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RARE BONE DISEASE (CB LANGMAN AND E SHORE, SECTION EDITORS)

Hypophosphatasia

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Abstract Hypophosphatasia is a rare disorder due to a mutation in the *ALPL* gene encoding the alkaline phosphatase (ALP) leading to a diminished activity of the enzyme in bone, liver, and kidney. Hypophosphatasia is a heterogeneous disease, ranging from extreme life-threatening forms revealed at birth in young infants presenting with severely impaired bone mineralization, seizures, and hypercalcemia, to young adults with premature exfoliation of their teeth without any other symptom. We will review the challenges of the clinical, biochemical, radiological, and genetic diagnosis. Schematically, the diagnosis relies on low ALP levels and, in most cases, on the genetic defect in the *ALPL* gene. An enzyme replacement therapy is now developed for hypophosphatasia; early results in the severe form of the disease are extremely encouraging. However, multidisciplinary care remains the core of treatment of hypophosphatasia encompassing nutritional support, adjustment of calcium and phosphate intake, monitoring of vitamin D levels, careful and personalized physical therapy, and regular dental monitoring and care.

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Introduction

Hypophosphatasia (OMIM no. 241500, no. 241510, no.146300) is a rare disorder characterized by a mutation in the tissue non-specific alkaline phosphatase gene leading to a diminished activity of the enzyme in target tissues, hence a variety of symptoms from life-threatening severely impaired mineralization at birth to bone pain, leg bowing, recurrent fractures, muscular insufficiency, or tooth loss in adults. The expression of the disease is extremely variable, yet always involves a defect in the mineralization of the bone and teeth.

Pathophysiology of Hypophosphatasia

The tissue non-specific alkaline phosphatase (TNSALP) has numerous properties in mineralized tissues such as controlling the concentration of inorganic pyrophosphate (PP_i), contributing to the extra cellular matrix mineralization, and hydrolyzing ATP [1]. The extra-calcified tissue functions of TNSALP are poorly known. TNSALP metabolizes the lipopolysaccharides and participates to the LPS detoxification [2]. TNSALP is essentially expressed in cells resident in teeth and bone, in the liver, the kidney, and the brain. The latter likely contributes to the neurologic phenotype of mice and human babies lacking TSNALP. The enzyme is present both anchored at the outer surface of cells like osteoblasts and in the circulation. Two other isoforms contribute to the measured circulating levels of total alkaline phosphatase (ALP). The placental ALP is negligible; the intestinal ALP represents about 20 % of the adult serum ALP [3]. In children, especially infants and

adolescents who are growing rapidly, the bone isoform is predominant when measured in the serum; in adults, both liver and bone TNSALP are present in approximately equivalent amounts [4].

Three substrates of the TNSALP enzyme are well characterized so far: PP_i, pyridoxal-5-phosphate (PLP), and phosphoethanolamine (PEA).

The TNSALP enzyme initiates the process of the extracellular matrix mineralization through the enzymatic degradation of extracellular PP_i, a strong inhibitor of mineralization, into P_i [1]. TNSALP can also dephosphorylate osteopontin thereby diminishing its mineralization-inhibiting function [5]. The enzyme is expressed at the outer surface of osteoblasts and chondrocytes, as well as on their matrix vesicles.

Hypophosphatasia is characterized by a deficient enzymatic activity of the TNSALP. In absence or reduced activity of TNSALP, increased PP_i level in the bone matrix is the cause of rickets and osteomalacia and results in hypophosphatasia [6].

PLP is the biologically active form of vitamin B6, used as a cofactor in several enzymatic reactions, including transamination, and in the metabolism of certain neurotransmitters. TNSALP hydrolyzes PLP to form pyridoxal (PL). Elevated levels of PLP are found in the serum of patients affected with hypophosphatasia. The accumulation of this metabolite is likely contributing to the epileptic seizures observed in some babies affected by hypophosphatasia and in the experimental knockout animals, $Alpl^{-/-}$ mice. The administration of pyridoxine in $Alpl^{-/-}$ mice tends to end the epilepsy; however, this has not been clearly demonstrated in humans [7].

Among the other substrates of TNSALP, PEA has been found elevated in the urine of patients affected with hypophosphatasia. Its exact metabolism by TNSALP remains incompletely elucidated. However, PEA may serve as a diagnostic marker of the disease [8].

Most of the studied pathophysiology of hypophosphatasia focused on bone and teeth, the major target organs of TNSALP. It is now obvious that the disease is indeed not limited to these hard tissues, which renders its clinical recognition difficult for the physicians. Many other tissues and organs are affected, but their underlying pathophysiology remains unknown. How does the reduced TNSALP activity lead to a proximal myopathy, described as "osteomalacia myopathy" [9, 10]? Which biological processes sustain the failure to thrive that we observe in young children, the bone pain with inflammatory clinical characteristics, or to the short stature that affect some patients?

