

Hypophosphatasia: an overview of the disease and its treatment

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Abstract This review presents the current knowledge on hypophosphatasia, a rare genetic disease of very variable severity (from lethal to mild) and clinical presentation, caused by defective production of tissue-non-specific alkaline phosphatase (TNSALP). Hypophosphatasia can affect babies in utero as well as infants, children, and adults. The article first presents the genetics of TNSALP and its many known mutations underlying the disease. Then, it presents the epidemiology, classification, and clinical presentation of the six different forms of the disease (perinatal lethal, prenatal benign, infantile, childhood, adult, and odontohypophosphatasia) as well as the essential diagnostic clues. The last section on treatment presents a survey of the therapeutic approaches, up to the ongoing phase 2 studies of enzyme replacement therapy.

Keywords Alkaline phosphatase · Bone · Fractures · Hypophosphatasia · Hypomineralization · Teeth

Introduction

Hypophosphatasia (HPP) was first described in 1948 by Rathbun [1], who differentiated it from the “well-known clinical entities of osteogenesis imperfecta and achondroplasia”

for some unusual findings, notably extremely low levels of alkaline phosphatase (ALP) and seizures.

Now we know that HPP is a rare and heterogeneous inherited disorder of bone and mineral metabolism, caused by a number of loss-of-function mutations in the *ALPL* (alkaline phosphatase liver type) gene, with generalized reduction of activity of the tissue-non-specific (liver/bone/kidney) isoenzyme of ALP (TNSALP). There are several forms of the disease ranging from lethal to mild. The clinical expression is highly variable, depending on the type of mutation, inheritance mechanism, and age of appearance. The most severe forms are those affecting infants and children, which may even manifest prenatally in utero. The main clinical signs are related to defective bone and teeth mineralization (rickets, osteomalacia, fractures, teeth loss), but other systemic manifestations can be present in the severe forms (seizures, respiratory and kidney problems, chronic pain, weakness, etc.). The main diagnostic laboratory abnormalities are low serum ALP and TNSALP activity (hypophosphatasemia) and increased levels of ALP substrates (inorganic pyrophosphate (PPi), pyridoxal-5'-phosphate (PLP, the active metabolite of vitamin B6), and phosphoethanolamine (PEA)) [2–7].

Alkaline phosphatase

ALP is a membrane-bound phosphomonoesterase, anchored to the plasma membrane by a phosphatidylinositol glycan moiety attached to its carboxyl terminus. It catalyzes dephosphorylation reactions and is essential in the catabolism of PPi and other molecules. In humans, ALP is present in all tissues, with higher concentrations in the liver, kidney, bone, intestinal mucosa, and placenta [3, 4].

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There are at least four ALP isoenzymes, encoded by four separate genes: three tissue-specific ALPs (“intestinal,” “placental,” and “germ cell”) and the ubiquitous TNSALP, especially abundant in the liver, bone, and kidney [2, 6, 8, 9], but also expressed in the brain, particularly in the cortical sensory areas [10]. TNSALP accounts for approximately 95 % of the total serum ALP activity, and PPi and PLP are currently considered its main physiological substrates. “Bone-specific ALP” (BSALP) is often imprecisely used as a synonym of TNSALP, while it is only its most abundant isoform. Both circulating and membrane-bound ALP enzymes are normally homodimeric, i.e., composed of two identical subunits. There is some evidence that membrane-bound TNSALP may exist in homodimeric or homotetrameric forms. Each monomer has one active site and its tertiary structure is stabilized by two Zn atoms. Mg^{2+} is necessary as a cofactor for catalytic activity [4, 6, 9].

In bone, TNSALP activity is present, at different levels, in pre-osteoblasts, osteoblasts, matrix vesicles, osteoid in areas of new bone formation, lining cells on the surface of the trabeculae, some newly embedded osteocytes, endosteal cells, subperiosteal cells, and bone marrow cells; in articular cartilage and growth plates, TNSALP is present in chondrocytes and cartilage matrix; in teeth, in ameloblasts and odontoblasts [8, 11]. TNSALP activity is essential for bone and teeth mineralization, and loss-of-function mutations are associated with skeletal hypomineralization, which is the predominant clinical manifestation of HPP.

The physiological role of TNSALP in tissues other than bone is poorly known. TNSALP is certainly involved in vitamin B6 metabolism, and PLP, the active metabolite of vitamin B6, is an essential cofactor in several biochemical processes, including the synthesis of major neurotransmitters (serotonin, dopamine, epinephrine, norepinephrine, gamma-aminobutyric acid).

The seizures observed in some HPP forms can be attributed to defective dephosphorylation of PLP to pyridoxal (PL), as demonstrated by studies on knockout mice. This is a key reaction, because only PL, not PLP, can cross plasma membranes and enter into cells, where it is again phosphorylated to PLP [12–16]. It should be noted that the only clinical sign of altered vitamin B6 metabolism in HPP is the seizures observed in the perinatal/infantile forms. Older children or adults have no signs of vitamin B6 deficiency or toxicity. For unclear reasons, in severe infantile HPP, seizures responding to vitamin B6 treatment seem to be an ominous sign [6, 9, 14].

According to recent evidence, TNSALP seems involved in central nervous system development (e.g., proliferation and differentiation of neural stem cells, maturation and function of synapses, myelination) and in different brain functions [4, 10, 17], and these actions may have a role in the variety of neurological and psychological signs and symptoms (from

neonatal seizures to chronic pain, anxiety, restlessness, and depression) that are frequently seen in the different forms of HPP. The occurrence of inflammation (osteomyelitis, arthritis) and muscle, bone, and cartilage damage in HPP also suggests a possible role of TNSALP in inflammatory processes [4]. Whether TNSALP deficiency has a role in the development of other symptoms of HPP (e.g., gastrointestinal symptoms) is not known.

The current knowledge about the functions of ALPs in the different tissues and organs is presented in detail in a recent book chapter by Buchet et al. [18].

Genetics

The *ALPL* gene (OMIM #171760) is located on the short (p) arm of chromosome 1, in the chromosome bands 1p36.1-p34, and is genetically linked to the Rh and fucosidase A loci. The *ALPL* gene consists of 12 exons distributed over 50 kilobases [19, 20]. Human TNSALP is a chain of 524 amino acid residues [21]. The genes of the other, tissue-specific, ALP isoenzymes are located on the long arm of chromosome 2 (2q34–37) [22].

