysis present in the proband at onset. NB: the tibial curvature was clinically normal, not suggesting soft bone. **b** Fracture of the right femoral neck discovered on X-ray analysis performed because of hip pain in the proband at onset. **c** Copper-beaten appearance of the skull in the mother, highly suggestive of a primitive mineralisation defect

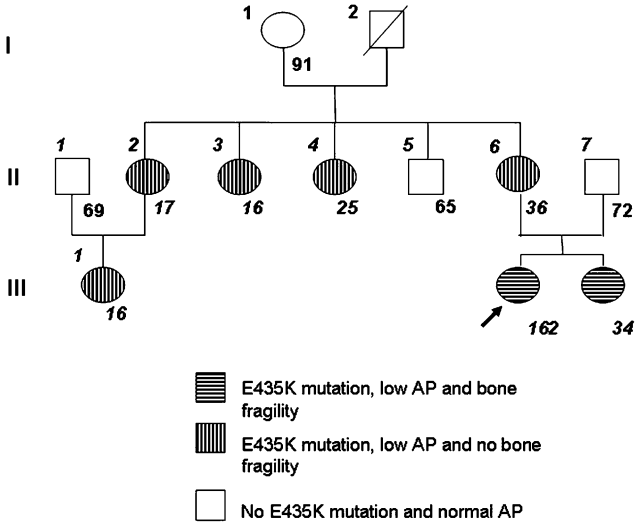

Fig. 2 Alkaline phosphatase (AP) phenotypes and genotypes in the family and cases of hypophosphatasia due to the E435K mutation. The numbers beneath the symbols indicate AP activity levels; low levels are in italics. AP level in the proband is at onset. AP = bone alkaline phosphatase activity, normal range: 335–935 UI/L (paediatric ranges) and 50–126 UI/L (adult ranges). All individuals had normal final height, within 2 SDs

dismiss low AP values. AP levels may also fluctuate in the course of the disease, so repeated measurement may be necessary for a definitive diagnosis. Lastly, hypophosphatasemia may be masked when liver, placental or intestinal isoenzymes are induced by various medications or disorders [14]. Nevertheless, taking into account the age-related reference values and the reliability of their measurement, the AP level, which is low even in mild forms of hypophosphatasia, is the biological hallmark which calls for genetic analysis. Phosphoethanolamine, a marker of AP deficiency, is elevated in severe hypophosphatasia, but is unreliable in mild cases, where it may be normal [14]. We observed a slightly elevated phosphoethanolamine level only in the proband's sister. The variation of phosphoethanolamine levels over time may explain the normal values found in the proband and the mother.

Second, bone pain and fractures are erroneously considered as "normal" in children in the context of rapid growth and frequent falls, and are not extensively investigated. Here, the proband was admitted to hospital and carefully examined because of repetitive fractures occurring over a limited period of time and due to minimal traumas. Her sister, however, had had two fractures in 5 years without any biological assessment, although the circumstances of trauma were similarly unclear.

Third, in mild forms, X-ray abnormalities may be moderate and, apart from fractures or pseudo-fractures, careful analysis is necessary to recognise the mineralisation defect. This was not the case in our patients, where "adult-like" abnormalities were present [3, 5, 13–15].

Our report shows a variability of expression of hypophosphatasia within the family [14]. Several individuals with low AP values and carriers of the E435K mutation did not experience low-energy fractures (Fig. 2). II-2 and II-4 and II-6 (the mother) had premature tooth loss, but not II-3. Quite strikingly, the mother had no history of bone fragility, although she had low AP levels and X-ray abnormalities suggestive of hypophosphatasia (Fig. 1). Fractures, however, occurred similarly during the prepubertal period in both sisters. Nevertheless, the heterozygous E435K mutation transmitted by the mother was probably implicated in the bone fragility phenotype in the two sisters. Glutamate at position 435 is conserved in phylogeny, present in the *E. coli* protein, which suggests a functional role. When recessively transmitted, this mutation is responsible for a severe perinatal form of hypophosphatasia [6, 9, 10, 12]. Based on the radiographic phenotype of the mother, a dominant effect of the E435K mutation is also suggested. Therefore, hypophosphatasia is indubitably a component of

Table 2 Total and lumbar spine bone densitometry values in the proband, her sister and mother