Hypophosphatasia Is a Genetic Disease

The *ALPL* gene, located on chromosome 1, encodes TNSALP. In 1988, M. Weiss and colleagues demonstrated that the disease was due to loss of function mutations in the *ALPL* gene [11], either heterozygous or homozygous [12]. More than 300 mutations of all sorts (missense, nonsense, splice site mutations, frame shift deletions, or insertions) have been described and collected so far in the *ALPL* gene mutation database [13•].

Diagnosis of the disease is therefore achieved by sequencing the *ALPL* gene. The identification of a variant is ultimately confirmed through functional studies to demonstrate the deleterious effect of the mutation on the TNSALP enzymatic activity. Several mutations have been already studied and reported, and in silico softwares are of great help to decipher the importance of the mutated residues when in vitro analyses are not accessible [13•].

Hypophosphatasia can be inherited as a recessive disorder due to homozygous mutations, compound heterozygous mutations [13•], or, in rare cases, to uniparental disomy [14]. The disease may also be dominantly inherited. In that case, heterozygous mutations are present; a dominant effect of the mutated allele is usually suspected to be the cause of the disease [15]. Dominant forms of hypophosphatasia are often associated with a mild phenotype. The extreme variability of the phenotype (severity, mode of presentation, affected tissues) is therefore due to these different modes of inheritance and to this strong allelic heterogeneity.

It is worth to note that genotype-phenotype studies have demonstrated the predictive power of the genotype; i.e., about 90 % of the patients sharing an identical—or almost identical—genotype also share a common phenotype. This finding eases considerably genetic counseling in the context of hypophosphatasia with a known genetic defect [16].

The prevalence of the disease has not been clearly determined because of the difficulty to establish the diagnosis in patients with mild forms (dominant forms with isolated dental or bone phenotype, for example) or prenatal benign forms. In the Mornet et al. study, the prevalence of severe patients diagnosed through molecular analysis in Europe was 1/297,000 [17]. The prevalence of patients with milder symptomatic forms due to heterozygous mutations was 47 times higher, i.e., 1/6370. Through a completely different approach, McKiernan et al. found that 1/5444 adult patients explored in a rural multidisciplinary clinic had a persistent low alkaline phosphatase level; this group of patients presented with rheumatologic complications reminiscent of the mild adult form of hypophosphatasia [18]. It is estimated that the current genetic techniques allow the detection of approximately 95 % of the mutations in patients affected with hypophosphatasia [19]. Yet, unidentified genes might present as an hypophosphatasia-like phenotype.

Clinical Presentation

Hypophosphatasia is a heterogeneous disease; i.e., it can reveal itself at any age, through a wide range of symptoms. The

diagnosis can be made in utero, during childhood, adulthood, or facing an atypical post-menopausal fracture. Overall, the most severe forms of the disease are associated with earlier symptoms and diagnosis. Milder forms are usually diagnosed later in life. These latter considerations are generally speaking applicable to hypophosphatasia; in fact, Fraser et al. [20], then Whyte et al. [21•], have dissected the disease based on the appearance of the first symptom (before 6 months of life: perinatal hypophosphatasia, in infancy: infantile hypophosphatasia, in childhood: juvenile hypophosphatasia, in adulthood: adult hypophosphatasia, symptoms limited to the teeth: odonto-hypophosphatasia). This classification is very useful because of its simplicity; however, it does not reflect the phenotypic continuum of the disease and often reduces adult patients to "mild" forms, though they might experience disabling complications of their disease. In addition, this classification omits the evolution of the disease, i.e., the variation of the disease's burden throughout life. Patients seemingly unaffected during their childhood might develop later on rheumatologic, orthopedic, and metabolic conditions leading to a profound handicap. In this chapter, we choose to avoid this classification to emphasize these two key elements of the disease: the continuum and the evolutivity throughout life. When possible, the overlapping pictures of hypophosphatasia will be provided (Table 1).

Another issue is the prenatal benign form of hypophosphatasia. As mentioned above, in utero diagnosis of hypophosphatasia usually suggests a severe disease that may lead to either an interruption of the pregnancy, stillbirth, or the birth of a baby affected with a severe form of the disease. Several cases of spontaneous improvement during the third trimester of the pregnancy, or in the first months or years of life, have however been described. The careful analysis of X-rays and biochemical and molecular markers is necessary to discriminate a benign perinatal hypophosphatasia from a lethal form of the disease [22–24] (Table 1).

In utero, the diagnosis of hypophosphatasia may be suspected as soon as the second trimester of the pregnancy with the finding on ultrasound of asymmetric bone anomalies and polyhydramnios; chest and abdominal circumferences are normal [22, 25•]. Stillbirth is common. Severely affected fetuses display dramatic impaired mineralization, short-limb dwarfism, bowed bones, skin-covered osteochondral spurs protruding from legs or arms, hypoplastic lungs, and defective mineralization of the skull and spine (Fig. 1a) [26]. However, clinical signs may appear later in the pregnancy and be limited to bowed long bones. Birth usually occurs between 37 and 40 weeks of gestation [22].