HPP derives from any mutation in the *ALPL* gene that causes decreased TNSALP activity and increased levels of its substrates. About 275 mutations have been identified and are reported in the *ALPL* gene mutation database (http://www.sesep.uvsq.fr/03_hypo_mutations.php). The inheritance mechanism can be autosomal recessive or dominant. About 80 % of the known mutations are missense point mutations, 10 % are small deletions, 4 % splicing mutations, 3 % nonsense mutations, 2 % small insertions, and ≤ 1 % complex insertion/deletions, large deletions, or mutations in the regulatory sequence [21, 23, 24].

The high clinical variability of the disease is correlated to the pattern of inheritance and depends on the large number of missense mutations and their variable effect on TNSALP activity. The more severe forms of HPP are transmitted as autosomal recessive traits and are due to *ALPL* mutations that suppress TNSALP activity almost completely. The milder forms can be transmitted as either autosomal recessive or autosomal dominant traits and are due to mutations that reduce but do not suppress enzyme activity. In heterozygotes, some severe mutations may cause mild forms of HPP because of a dominant negative effect [5, 6, 25–28]. Significantly different phenotypic presentations of the disease may even occur within a family [29].

It has been suggested that some genetic variants of TNSALP (not necessarily related to the presence of HPP) might turn out to be particularly interesting for future bone research: Goseki-Sone et al. [30] found a significant association between the 787T>C (Tyr246His) gene polymorphism and bone mineral density (BMD) in a population of 501

postmenopausal women. Moreover, the MrOS study led to the identification of some rare, non-synonymous *ALPL* gene mutations associated with low serum ALP activity and low BMD in elderly males [31]. Future investigations might identify some TNSALP polymorphisms as determinants of BMD variability and as predisposing genetic factors for low bone mass and osteoporosis.

Epidemiology

HPP is distributed worldwide and affects all races, but with a highly variable prevalence. It is particularly frequent in Canada, where the prevalence of the severe form was estimated to be 1:100,000 [32]. In particular, the prevalence is very high (up to 1/2500) in the Mennonite Canadian population, in which 1:25 individuals may be a carrier [33, 34]. In European populations, the prevalence of severe HPP has been estimated at 1:300,000 [35]. In Japan, the perinatal lethal form has an estimated prevalence of 1:900,000 and is often due to homozygosity for *ALPL* mutation c.1559delT, a mutation found only among the Japanese, with an estimated carrier frequency of 1/480 [36]. In African-Americans, HPP seems to be “particularly rare” [37].

The prevalence of the milder forms of HPP, including those of adults, is extremely difficult to estimate, because of the variety of symptoms and the frequency of undiagnosed cases. Mornet et al. have proposed a genetic model, based on the proportion of dominant mutations among the “severe” alleles and on the estimated penetrance of the disease in heterozygotes for dominant mutations. According to this model, the prevalence of dominant “mild” HPP in the European population is about 1/6370 [35].

Pathogenesis of bone hypomineralization in HPP

The most characteristic and more severe consequences of the deficiency of TNSALP activity are due to defective mineralization of bone and teeth. The ratio of phosphate (Pi) to PPi is crucial in the mineralization process. Pi is needed for the formation of hydroxyapatite crystals, while PPi inhibits it.

The physicochemical and biochemical processes leading to mineralization of cartilage, bone, and teeth are presented in detail by Millán et al. [38]. The final result of these processes is the deposition of hydroxyapatite in specific areas of the extracellular matrix, along collagen fibrils [39] and within the matrix vesicles produced by chondrocytes and osteoblasts [40]. The early phase of mineralization seems to begin inside the matrix vesicles, where Ca^{2+} and Pi accumulate. In a second phase, the vesicles’ membranes break down and the hydroxyapatite crystals are exposed to extracellular fluids, allowing their

propagation and deposition in the collagenous matrix [41–43]. This process is regulated by the interplay of promoting and inhibiting factors, including three phosphatases—TNSALP, phosphatase orphan 1 (PHOSPHO1), and nucleoside pyrophosphohydrolase-1 (NPP1)—that play a crucial role in the regulation of the Pi to PPi ratio [38].

In HPP, the reduction of TNSALP activity and the accumulation of PPi in excess inhibit the calcium/phosphate crystal nucleation, retarding the growth of hydroxyapatite crystals in the bone matrix vesicles [44–47], although the function and differentiation of osteoblasts seem to be unaffected [48]. This hampers the whole process of bone mineralization, with the characteristic consequences of hypomineralized bone, spontaneous fractures, tooth loss, as well as soft tissue calcifications (osteoarthritis, arterial calcification) [38].

Dental problems in HPP

All forms of HPP (see below, *Classification*) are characterized by premature exfoliation and structural alterations of the primary (deciduous) teeth and by the increased occurrence of severe dental caries. The anterior teeth are more frequently affected, and incisors are more frequently lost.

Dental X-rays show loss of alveolar bone and enlarged pulp chambers and root canals. The excess PPi severely hampers the mineralization of cementum, and histological studies have identified lack of cementum as the cause of premature tooth loss. Impaired dentinogenesis is also observed, as revealed by large pulp chambers. The enamel seems not to be directly affected in HPP patients, although enamel hypoplasia, predisposing to dental caries, has been reported. Even if the primary teeth are most characteristically affected, HPP may also cause alterations in the permanent teeth, requiring counseling and early interventions [49–53].

Classification

The clinical presentation of HPP is highly variable, ranging from death in utero with almost a total absence of skeletal mineralization to adult dental problems and osteopenia. The classification of HPP is essentially unchanged since its first proposal in 1957. Five principal subtypes of HPP were considered: perinatal lethal, infantile, childhood, adult, and odontohypophosphatasia. Recently, a sixth subtype—the prenatal (or perinatal) benign HPP—has been recognized (Table 1).