	Proband				Sister	Mother	Father
Age (years)	9	10	13	14	19	40	42
Height (cm)	133	147	158	162	161	165	173
SDS	+0.8	+0.9	+0.6	−0.1	−0.2	+0.1	−0.1
Total body BMD							
BMD (g/cm^2)	0.857	0.852	0.940	0.954	0.875	1.026	
BMD (*Z*-score)	−0.7	−0.8	−0.9	−1.8	−1.5	−0.8	
BMD (*T*-score)	−1.2						
Lumbar (L2–L4) spine BMD							
BMD (g/cm2)	0.699	0.742	0.978	0.992	1.053		
BMD (*Z*-score)	−0.5	−0.2	−0.9	−1.7	−0.9		
BMD (*T*-score)	−1.2						

Bone mineral density (BMD) was evaluated by dual-energy X-ray absorptiometry (Lunar Prodigy, Lunar Corporation, Madison, WI)
Z-scores were calculated for age from data provided by Boot et al. [1]
T-scores were calculated from data provided by the Lunar Corporation

the bone fragility observed in our patients. However, there was no other clinical event suggesting bone fragility in the rest of the pedigree. The information regarding bone fragility in the other members with low AP levels (II-2, II-3, II-4 and III-1) was only indirect. However, from the information given by the mother (II-6), it is clear that there were no low-energy fractures in these individuals, although II-2 and II-4 had relatively premature tooth loss. Therefore, the E435K mutation and low AP activity are certainly not the sole determinant of the phenotype observed in the two sisters. Indeed, although improbable, a second undetected transmitted mutation of the *ALPL* gene cannot be formally excluded. More generally, digenic inheritance of another gene involved in bone pathophysiology is a possibility. It is more likely that the overall genetic background and the environment, including nutritional factors, may have amplified the effect of the *ALPL* mutated allele. In this regard, it is interesting to note that the proband had low urine calcium levels at onset (Table 1), suggestive of some degree of calcium deficiency which, like vitamin D deficiency, is frequent in the French population of adolescents [7]. Unfortunately, no accurate information was available regarding the proband's calcium intake at onset. However, she had not been exposed to medications that could have an impact on bone homeostasis, such as glucocorticoids or anti-epileptics. In addition, protein and energy intake was normal, with a body mass index at the 25th percentile, which does not suggest any chronic disease, coeliac disease in particular. As mentioned above, the immobilisation period also contributed to significant BMD decrease in the proband, underlining the importance of physical activity in lowering the impact of genetic background on bone health. However, it was noteworthy that both sisters had significant sports activity.

In summary, while the diagnosis of mild hypophosphatasia was obvious, additional factors, calcium deficiency in particular, contributed to the bone fragility observed in the two sisters. They probably explain the variable expression in the family. The relatively low mineral density (BMD *Z*-scores) observed in the proband and her sister certainly increased the likelihood of fractures. DEXA values are difficult to interpret when osteomalacia is present, which could be the case in our patients.

It is also noteworthy that, in both sisters, the fractures occurred before puberty. The age of menarche was 13 years in the proband and 14 years in her sister. Therefore, the end of the "brittle period" corresponded to the beginning of puberty, when the proband was 10 and a half years old. The increase of serum AP level was modest during puberty, hence, other parameters, such as oestrogen secretion, probably contributed more to increase bone strength.

The mother had no history of fractures. However, the risk factor represented by hypophosphatasia in aged individuals [4] will probably have an increased effect during the menopause, justifying specific follow-up. Recombinant PTH 1-34 (teriparatide) has recently been found to be effective in improving mineralisation and bone pain in one case of adult hypophosphatasia [16]. Such a treatment is only exceptionally indicated in children in view of concern about bone sarcoma. However, it could be considered in the future in severe cases of hypophosphatasia after growth plate closure.

Conclusion

In these two young sisters with childhood hypophosphatasia and repetitive fractures, but without bone deformity or growth delay, the only obvious hallmark sign was premature tooth loss in the past. However, radiological signs clearly indicated hypophosphatasia. We stress that hypophosphatasia must be considered in children with repeated fractures and when the radiographic signs are detected. The AP level must be accurately assessed and, if the values are low, genetic analysis must be performed. A preventive approach must be taken to associated factors of bone fragility, in particular, calcium and vitamin D deficiency and the lack of physical exercise.

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