Patients who are already symptomatic at birth or during the first weeks of life present with a very severe form of the disease, which may affect many organs. In most infants, the lack of mineralization of the chest cavity combined with muscle insufficiency leads to a respiratory failure, requiring ventilator support. Neurological complications are frequent, but not well understood. Epileptic seizures may arise due to the deficient metabolism of the PLP in PL. They start after a free interval of a few days after birth and are usually of very poor prognosis [27, 28]. Intracerebral hemorrhages, central apnea, and episodes of bradycardia may occur. In addition, patients usually present with hypercalcemia and hyperphosphatemia; they quickly develop nephrocalcinosis and renal damage. Multiorgan failure is the common consequence, leading to the observed death rate of 50 to 90 % in the absence of targeted enzyme replacement therapy [25•]. In rare cases, the neurological disease is the leading symptom, with a moderate skeletal defect [27, 28].

Later in infancy or childhood, several various symptoms may reveal the diagnosis of hypophosphatasia. Skeletal symptoms encompass bone pain, leg bowing, joint enlargement similar to that of rickets, and fractures. X-rays show low bone mineralization, flared metaphysis that could be misdiagnosed with rickets, area of radiolucencies at extremities of the long bones (tongues), and bowed and gracile bones (Fig. 1b–d). Metabolic abnormalities are common, but may be transient: hypercalcemia, hyperphosphatemia, low parathyroid hormone (PTH) levels, and elevated hypercalciuria leading to nephrocalcinosis. Walking delay, waddling gait, and the necessity to use assisting walking devices are attributed to the muscle hypotonia. Premature cranial suture closure is extremely frequent (craniosynostosis) in young affected children and may lead to cranial hypertension and ocular and neural complications. From birth to the age of 4–5 years, careful attention must be paid to the growth of the head in affected babies to screen for craniosynostosis and allow early surgery [29]. Cranial sutures may be already fused at birth. Deafness may occur in severe patients even in the absence of cerebral damage; its exact origin is still unknown as it is not present at birth and may develop over time. Failure to thrive and reflux is frequent when the disease is symptomatic before the age of 3 years. Statural growth is always affected in young children with a severe disease or in infants requiring nutritional support [25•]. Patients with mild symptoms of the disease or diagnosed later in life display a near-normal height [21•]. Growth hormone deficiency has been identified in a subset of patients [25•].

A constant feature of the disease is the premature, painless loss of the primary teeth with intact roots (in the absence of evident trauma). The premature loss is preceded by an increased mobility of the teeth; the first tooth is usually gone by the 2nd year of life because of the mineralization defect of the tissues that anchor the tooth to the periodontal ligament (cementum and alveolar bone) [30, 31]. As for other symptoms, young patients loose more teeth, starting with the lower and upper anterior teeth then followed by the posterior teeth, at an earlier age than do patients with a milder form of the disease (Fig. 1e, g). Other features of hypophosphatasia

Table 1 Clinical presentation of hypophosphatasia, differential diagnosis

	In utero Stillbirth	Neonatal period/ infancy Death	Childhood	Adolescence	Adulthood/odonto
Bone	Short bowed bones, osteochondral spurs, narrowed chest, hypomineralized chest and skull	Hypomineralized bones and chest	Fractures	Fractures	Fractures, pseudofractures
	Prenatal benign form of hypophosphatasia	Fractures	Bowed bones	Bowed bones	Bone/joint pain
		Bowed bones Bone pain	Bone/joint pain	Bone/joint pain	Chondrocalcinosis Periarticular calcifications
Pulmonary		Respiratory failure Infection			
Neurology		Seizures Intracranial hemorrhages Deafness Craniosynostosis	Craniosynostosis Deafness		
Muscle	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia
General		Failure to thrive	Failure to thrive Short stature	Short stature	Fatigue
Teeth			Early lacteal teeth loss[b]	Permanent teeth loss[b]	Permanent teeth loss[b]
ALP	Low in the amniotic fluid	Low ALP[c] in the serum: 0–150 IU/L (Taketani et al. 2014); 2–15 IU/L (Whyte et al., 1996)	Low ALP in the serum: 75–250 IU/L (Taketani et al. 2014); girls 53.6 IU/L, boys 37.5 IU/L (Whyte et al. 2015); 15–90 IU/L (Whyte et al. 1996)	Low ALP in the serum	Low ALP in the serum: 150–325 IU/L (Taketani et al. 2014); 15–98 IU/L (Berkseth et al. 2013); 4–25 IU/L (Whyte et al. 1996)
	Case report: <4 IU/L in cord blood (Watanabe et al. 2014)	Elevated levels of PLP in the serum	Elevated levels of PLP in the serum	Elevated levels of PLP in the serum	Elevated levels of PLP in the serum
		PEA elevated in the urine	PEA elevated in the urine	PEA elevated in the urine	PEA elevated in the urine
Biochemistry		Hypercalcemia	Hypercalcemia		Triggered by exogenous events[a]
		Hyperphosphatemia Hypercalciuria Low PTH	Hyperphosphatemia Hypercalciuria Low PTH		Hypercalcemia Hyperphosphatemia Hypercalciuria Low PTH
Differential diagnosis	Osteogenesis imperfecta (OI) type II Hypochondrogenesis	Achondrogenesis Osteogenesis imperfecta (OI)	Rickets Leukemia		Chondrocalcinosis Osteoarthritis and pseudogout
	Campomelic dysplasia	Rickets	Chronic recurrent multifocal osteomyelitis (CRMO)		Osteopenia/osteoporosis
	Caffey disease	Thanatophoric dysplasia (Millán and Plotkin, 2012)	Idiopathic juvenile osteoporosis (IJO) (Whyte 2010) (Mornet et al. 2014)		Forestier disease, osteo-arthropathy
	Kyphomelic dysplasia (Matsushita et al. 2014)				Periodontal disease
	Chondrodysplasia with bone mineralization defect (Wenkert et al. 2011) (Sinico et al. 2007)				Dentin dysplasia type I (Wendling et al. 2001)