It must be underlined that some forms significantly overlap in their clinical features. The infantile and childhood forms are a continuum and are sometimes difficult to distinguish. Pain, stiffness, and weakness of the lower limbs, probably due to microfractures of a

Table 1 Main characteristics of the different forms of hypophosphatasia

Form	Inheritance	Clinical course and features	Dental features	Diagnosis
Perinatal lethal	AR	Most severe form. Stillbirth or death within days/weeks after birth Severe hypomineralization. Derangements in calcium/phosphate metabolism Osteochondral spurs in forearms, legs Severe lung hypoplasia (chest deformities, rib fractures) Seizures	–	Radiography and ultrasonography Laboratory: low ALP (umbilical cord blood)
Prenatal (or perinatal) benign	AR/AD	Benign evolution (but long-term course unknown) Limb shortening and bowing of long bones observed in utero Spontaneous improvement of skeletal defects after birth	–	Ultrasonography Clinical evaluation Laboratory: low ALP (umbilical cord blood)
Infantile	AR	First symptoms during the first 6 months of life Poor prognosis during the first year of life Severe hypomineralization (rachitic ribs) Premature craniosynostosis (Chiari I malformation, hydrostatic hydrocephalus, hydrosyringomyelia), swallowing disorders, irritability, seizures Severe muscular hypotonia Hypercalciuria, nephrocalcinosis	Premature loss of deciduous teeth	Radiography Clinical evaluation Laboratory: low ALP; high PLP, PEA, PPi
Childhood	AR/AD	First symptoms after 6th month of life Rickets-like signs of hypomineralization Short stature, failure to thrive. Delayed walking. Repeated fractures. Waddling gait due to bone deformities. Chronic bone pain (lower extremities). Muscular hypotonia Lack of appetite, nausea, gastrointestinal problems	Premature loss of deciduous teeth Caries	Radiography: Bowdler spurs, shortening of long bones Clinical evaluation Laboratory: low ALP; high PLP, PEA, PPi Bone densitometry: low BMD
Adult	AR/AD	Stress fractures of metatarsals, tibia. Femur pseudofractures. Fragility fractures. Osteomalacia, osteoporosis History of delayed fracture healing in childhood; often mild “rickets” Chondrocalcinosis, osteoarthritis. Myopathy, weakness Renal abnormalities, reduced GFR. Nephrocalcinosis and kidney stones Psychiatric symptoms (insomnia, restlessness, anxiety, depression)	Loss of permanent teeth at 40–60 years of age	Radiography. Clinical evaluation Laboratory: low ALP; high PLP, PEA Bone densitometry: low BMD
Odonto HPP	AR/AD	Not associated with bone, articular, or muscular problems	Premature exfoliation of deciduous and/or permanent teeth (mainly incisors) Severe dental caries. Reduced thickness of dentin. Enlarged pulp chambers Alveolar bone loss	Clinical and dental evaluation Laboratory: low ALP; high PLP, PEA

Legend: *AD*=autosomal dominant, *AR*=autosomal recessive, *ALP*=alkaline phosphatase, *BMD*=bone mineral density, *GFR*=glomerular filtration rate, *PEA*=phosphoethanolamine, *PLP*=pyridoxal-5-phosphate, *PPi*=inorganic pyrophosphate

Measurement of PPi is not always available

hypomineralized bone, can be present in both children and adults. Dental problems (abnormal tooth shape and structure, abnormal enamel, dentin and cementum, abnormal color, anomalies in eruption and exfoliation, with precocious tooth loss) are also common in both childhood and adult forms.

The clinical nosology of HPP in children has been evaluated and expanded in a very recent article by Whyte et al. [54].

As a rule, the prognosis is linked to the skeletal complications, and a worse prognosis is usually associated with earlier signs and symptoms [3–6, 55, 56].

Perinatal lethal HPP (OMIM #241500)

This is the most severe form of HPP, almost always lethal immediately after birth or in the first few days of life.

Inheritance is autosomal recessive. The disease is already evident in utero as severe skeletal hypomineralization. Bones are almost completely devoid of minerals and, in the skull membranous bones, only the central area is calcified. Skin-covered osteochondral spurs are present in forearms or legs and are diagnostic. Babies who survive delivery show rapidly worsening alterations of calcium/phosphate metabolism (hypercalcemia), apnea, seizures (very difficult to treat), and progressive encephalopathy. Severe respiratory problems, due to chest deformities and lung hypoplasia, are the direct cause of death [4, 57, 58].

Prenatal diagnosis is possible by analysis of the fetal DNA of cells obtained from the amniotic fluid. Watanabe et al. identified a lethal homozygous mutation at the 15th week of gestation and observed marked hypomineralization of the fetal skeleton with ultrasonography 4 weeks later [59].

Prenatal (or perinatal) benign HPP

This form was first described in 1999, with four cases reported by Moore et al. [60] and one more case by Pauli et al. [61]. Ultrasound examination of the fetus shows evident pathological signs, such as short and severely bowed legs, but beginning with the third trimester of pregnancy, there is a spontaneous improvement and at birth, the newborn may be almost normal. The prognosis appears favorable, but long-term follow-up data are still lacking. It has been suggested that these cases might only be a casual early identification of a mild form of HPP [4, 6, 62].

Prenatal (perinatal) benign HP was initially attributed to dominant mutations, D361V and G46V in particular [63]. However, a recent article by Wenkert et al. [64] analyzed 17 new cases in depth and observed both autosomal dominant and autosomal recessive inheritance. These authors also reviewed the available literature and summarized the findings about 24 more published cases. They strongly emphasize that, contrary to previous opinion, prenatal presentation of HPP cannot be considered lethal anymore. In particular, ultrasound findings (poor skeletal mineralization, limb bowing, short femurs) cannot reliably differentiate the benign from the lethal form.

Differential diagnosis of perinatal HPP

The differential diagnosis is with severe forms of osteogenesis imperfecta, congenital dwarfism, campomelic dysplasia, and chondrodysplasias with defective bone mineralization.

Infantile HPP (OMIM #241500)

Infants affected by this form of HPP may be apparently normal at birth, but characteristic clinical signs appear in the first 6 months of life. The major presenting signs are failure to

thrive, generalized hypomineralization with rachitic features, chest deformities and rib fractures, severe muscular hypotonia and weakness, hypercalcemia (possibly explaining the irritability, anorexia, and vomiting observed in these patients), nephrocalcinosis secondary to hypercalciuria, swallowing problems, and seizures. Premature closure of the cranial sutures frequently occurs, with problems of intracranial hypertension (hydrostatic hydrocephalus, Chiari I malformation, hydrosyringomyelia) [3, 4, 6].

The diagnosis is clinical and radiological. Rickets, demineralization, and fractures soon become evident. Hypomineralization of the cranial bones may look like wide open sutures, while premature synostosis is common.

Prognosis is very poor in the severe forms of infantile HPP. Respiratory problems, including pneumonia, are a consequence of chest deformities and rib fractures and are the main cause of death. Epilepsy responding to vitamin B6 is also an ominous sign, predicting a lethal outcome [6, 9, 14].

Some patients with infantile HPP show spontaneous improvement, with increased mineralization and regression of most clinical problems. They will often have short stature and premature loss of deciduous teeth, as well as the possible complications of premature craniosynostosis, but the long-term prognosis is favorable [6, 9].

Childhood HPP (OMIM #241510)

This is the form of HPP with the greatest clinical variability. Its clinical manifestations are a continuum with the infantile form described above, and the diagnosis is made when bone alterations appear after 6 months of age. Genetic analysis showing mutations of the *ALPL* gene is essential to confirm the diagnosis in doubtful cases [5, 65].

In most cases, childhood HPP becomes evident after 1 year of age, with a variety of presenting signs and symptoms, such as rickets, skeletal deformities (such as dolichocephalia and enlarged joints), fractures, lower-than-average stature, delayed walking, waddling gait due to bone deformities, appendicular muscular hypotonia, and weakness apparently due to non-progressive myopathy, chronic bone and joint pain of the lower extremities, chronic non-bacterial osteomyelitis, premature closure of cranial sutures with possible signs of intracranial hypertension, and failure to thrive with anorexia, nausea, and gastrointestinal problems. Painless premature loss of primary teeth before 5 years of age, due to hypoplasia of the cementum, and dental caries are characteristic and common and may be a clue to diagnosis [4, 6, 9, 65]. Bone problems sometimes undergo spontaneous remission after puberty, but the disease may reappear in adulthood [6, 9].

Recently described clinical features of infantile and childhood HPP

Scoliosis A few cases of scoliosis associated with HPP have been reported. The deformity appears in the first year of life and the curve progression is very rapid. Orthopedic examination of infants with HPP is recommended [66].

Recurrent fractures as the presenting sign of childhood HPP Recurrent fractures are normally observed in adult patients with HPP, but Moulin et al. have recently reported the case of a 9-year-old girl presenting with recurrent fractures after minimal trauma. Her sister had also had fractures. Low total ALP and bone-specific ALP were thereafter discovered in both girls and their mother, and an *ALPL* gene mutation (E435K) was identified [67].

Differential diagnosis of infantile and childhood HPP

The differential diagnosis of the infantile and the childhood forms of HPP is with inborn errors of metabolism, organic aciduria, primary and secondary rickets, osteogenesis imperfecta, dentinogenesis imperfecta, achondrodysplasia, periodontitis, cleidocranial dysostosis, Cole-Carpenter syndrome, idiopathic juvenile osteoporosis, renal osteodystrophy, chronic non-bacterial osteomyelitis and osteosarcoma. Neglect and non-accidental trauma should also be considered as possible differential diagnoses.

Adult HPP (OMIM #146300)

The great variability in clinical presentation of adult HPP is well described in an interesting recent retrospective study [68]. This form appears after middle age and often presents with pain (due to stress fractures or pseudofractures), osteomalacia, osteoarthropathy, and/or chondrocalcinosis. Some patients are asymptomatic and a diagnosis is sometimes made by chance on the basis of typical laboratory findings. Sometimes they will recall a history of mild rickets and premature loss of primary teeth in childhood, but were apparently normal in their youth and young adulthood [6, 68].

Foot pain due to recurrent stress fractures of the metatarsal bones or, with more advanced disease, thigh pain due to femoral pseudofractures is typical of this form. Refractory pain of the extremities can also be a presenting sign. The femoral pseudofractures (Looser's zones) observed in this form of HPP are often bilateral and occur in the lateral cortex in the subtrochanteric diaphysis, while in classical osteomalacia they are observed in the medial cortex of the femoral neck [69, 70]. Yavuz et al. have recently reported the case of a 45-year-old male archer with stress fractures on both femoral necks and a non-displaced stress fracture on the proximal metaphysis of

the left ulna [71]. Fracture healing is delayed and difficult, and these patients often experience problems with implants for fracture fixation or joint replacement [4, 69]. Bone mass is often low [72, 73].

Low bone mass and fractures due to mild forms of adult HPP may lead to a wrong diagnosis of primary osteopenia or osteoporosis and also to inappropriate treatment with bisphosphonates. In such cases, subsequent findings of low serum ALP may be misinterpreted as an effect of antiresorptive therapy. This problem underlines the importance of a careful differential diagnosis (including measurement of serum ALP and, if necessary in doubtful cases, genetic analysis) before starting any therapy for "osteoporosis."

The current concern about the occurrence of "atypical" femur fractures in patients treated with long-term bisphosphonates for osteoporosis should call attention to the possibility of a wrong diagnosis of osteoporosis in a subject with HPP [74, 75].

Degeneration of articular cartilage and articular deposition of calcium PPI dihydrate (CPPD), with pseudogout attacks, are also observed in adult HPP. The signal transduction pathway that is activated in the inflammatory response to precipitation of CPPD crystals (CPPD-induced arthritis) has been recently investigated [76]. Calcific periarthritis has recently been reported as the only clinical manifestation of HPP in three middle-aged sisters with recurrent episodes of pain at shoulders, elbows, wrists, hips, and tendons of Achilles, with no other signs of disease, such as dental disease, fractures, or pseudofractures. Low serum levels of total ALP and bone-specific ALP, hyperphosphatemia, and increased serum concentrations of PPL were discovered as well as a specific mutation [77].

Renal alterations and stones have also been reported as well as early loss of permanent (secondary) teeth [4]. Finally, pain, depression, and anxiety, more severe than in a normal reaction to disease, are sometimes present in adult HPP patients [4] and might reflect some effect of TNSALP deficiency on central nervous system function.

Differential diagnosis of adult HPP

The differential diagnosis is with osteopenia/osteoporosis, osteoarthritis, and pseudogout.