[a] Rare and such as immobilization, recurrent fractures

[b] See text for other dental features

[c] ALP may be not as low as expected in the context of neonatal intensive care or following a fracture

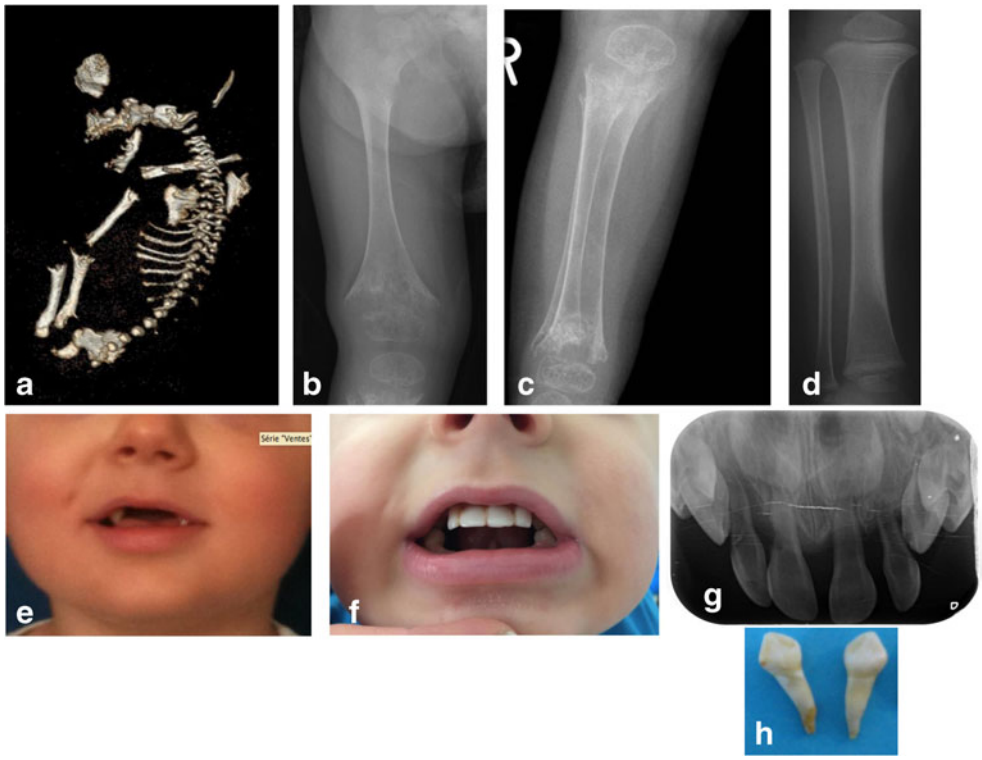

Fig. 1 **a** Scannographic image at 30 weeks of pregnancy in a fetus affected with hypophosphatasia and a homozygous mutation in the *ALPL* gene. Note the lack of mineralization of the skull, the thin ribs, but most of all, the abnormal distal femoral metaphyses with eperons and irregularities. **b** Femur and **c** tibia images on standard X-rays in an 18-month-young boy affected with hypophosphatasia and a compound heterozygous mutation in the *ALPL* gene. **d** For comparison, an X-ray of an unaffected 20-month-old boy is shown. **e** Premature loss of the anterior upper and lower teeth before the age of 2 years. **f** A nice smile with a fixed partial denture in the same child 1 year later. **g** Retroalveolar radiograph of the same patient before he lost upper incisors and canines during the following year. Note the reduced alveolar bone level around the incisors. **h** Pictures of exfoliated teeth (decidual canines) with their entire roots

comprise cementum aplasia or hypoplasia, large pulp chambers, abnormal tooth shape, enamel hypoplasia, delayed eruption, retained teeth, and alveolar bone loss. The dental exfoliation is often the "trigger sign" that will lead to the diagnosis of hypophosphatasia, as it is very unusual in children [32]. Adults may also present a premature loss of the permanent dentition [33]. Because tooth loss is common in the general adult population, this usually does not prompt further investigations. In some patients, dental features are the main manifestation of the disease. A careful and thorough clinical investigation is required and may reveal history of fracture, or unexplained periods of bone and joint pain, or even familial history of unexplained neonatal death of siblings [21•, 32, 34, 35].