Odontohypophosphatasia (OMIM #146300)

This is the least severe form of HPP and may affect both children and adults. It can be inherited as an autosomal recessive or autosomal dominant disease [51, 78, 79]. The clinical presentation is with dental alterations (premature exfoliation of primary teeth and/or severe dental caries) without evidence of bone disease such as rickets or osteomalacia. Dental X-rays show reduced alveolar bone and enlarged pulp chambers and

root canals. Biochemical findings are essentially similar to those of patients with mild forms of childhood or adult HPP.

Diagnosis

The diagnosis of HPP, at least for the more clinically relevant forms, is based on medical history, physical examination, laboratory findings, and typical X-ray skeletal alterations [3–6, 9]. The diagnosis of mild HPP forms, particularly in adults, can be more difficult due to less marked clinical features. Genetic analysis and identification of the *ALPL* gene mutations are now possible to clarify doubtful cases.

Reduced serum ALP activity (hypophosphatasemia, i.e., low, or inappropriately low for age, ALP levels) is characteristic of HPP, but by itself is not sufficient for a diagnosis, as it can occur in several conditions unrelated to bone, such as celiac disease, severe anemia, hypothyroidism, Wilson's disease, malnutrition, massive transfusion of blood or plasma, early pregnancy, and drugs [4, 5]. It must also be remembered that serum ALP levels do significantly vary with age and sex. Hypophosphatasemia has also been reported in severe osteogenesis imperfecta [80], cleidocranial dysplasia [81], and osteochondrodysplasia [82].

Biochemical tests

Subnormal serum ALP activity for age and sex is the main laboratory alteration that should arise a suspect of HPP. As a rule, HPP severity inversely correlates with serum ALP activity for age. Reliable laboratory methods used for ALP or TNSALP measurement provide both upper and lower limits of normal for the various age ranges [4, 6, 9]. Unfortunately, clinicians are still more likely to pay attention to higher-than-normal values than to low values, and the presence of mild forms of HPP may go unnoticed. In particular, both children and adults with ALP values around or slightly below the lower limit for age may have mild HPP and deserve further investigation.

Accumulation of ALP substrates (PPi, PLP, or PEA) is an indicator of low ALP activity. Most HPP patients have increased urinary excretion of PPi, but this may not be evident in milder forms [2]. Moreover, in many cases, PPi levels can only be measured in research settings, because commercial assays are not widely available yet. High PEA level in urine or plasma can suggest a diagnosis of HPP but is not pathognomonic, and this test is now considered of secondary importance. A more sensitive marker is an elevated plasma level of PLP, whose values correlate well with disease severity in children. High PLP levels are also observed in odontohypophosphatasia. Some commercial assays do not distinguish between PLP and its dephosphorylated metabolite (pyridoxal).

In infantile HPP, the impaired skeletal mineralization brings about an elevation of serum phosphate and calcium levels, with consequent hyposecretion of parathyroid hormone (PTH). Hypercalcemia and hypercalciuria may be present in childhood HPP, but PTH and 25-hydroxy-vitamin D are usually normal or only slightly decreased, except in the presence of hypercalcemia or renal insufficiency [4, 9]. Hypovitaminosis D is often found in adult HPP [4].

Genetic analysis

It is now possible to confirm a diagnosis of HPP by sequencing the *ALPL* gene. Genetic analysis is useful when the clinical and biochemical data are not conclusive for the prenatal diagnosis of severe HPP in pregnant women who already have an affected child. Genetic consultation is necessary prior to genetic testing. As already stated, inheritance can be autosomal dominant or recessive, but de novo mutation has also been described. For example, Taillandier et al. [83] reported a case of childhood HPP due to heterozygosity for a moderate maternal mutation (E174K) and a severe de novo mutation (M45I).

Prenatal diagnosis

Prenatal diagnosis is technically possible, but its usefulness is questionable, considering that the “perinatal lethal” and the “prenatal benign” form cannot be reliably differentiated. A recent article presents the results of the genetic analysis of 19 cases of fetal HPP revealed by ultrasonography: none of these cases could be diagnosed as prenatal benign HPP [84].

Imaging techniques

X-rays may show different degrees of hypomineralization and rickets according to the HPP form. The most impressive images are observed in the perinatal lethal form, in which an almost total absence of bone structure may be present. In infantile and childhood HPP, a wide variety of alterations—e.g., severe generalized osteopenia, absent bones, gracile bones, abnormal growth plates, fractures, “tongues” of radiolucency, patchy areas of osteosclerosis—may be observed [4, 6, 9, 85]. Skull X-rays may reveal hypomineralization of cranial bones with apparently wide open sutures, as well as increased convolutional markings suggesting the premature fusion of cranial sutures [4, 9, 86, 87]. Kozłowski et al., in a detailed radiographic study of 24 children with HPP (3 perinatal lethal, 18 infantile, 3 childhood), in addition to the most characteristic findings (osteoporosis, metaphyseal changes, craniostenosis or widened cranial sutures, bowing of the long bones) reported several other anomalies, such as spurs of the long bones (Bowdler sign), distal femoral defects, tibial deformities, abnormal distal phalanges of fingers, multiple rib

fractures, wedging of the lower thoracic and upper lumbar vertebrae, partial premature epiphyseal fusion, nephrocalcinosis, and loss of the lamina dura around the teeth [88].

In adult HPP, X-rays may show metatarsal stress fractures, pseudofractures of the femur, osteopenia, and chondrocalcinosis [68].

MRI or whole-body MRI is a sensitive tool to reveal early changes of bone structure or signs of musculoskeletal inflammation in both children and adults [89–91]. In HPP patients with chronic pain, MRI may reveal hyperemia and edema, more often localized in long-bone metaphyses [91]. Ultrasonic study of the kidneys can be useful to reveal and monitor nephrocalcinosis or kidney stones [92].

BMD evaluation

Few data on BMD in HPP are available. BMD is low in adult HPP and might lead to a wrong diagnosis of primary osteopenia or osteoporosis [4, 72, 73] and a contraindicated treatment with bisphosphonates. In a 4-year prospective evaluation of BMD (with both pQCT and DXA) in 6 subjects with childhood HPP, Girschick et al. [93] observed a decreasing trend of BMD Z-score. A very recent article by Whyte et al. [54] presented cross-sectional data on BMD measured by DXA at the lumbar spine and hip and observed lower values in the more severe cases of HPP.

The pQCT study by Girschick et al. [93] also reported hypermineralization of the metaphyseal trabecular bone and a significant hypomineralization of the metaphyseal cortical bone of distal radius. The hypermineralization of the trabecular bone was present at diagnosis and was interpreted as “a compensation for a mechanically incompetent bony structure,” but showed a decreasing trend over time.

Histopathological findings

The main bone finding in all forms of HPP except odontophosphatasia is defective mineralization, with osteoid accumulation reflecting the decrease in ALP activity. The TNSALP-producing cells, chondrocytes and osteoblasts, are present in bone and growth plates, but TNSALP activity is reduced or virtually absent [48, 55, 94].

Histomorphometry and quantitative backscattered electron microscopy of the iliac crest biopsies from 8 patients with adult HPP confirmed an increase in non-mineralized osteoid, as expected, but also demonstrated altered trabecular microarchitecture, increased number of osteoblasts, and impaired calcium distribution within the mineralized bone matrix, in comparison with specimens from healthy donors and subjects affected by other types of osteomalacia [72].

Treatment

Therapeutic interventions in HPP must address very different problems—chronic pain, musculo-articular-skeletal alterations, fractures, renal problems, psychological and neurological problems, and dental problems. There are no established medical treatments for HPP, but only therapeutic interventions to alleviate symptoms and reduce complications, and patients should ideally be followed by an experienced multispecialty team. Only general rules can be outlined, the first of which is to carefully avoid the use of drugs that are contraindicated and potentially dangerous for HPP patients, such as bisphosphonates or too high doses of vitamin D. New therapies are being evaluated [95], and in particular, phase 2 studies of enzyme replacement therapy (ERT) with a recombinant TNSALP for severe infantile forms are currently ongoing.

General management

Nutrition and physical activity

Dietary phosphate (Pi) restriction and/or pharmacologic binding of dietary Pi have been proposed for hyperphosphatemic patients, remembering that Pi competitively inhibits TNSALP activity and gene expression [96].

In children, adequate nutrition is recommended, especially in the presence of persisting growth retardation. Vitamin D insufficiency is common and standard supplementation, according to the guidelines for healthy subjects, is recommended. High doses could aggravate the hypercalcemia/hypercalciuria in infantile HPP [97]. Supplementation with active metabolites (such as 1 α -hydroxy-vitamin D or 1, 25-dihydroxyvitamin D) should be avoided, because they can increase intestinal Pi absorption and serum Pi levels. Calcium supplementation is to be avoided except in well-selected cases, due to the risk of hypercalcemia and kidney stones. In adults, appropriate supplementation with calcium and vitamin D can prevent secondary hyperparathyroidism [97].

Maintaining good physical function is very important. Regular low-impact physical exercise is recommended at any age, as muscular activity physiologically helps in building and maintaining bone mineral mass and bone strength. The extent this will succeed in HPP is probably dependent on the severity of the disease and remains to be established. In some cases, physiotherapy may be required. At least in the severe forms of HPP, physical activities carrying a significant risk of trauma must be avoided, as these patients are at high risk of fragility fractures that will heal with great difficulty and in a long time, requiring prolonged casting or orthopedic interventions.

Inflammation and musculo-articular-skeletal alterations

NSAIDs have been reported as effective in patients with pain and inflammatory symptoms, and significant subjective and functional improvement, as well as normalized prostaglandin levels, have been observed. Although prolonged high-dose NSAID use is inappropriate, intermittent use can help and anti-inflammatory effects may persist for 3–6 weeks after withdrawal. In adults with impaired renal function, NSAID use is risky and requires close monitoring of renal function [4]. NSAIDs have also been successfully used for chronic bone and joint pain in childhood HPP [98].

Osteoporosis and fractures

Bisphosphonates are theoretically contraindicated, considering their PPI-like chemical structure and the fact that they can further suppress TNSALP activity [73]. Unlike classical osteoporosis, HPP is a disease of deficient bone mineralization, not one of excess bone resorption. It is thus unlikely to benefit from bisphosphonates, and there is some risk of untoward side effects. In adults with undiagnosed mild HPP and treated with bisphosphonates for “osteoporosis,” atypical (lateral subtrochanteric) femur fractures have been observed [74, 75, 97]. For example, Sutton et al. [74] described the case of a 55-year-old woman who sustained atypical subtrochanteric femoral fractures after 4 years of bisphosphonate treatment for “osteoporosis.” Only after these fractures the correct diagnosis of adult HPP was made.

It is also likely that a significant number of individuals with mild forms of HPP are mistakenly treated with bisphosphonates for “osteoporosis,” and some of them may sustain femoral pseudofractures, but the number of these cases and the frequency of this complication cannot be estimated [97].

There are some case reports, with inconsistent outcomes, on the use of teriparatide (recombinant human parathyroid hormone) in adults to treat stress fractures. The theoretical risk of osteosarcomas advises against its use in children. The rationale for this therapy is that PTH stimulates the synthesis of TNSALP in osteoblasts. Whyte et al. have reported the healing of metatarsal stress fractures and proximal femur pseudofractures with a 18-month treatment [99]. Positive results were also observed in a 75-year-old woman with multiple low-trauma fractures: skeletal mineralization and bone turnover markers improved, and no more fractures were sustained, during a 24-month treatment [100]. Pain, mobility, and fracture repair were all improved in two sisters, respectively, treated for 7 and 18 months [101]. In a 53-year-old woman with painful non-healing femoral pseudofractures, bone biopsy showed increased amount of osteoid and number of osteoblasts after 5 months of teriparatide, and 3 months later, the pseudofracture of the right femur was completely

healed, bony callus had appeared in the left femur, pain had resolved, and mobility was improved. However, the response was not sustained and serum ALP and PLP and urine PEA returned to baseline between 8 and 13 months of treatment [102]. Teriparatide had no effect on bone density, fracture healing, and TNSALP levels in a 43-year-old woman [103]. These inconsistent results have suggested that the response might depend on the specific gene mutations [102].