Non-specific signs such as fatigue, absence of a pubertal growth spurt, or bone pain relieved by non-steroid anti-inflammatory drugs may reveal the disease in adolescents or young adults. In patients around 50 years of age, hypophosphatasia has been diagnosed in the context of chondrocalcinosis; pseudogout; joint swelling; subtrochanteric, vertebral, and foot fracture; non-healing fractures; pain; and musculoskeletal complaints [36•]. Some patients do report a history of childhood rickets or teeth anomalies followed by an uneventful youth. Adult patients with a known diagnosis of hypophosphatasia may experience severe complications when they age: atypical fractures, stress fractures, pseudofractures, fracture healing difficulties, muscle fatigue, hypercalcemia, and renal insufficiency secondary to immobilization, alteration, and loss of permanent teeth [36•, 37–39]. These complications may occur spontaneously triggered by trauma or environmental exposition. It is worth to note that pregnancy and menopause are physiological conditions that will require outstanding attention in these fragile patients.

Biochemistry

A reduced enzymatic activity of TNSALP (commonly termed alkaline phosphatase or ALP activity) measured on a blood sample is the key marker of the disease. It is therefore of major importance to interpret the ALP results within the clinical context, i.e., knowing the age reference range for ALP, which varies widely throughout life. ALP values are physiologically more elevated during periods of life with greater growth velocity and bone metabolism, i.e., infancy and adolescence [40, 41].

ALP may be elevated in stressed condition such as intensive care or following a traumatism or a fracture. On the contrary, a single low value of ALP is not sufficient to establish a diagnosis of hypophosphatasia. Indeed, several clinical conditions are known to be associated with low levels of serum ALP, including disorders with low bone turnover such as celiac disease; severe anemia; hypothyroidism; malnutrition; Wilson's disease; vitamin C, folate, or vitamin B12 insufficiency; hypomagnesemia; hypoparathyroidism; or drugs (bisphosphonates, denosumab, glucocorticoids, estrogens, omeprazole, clofibrate, high doses of vitamin D). It has been also described with other conditions like massive transfusion of blood or plasma or early pregnancy [8, 37].

In hypophosphatasia, levels of ALP are approximately inversely correlated with the severity of the disease (the more severe the disease, the lower the ALP) [3]. However, no cutoff level could be identified to individualize the different forms of the disease (Table 1). As an example, in a large Japanese series of affected patients, serum ALP ranged approximately between 0 and 100 IU/L at birth and between 10 and 150 IU/L in babies with the perinatal lethal form and with the benign form of hypophosphatasia, respectively [25•]. A retrospective review of young patients (below the age of 10 years) suggested recently that boys had lower serum ALP levels than girls [21•]. For prenatal diagnosis, a few teams have measured ALP activity in cord blood and found low levels in affected babies [14]. However, only severe forms have been reported and the overlap with perinatal benign hypophosphatasia is highly plausible.

In conclusion, serum ALP activity (or specific bone ALP in adults) is of great aid to the diagnosis of hypophosphatasia, but should be measured several times in stable clinical conditions to corroborate the diagnosis.

The accumulation of the other TNSALP substrates, serum PLP and urinary PEA, may also contribute to confirm the diagnosis of hypophosphatasia when the diagnosis is unclear. However, assays are not easily accessible and have their limitations. In particular, levels of substrates, as for ALP, do not predict hypophosphatasia severity [6, 42]. Now that the genetics of *ALPL* is so contributive, TNSALP substrates are now only considered in difficult clinical cases or as biomarkers of the disease activity in clinical research settings [37, 43].

Hypophosphatasia is sometimes described as "rickets." However, two main biochemical features distinguish hypophosphatasia from the definition of rickets: the low ALP and the elevated blood phosphate (and often, calcium) level. Different from all other rickets conditions, the endocrine regulation of the phosphate and calcium homeostasis—including PTH and FGF23—is normal and adequate in hypophosphatasia; the defect lies in the mineral matrix. Schematically, as the Ca++ and P_i cannot be integrated into hydroxyapatite crystals and form mineral, they accumulate in the bloodstream, causing hyperphosphatemia and hypercalcemia. The increased calcium load through the nephrons hence leads to hypercalciuria, nephrocalcinosis, and renal failure. PTH and $1,25(OH)_2$vitamin-D are adequately suppressed by the hypercalcemia. These metabolic features are always present in the neonatal severe forms of the disease and may even be the revealing symptom of the disease. They are often present in young children, may be transient, and are likely underdiagnosed. In mild forms of hypophosphatasia, a low PTH level is suggestive of an undiagnosed, asymptomatic past moderate hypercalcemia [44] (our experience).