Doshi et al. reported an interesting case of bisphosphonate-related atypical femur fractures successfully treated with teriparatide after the correct diagnosis of HPP was made. A 50-year-old woman sustained bilateral stress femur fractures that did not heal with a 2.5-year calcium, vitamin D, and risedronate treatment for “osteoporosis.” After surgical fracture stabilization, HPP was diagnosed. She had significant fracture site pain and generalized bone pain (both weight-bearing and non-weight-bearing pain) and was unable to walk. Teriparatide treatment was started, and after 8 weeks, pain was significantly reduced and she could again walk unassisted. At 4 and 16 months of treatment, radiographs demonstrated fracture healing [104].

Calcitonin followed by a bisphosphonate (clodronate) was unsuccessfully tried in a 7-month-old girl affected by infantile HPP, presenting with failure to thrive, hypomineralization, nephrocalcinosis, hypercalcemia, and continuous vomiting. The clinical picture, including skeletal mineralization, continued to deteriorate and she died of respiratory failure 7 months later [105].

Jakob et al. [95] reported that a phase 2 clinical study using an anti-sclerostin antibody (primarily developed to treat osteoporosis through enhancement of osteoblast activity [106]) to stimulate bone formation in adults with mild HPP was ongoing in 2012, but no results have been published so far.

Neurological and psychological problems

In severe neonatal HPP, seizures are apparently due to altered PLP metabolism. In some cases, these seizures respond to vitamin B6 or pyridoxine treatment, but this is not considered a favorable sign and may on the contrary indicate a lethal condition [6, 9, 14].

The neurological or psychological symptoms that are commonly seen in the forms that allow long-term survival (chronic pain, irritability, restlessness, insomnia, anxiety, depression) can only be treated symptomatically. Recent advancements on TNSALP role in nociception and purinergic signaling may open the way to novel and more specific approaches [4].

Renal problems

Hypercalcemia and the resulting hypercalciuria can cause renal stones. Hyperphosphatemia may require treatment with phosphate binders. Nephrocalcinosis can cause

worsening of renal function. Antihypertensive treatment may also be necessary. Prudent, short-term use of NSAIDs should be considered, possibly in cooperation with a nephrologist.

Neurosurgical problems

Premature fusion of cranial sutures is common in the infantile and childhood forms, and complications like secondary ectopia of the cerebellar tonsils (Chiari I malformation) or formation of hydrosyringomyelia may occur. The risk of premature craniosynostosis must always be considered, and periodic radiological, neurological, and ophthalmoscopic assessment is recommended until adolescence. In the presence of Chiari I malformation or hydrosyringomyelia, lifetime surveillance is needed [107]. Elevated intracranial pressure may be revealed by neurologic signs and symptoms, like continuous headache, seizures, numbness of extremities, paralysis, and papilledema, and requires neurosurgical intervention.

Dental problems

The premature loss of primary teeth can be the presenting sign of HPP and must not be ignored. Children affected by HPP need regular supervision by a dentist, and accurate oral hygiene is of course even more important than usual. Premature exfoliation of teeth may require prosthetic interventions to avoid abnormal transversal development of the jaw and non-alignment. Other strong reasons for teeth replacement are helping the normal development of speech and avoiding self-image or relational problems.

Bone marrow and mesenchymal stem cell transplantation

Bone marrow transplantation has been tried with some success in a few patients with severe infantile HPP. In 2003, Whyte et al. described an 8-month-old girl in whom some improvement of bone mineralization was observed 3 months after transplantation of her sister's T-cell-depleted bone marrow. The patient received a boost of marrow cells at age 21 months, with further improvement, and was alive and relatively well at 6 years of age, although the hypophosphatasemia was never corrected [108]. In 2007, Cahill et al. reported another successful case of transplantation of bone fragments and cultured osteoblasts by multiple routes in a 9-month-old girl, with clinical improvement persisting 7 years later [109]. A Japanese team has reported about bone marrow and mesenchymal stem cell transplantation (obtained from the father) in an 8-month-old girl with infantile HPP. The initial success was followed, at age 32 months, by the development of Ph⁺ leukemia that was attributed to the effect of immunosuppressive treatment on the transplanted stem cells. After another transplantation at age 40 months, complete

histological and molecular remission was observed [110, 111]. The same authors have recently reported about two more cases of severe HPP, in whom bone marrow transplantation followed by multiple infusions of mesenchymal stem cells led to improvements in bone mineralization, muscle mass, respiratory function, and mental development. The patients are surviving at 3 years of age [112].

Enzyme replacement therapy

The early attempts consisted in the intravenous administration of normal plasma, ALP-enriched plasma from patients with Paget bone disease, purified human liver ALP, or purified human placental ALP in infants with severe HPP, but were not successful [113–115].

Then in 2008, a bone-targeted form of recombinant TNSALP (sALP-FcD10, first renamed ENB-0040, and then Asfotase alfa, the current name) became available and was since investigated, first in knockout mice models and then in infants with life-threatening HPP. Some phase 2 clinical studies in children are currently ongoing.

The preliminary studies of ENB-0040, with subcutaneous injections of high dose (8.2 mg/kg/day) in newborn TNSALP-null (Akp2^{-/-}) mice, that mimic human infantile HPP, reported normal growth, absence of skeletal or dental alterations, and no epilepsy [116, 117]. Another study [118] investigated the optimal dose-response relationship: Akp2^{-/-} mice with various phenotypic traits were treated with subcutaneous ENB-0040 at different doses (0.5, 2.0, or 8.2 mg/kg/day) from birth to 43 days of age. Using X-rays, micro-computed tomography, and histomorphometry, it was shown that the doses preventing bone defects in 80 % of mice were 2.8–3.2 mg/kg/day depending on the skeletal site. There was a positive correlation between survival and ENB-0040 dose. Urinary PPi concentrations remained elevated independently of dose. Other studies on Akp2^{-/-} mice reported the effects of therapy starting immediately after birth: ENB-0040 prevented the hypomineralization of alveolar bone, dentin, and cementum, as well as enamel defects [117, 119].

On the basis of the positive results in mice models, ENB-0040 was investigated in some patients with very severe neonatal forms of HPP. Rodriguez et al. treated a preterm 3-week-old infant with HPP, mechanically ventilated since birth because of severe chest wall insufficiency, and after 12 weeks of ENB-0040 reported improvement in lung function and chest wall mechanics (with respect to a baseline evaluation at age 8 weeks, after 5 weeks of treatment) and a lower request of oxygen. The child was discharged to home at age 32 weeks, but 2 weeks later he developed fever, hypoxemia, pneumonia, and shock. He was again hospitalized, ERT was suspended, and he died a few days later from septic shock and multiorgan failure, disseminated intravascular coagulopathy, hypoxemic

respiratory failure, and acute respiratory distress syndrome [120].