As hypophosphatasia patients age, these symptoms usually disappear after the age of 5–8 years. However, several groups have reported episodes of hypercalcemia associated with suppressed PTH following immobilization or fractures in adolescents or adults [39]. An acute immobilization, combined to the "adynamic bone" associated with the low ALP, worsened by an increased intestinal absorption of calcium from the gut (vitamin D excessive load, for example) may facilitate the occurrence of hypercalcemia and its systemic consequences in adults. In their systematic study of adult patients, Berkseth and colleagues found that 3 and 4 out of 22 adults displayed moderate hypercalcemia and hyperphosphatemia, respectively [36•].

Imaging

Fetal ultrasonography detects skeletal undermineralization, short and/or bowed long bones, and spur-like projections of the long bones. Other features have been described above [22, 26, 43]. Intrauterine 3D helical computer tomography may improve the diagnosis accuracy; however, it cannot be done until the 28–30 weeks of gestation (Fig. 1a).

X-rays performed on stillborn fetuses or severely affected newborns show irregular, very short, and deep cup-shaped metaphyses, irregular and bifid. The diaphyses are thin. Some bones may be hypoplastic or absent (including missing vertebrae); the cranial vault displays a severe default of mineralization. Clavicles are usually visible and preserved.

In children, the skeleton gives a global impression of bone demineralization. Metaphyses are irregularly enlarged, simulating rickets. Focal defects at the central zone of the metaphyses of the long bones—termed "tongues of radiolucencies"—are typical of the disease (Fig. 1b–d). These features predominate at the metaphyses and therefore heal and disappear by the end of adolescence and growth (except for the mineralization defect). The skull is dolichocephalic. The diaphyses may show fractures and/or patchy regions of various radiointensities with zones of osteosclerosis [4, 43, 44]. The skull is undermineralized as a whole, then sutures fuse leading to the typical aspect of copper beaten skull, a sign of raised intracranial pressure.

In adults, roentgenograms may reveal numerous stress fractures of the metatarsals, femoral pseudofractures, delayed healing of fractures, focal osteolysis, chondrocalcinosis, sequealae of the pediatric disease (leg bowing, epiphyses anomalies), and periarticular calcifications [35, 36•, 45, 46]. Around teeth, standard retroalveolar X-rays may show a reduced alveolar bone level in children or adults.

Bone scintigraphy may help to screen for stress fractures.

Cranial computed tomography is not necessary for the diagnosis of craniosynostosis. However, it clarifies the states of the sutures prior to surgery.

Beck and colleagues performed whole-body MRI in four children affected with hypophosphatasia and showed local fluid accumulation in metaphyseal defects of the femur, corresponding to the radiolucencies seen on conventional X-rays. They evidenced hyperhemia and edema in the metaphyses of long bones, sustaining the hypothesis of an inflammatory process in hypophosphatasia [47].

The mineralization defect can be quantified in certain conditions by densitometry. Indeed, Whyte and colleagues have shown that the bone mineral density measured through dual X-ray absorptiometry (DXA) at the hip or the lumbar spine in a series of almost 100 patients (5–21 years) correlated with the severity of the disease (i.e., with the classification) [21•]. In a different report, Girschick and colleagues have observed a trend for decreased BMD over time in affected children [44]. However, one should keep in mind that a low BMD is not specific to hypophosphatasia and should not orient toward a false diagnosis of osteoporosis in the context of fractures [37].

Establishing the Diagnosis of Hypophosphatasia (See Table 1 for Differential Diagnosis)

In most cases, the bone and teeth mineralization defect is associated with a reduced TNSALP enzyme activity. Serum calcium and phosphate might be normal or slightly increased. Urinary calcium excretion might be above the normal range. The diagnosis of hypophosphatasia is clear. Sequencing of the *ALPL* gene is of great contribution to confirm the diagnosis when clinical features and biochemistry are not clear enough. In those cases, genetics should be viewed as a confirmation of diagnosis, not as a requirement.

Prenatal assessment of hypophosphatasia in couples with a previously affected child or pregnancies diagnosed with features suggestive of hypophosphatasia is perhaps the most difficult situation. In utero bone scans are of great help to identify the images specific of hypophosphatasia after weeks 28–30. Since 1995, sequencing of the *ALPL* gene on DNA extracted from amniocytes is performed, allowing antenatal diagnosis of the disease [48]. It is perhaps the only situation where genetics is essential for the diagnosis of the disease. Recently, exome sequencing identified heterozygous compound *ALPL* mutation fetuses with unexplained sonographic abnormalities and mineralization defects [49, 50]. However, several cases are still molecularly undefined despite features reminiscent of hypophosphatasia and low serum ALP [51].

Symptomatic Management of Hypophosphatasia (Table 2)

Therapeutic intervention in hypophosphatasia must be comprehensive and carefully planned with the family and caregivers. Due to the extreme rarity of the disease, patients should be managed by an experienced reference center or in coordination with this center.

In severe neonatal forms, patients are in the neonatal intensive care unit; their management is essentially symptomatic. They may require ventilator support because of the weak mineralized chest. Antalgics are mandatory as bone pain is constant and discomfort is a predominant clinical feature in these babies. When neurological signs occur, vitamin B6 or pyridoxine should be administered in association with antiepileptics. However, it is often an indicator of a lethal condition [27, 28].

In the presence of hypercalcemia, hyperphosphatemia, and nephrocalcinosis, the diet—or parenteral infusion—should be restricted in calcium and phosphate [22]. Phosphate binders may help, but long-term studies are lacking to evaluate their impact. Vitamin D insufficiency is common, yet should not be tolerated as it will promote secondary hyperparathyroidism and bone resorption; therefore, vitamin D levels should be monitored to reach levels that do not trigger secondary hyperparathyroidism (25OHD levels ≈ 20 ng/mL). On the other hand, high doses of vitamin D should also be avoided to prevent increase in calcium and phosphate absorption and subsequent increases in urinary calcium excretion and nephrocalcinosis. When calcium and phosphate levels normalize and PTH is not suppressed, calcium and phosphate intake can be adjusted to recommended intake for the age of the patient (500 mg at the age of 5 years, 700 mg at the age of 7 years, 900 mg at the age of 9 years, and 1000 mg at the age of 10 years and older).

Nutritional support is a key point in the management of severe and mild forms of hypophosphatasia in childhood, although the pathophysiology of the leanness of the children is not clear. Antireflux therapy is often needed. Nutrition through gastric or jejunal tubes may be necessary for a couple of months or years to overcome a severe reflux or insufficient caloric intake. Protein intake should be adjusted to recommended intake for age to help build muscles and bone in coordination with the multidisciplinary care.

Table 2 Management and care

	Neonatal period/infancy	Childhood	Adolescence	Adulthood
Enzyme replacement therapy	Send the patient to a specialized team to manage accessibility to Asfotase alfa		[a]	[a]
	Fractures: prolonged casting or intramedullary rods			
Bone	Painkillers, immobilization	Corrective surgeries for leg bowing NSAID for acute periods of pain		NSAID for acute periods of pain
Muscle		Assisted devices		
		Low impact physical therapy		
Neurology	If seizures: antiepileptics/vitamin B6/pyridoxine		Psychological and psychiatric support for anxiety, insomnia and depression	
Pulmonary	Ventilatory support if needed			
Nutrition	-Phosphate binders -Limit calcium and phosphate intake	Adjust calcium and vitamin D intake to recommendation for age		Avoid overweight to prevent hip and joint arthrosis
	-No vitamin D supplementation if 25OHD is >20 ng/mL			
	-Antireflux therapy			
	-Feeding tube if necessary			
Teeth	Early education on rigorous oral hygiene to prevent periodontal disease.	Oral hygiene		Oral hygiene
		Removable prostheses		Dental implants
General	Social support		Prepare transfer to adult care	Adaptation of the workplace

[a] In the future, treatment regimens of asfotase alfa might be proposed for severely affected adolescents and adults

Neurosurgery is performed in patients with symptomatic craniosynostosis, i.e., with papillary edema or intracranial hypertension, or Chiari malformation.

Dental care is a major concern for the patients. As mentioned above, a tooth loosening in hypophosphatasia is likely to be the consequence of a constitutional defect of the mineralized tissues that anchor the teeth in the jaws. It is unknown if the inflammation associated with the accumulation of dental plaque contributes to this process, as it does in periodontitis. As a precaution, it is recommended to lower as much as possible the inflammatory challenge by carefully and regularly cleaning oral surfaces in children and adults. Frequent monitoring of the periodontal health is also recommended. In young children, the replacement of exfoliated teeth with removable or fixed prostheses is conceivable until the permanent dentition rises (Fig. 1e, f). It is necessary for the proper expansion of the jaw and eruption of permanent teeth, the acquisition of speech, the masticatory function, and the development and socialization of the child. In adults, dental implants have been successfully used to replace permanent lost teeth by trained specialists [32, 33].

Non-steroidal anti-inflammatory drugs are commonly used to treat pain and inflammatory symptoms, even during childhood. Their use requires a careful monitoring of the renal function. They are not to be administered continuously but rather on demand or through regular cycles with pauses to prevent the renal toxicity [52].

Management of Bone Fragility and Fractures

Physiotherapy and low-impact physical exercise adjusted to age, weight, and fatigue are highly recommended to strengthen the muscle-bone unit. Physical activity and sports should be practiced with experienced teachers to avoid trauma and fractures.