In the same year, Whyte et al. [121] published the first results of a phase 2, multinational, open-label study of ENB-0040 treatment in infants and young children with life-threatening HPP (ClinicalTrials.gov number, NCT00744042). Eleven patients (7 girls and 4 boys, aged 2 weeks to 3 years; 5 with perinatal HPP and 6 by infantile HPP) were enrolled: one withdrew, 10 completed 6 months of therapy, and 9 completed 1 year.

All children presented typical symptoms (failure to thrive, severe hypomineralization, fractures, and substantial motor delay or regression) before 6 months of age. Lost dentition was present in those with erupted teeth, and all but two patients had nephrocalcinosis or renal stones. Treatment consisted in a single intravenous infusion (2 mg/kg) of ENB-0040, followed by subcutaneous injections three times a week (1 mg/kg, to be increased up to 3 mg/kg in case of worsening pulmonary function, failure to thrive, or absence of skeletal improvement). Skeletal healing became evident within 24 weeks of treatment in all patients but one, with increased BMC, improved endochondral and membranous bone formation, fracture healing, reduced deformity, resolution of radiolucencies, and sclerosis. After 6 months of therapy, nine patients showed healing of rickets, together with improvement in pulmonary function and growth, and the plasma levels of PPI and PLP had decreased. Apparently, the therapy had no effect on craniosynostosis and two patients required cranial surgery. After 48 months of therapy, serum calcium and phosphate levels showed only transient minor fluctuations. Serum PTH showed an increasing trend in parallel with bone mineralization, and this led to a liberalization of dietary calcium intake. PPI, measured in 5 patients, significantly decreased over the 48 weeks of treatment. The most common treatment-related adverse event was mild, localized, transient erythema at the injection site. Nephrocalcinosis did not progress after the first 6 months of treatment and even improved in some patients. No ectopic calcifications were observed. The increased bone mineralization process did not lead to hypocalcemia (“hungry bones”). There were no signs of drug hypersensitivity or tachyphylaxis, and only four patients developed low titers of anti-ENB-0040 antibodies, with no evident clinical, biochemical, or autoimmune abnormalities after 48 weeks of treatment. Three serious adverse events in three patients (one each of respiratory distress, craniosynostosis, conductive hearing loss), however, were considered as possibly related to the treatment.

In 2014, Whyte et al. presented a 3-year follow-up report of the study at a medical conference [122]. In the meantime, one patient had died for a cause (sepsis) unrelated to the study drug. Treatment with Asfotase alfa (the ENB-0040 was given its new name in the meantime) for 3 years was well tolerated. Significant improvement in skeletal mineralization, that had

begun after 3 months of treatment, continued over the 3 years of follow-up. At study start, most patients were requiring some respiratory support, but only one continued to require supplemental oxygen after 3 years. Survival at 3 years was 90 %, while in prior studies in comparable populations it was less than 50 %.

At the same conference, Madson et al. presented the follow-up results of another study, actually an open-label extension study of a 6-month randomized, multinational, open-label study (ClinicalTrials.gov: NCT00952484) whose preliminary results had been previously presented [123]. The study objective was the evaluation of efficacy and safety of long-term Asfotase alfa treatment in children (aged 5–12 years) with rickets and impaired physical function due to HPP. Thirteen children were initially enrolled, one withdrew, 12 were continuing treatment after 36 months. Sustained improvement in bone mineralization and in physical motor function, without serious drug-related adverse events, was reported.

At another 2014 conference, Rockman-Greenberg et al. presented the results of another ongoing Phase II, open-label, global, multicenter trial (ClinicalTrials.gov: NCT01176266; EudraCT:2010-019850-42) on 15 children with HPP, aged 21.1 weeks (range 0.1–304; $N=8$ age <22 weeks; $N=7$ age 168–304 weeks) [124]. The 13 patients with at least 6 months of follow-up data were evaluated. The overall survival was 93 % (one patient withdrew and died on day 22, from disease-related complications, not attributed to the drug). After 24 weeks of treatment, a significantly improved bone mineralization was observed. There were no serious drug-related adverse events.

There is only one report on ERT with Asfotase alfa in adolescent and adult patients with HPP. In 6 adolescents and 13 adults, after 24 weeks of treatment, TNSALP substrate levels (serum PPI and serum PLP) were significantly decreased, motor function (6-min walking test) was improved and the treatment was well tolerated [125].

Adverse reactions to Asfotase alfa

In all the previously cited studies, the treatment emergent adverse reactions were common, in most cases mild to moderate, and were not attributed to the drug. They are well detailed in the respective publications [121–125]. The most common drug-related adverse events were injection site reactions, observed in 55 % to 92 % of the treated children. One patient withdrew from a study due to an adverse reaction (irritability, piloerection, fever, chills) to the first intravenous infusion [121]. Three serious, possibly drug-related, adverse events were reported by Whyte et al., as already mentioned above [121].

Conclusions

In summary, HPP is a rare inherited disorder of bone and mineral metabolism, caused by a number of loss-of-function mutations in the *ALPL* gene. It is characterized by extreme variability in clinical presentation, especially in the mild forms of both children and adults. For this reason, the diagnosis can be difficult, and is often missed or delayed, particularly in adults.

Recently, ERT with recombinant TNSALP has been developed and clinical phase 2 studies are ongoing, especially in the severe forms of infants and children, with promising results for bone and lungs.

Notwithstanding recent advances, further investigation is needed in particular for the following aspects: clarification of the different functions of TNSALP (e.g. in nervous tissue); phenotype–genotype correlation studies to predict the severity of the disease; randomized, controlled clinical trials to evaluate treatment strategies for mild and adult forms of HPP.

Compliance with ethical standards This article does not contain any studies with human participants or animals performed by the author.

Conflict of interests Maria Luisa Bianchi has received honoraria and travel reimbursement from Alexion Pharmaceuticals for consultation and for speaking at meetings.

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