Fractures, pseudofractures (stressed fractures), or acute bone pain should be managed by experienced orthopedist surgeons and often require prolonged casting or stabilization with intramedullary rods.

Orthopedist surgeons have a major role during growth in the follow-up of lower limb bowing and scoliosis. It is crucial to measure the functional impact of the bone deformity in order to prevent future rheumatologic sequelae, by using corrective surgeries, usually in the form of multiple osteotomies [53]. It is expected that the enzyme replacement therapy using the recombinant ALP will dramatically modify the attitude of surgeons and encourage them to postpone surgeries until the end of growth.

Therapies targeted to the bone have been reported with inconsistent outcomes and should be considered with extreme caution. Bisphosphonates are, in theory, contra-indicated in patients with hypophosphatasia, because they diminish the ALP enzymatic activity, and hypophosphatasia is mainly a disorder of bone mineralization, which is unlikely to benefit from a therapy acting on osteoclastic resorption. In fact,

several adult cases of atypical fractures in patients with hypophosphatasia receiving bisphosphonates have been reported [54, 55]. The use of teriparatide—only allowed in adults—is controversial. In theory, PTH should stimulate ALP in osteoblasts and might promote bone formation or fracture healing. As conflicting results have been reported, teriparatide should be used only by trained practitioners with careful monitoring of bone markers, in the absence of any available enzyme replacement therapy [37].

Enzyme Replacement Therapy: Asfotase Alfa

The disease manifestations result both from the enzymatic defect in the target tissue—the bone and teeth—and from the accumulation of substrates in other organs. Enzyme replacement therapy (ERT) was developed for hypophosphatasia by Enobia Pharma (now Alexion). They engineered asfotase alfa, a recombinant ALP fusion protein, comprising the ALP ectodomain, the human IgG1 Fc domain, and a terminal deca-aspartate motif that directly targets the molecule to the bone.

In preclinical animal studies, subcutaneous injections of asfotase alfa restored normal ALP levels and prevented skeletal and dental manifestations of hypophosphatasia or seizures in ALP-deficient mice [56••] (summary in [57]).

Asfotase alfa is now being evaluated in children and adults with hypophosphatasia (https://clinicaltrials.gov/ct2/results?term=asfotase+alfa&Search=Search). The first study was performed in 11 young infants with the most severe form of the disease. Asfotase alfa was administered subcutaneously three times per week at a dose of 1 to 3 mg/kg. One infant died out of the 11 affected with a life-threatening disease who were included in the study. A striking improvement in radiographic mineralization (primary endpoint of the study), followed by pulmonary (removal from ventilator support) and muscle function, was observed in these children. The survival advantage brought by asfotase alfa in young infants affected by the severe form of the disease is clear and dramatic. PTH levels increased, allowing improved calcium intake in the patients' diet. Local side reactions such as erythema, pruritus, and pain were the most frequent side effects. One patient died of pneumonia and multiorgan failure. Craniosynostosis and hearing loss did occur during the course of asfotase alfa therapy. From these preliminary results and the preclinical studies, it appears that, although the enzyme is targeted to the bone, its effect is spread to other organs like the lungs or the muscle [58••]. In infants and children, asfotase alfa allowed a significant improvement in the radiological rickets score, height *z* score, and musculo-skeletal performances [59]. We are eagerly waiting for longer-term results, as well as replication in older children, adolescents, and adults. The extreme variability in the clinical presentation of the disease, its unpredictability throughout time, and its multisystemic faces will render the definition of common criteria of efficacy difficult. It is therefore of major importance to document the natural history of the disease, because, in patients affected with non-lethal forms of hypophosphatasia, survival might not be affected, while quality of life certainly is. For these patients, one might consider different applications of asfotase alfa (lower doses, short periods of treatment during phases of increased growth velocity, or framing surgery).

The availability—already in some countries—of an enzyme replacement therapy that modifies early survival raises several questions. Should we develop a neonatal screening program for hypophosphatasia? During the course of the diagnosis of hypophosphatasia during a pregnancy, we will now inform the couple that a therapy is available.

Conclusion

Hypophosphatasia is an uncommon genetic disease, which may be diagnosed at birth because of a severe bone demineralization; during childhood because of teeth loss, joint pain, and growth impairment; and during adulthood because of repeated fractures or teeth loss. Patients usually experience a long delay before meeting a specialist who will recognize the low level of ALP and confirm hypophosphatasia. The major recent outcomes for hypophosphatasia are (i) the organization in rare disease networks, aiming at more visibility for patients and facilitating care and research, (ii) the easy access to the genetic diagnosis, and (iii) the development of the enzyme replacement therapy.

Compliance with Ethical Standards

Conflict of Interest Agnès Linglart reports personal fees from Alexion, before and during the period she was writing the current review. Martin Biosse-Duplan declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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