

# BEST PRACTICES IN:

## Recognizing and Diagnosing Hypophosphatasia

A supplement to CLINICAL ENDOCRINOLOGY NEWS®. A preprint to the November 2013 issue.

**Introduction:** Hypophosphatasia (HPP) is a rare inherited metabolic disease characterized by mineralization defects of the bones and teeth, and a number of systemic complications including impaired respiratory function, seizures, muscle weakness, bone pain, and nephrocalcinosis (Table 1).<sup>1,2</sup> HPP is caused by inactivating mutations in the gene encoding the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP), which lead to



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a deficiency in TNSALP enzymatic activity—the primary biochemical defect in HPP.<sup>1</sup> Disease severity is generally inversely related to the age at onset, and in its most severe form the condition causes death *in utero* or in early infancy.<sup>1</sup>

### Incidence and Prevalence:

Reports detailing the incidence and prevalence of HPP are scarce. Severe HPP has an estimated incidence of 1:100,000 in Toronto, Canada<sup>3</sup> and an estimated prevalence of 1:300,000 in France.<sup>4</sup> HPP is especially prevalent in the Mennonite population in Manitoba, Canada, owing to a particular

founder mutation estimated to have a carrier frequency of 1:25, and results in a predicted frequency of homozygous affected babies of 1:2500 in this population.<sup>5,6</sup> HPP primarily occurs in Caucasians but has been observed in Japanese, Hispanic, and Native American populations; HPP is very rare among the black population.<sup>1,7</sup>

**Genetic Basis of Disease and Etiology:** The human ALP isoenzyme family is encoded by 4 separate genes. Three of the genes each encode a single ALP isoenzyme specific to the intestines, placenta, and germ cells, respectively.<sup>8</sup> The fourth gene, *TNSALP*, encodes an ALP isoenzyme that is expressed ubiquitously; the highest levels of expression occur in the liver, bone, and kidney. Differences in catalytic activity of liver, kidney, and bone TNSALP result from varying posttranslational glycosylation modifications.<sup>8</sup>

The *TNSALP* gene is located on the short arm of chromosome (1p36.1-34) and comprises 12 exons distributed over 50 kb.<sup>9,10</sup> To date, 267 distinct mutations

and 16 polymorphisms in *TNSALP* have been associated with HPP.<sup>7</sup> The majority (74.5%) of mutations are missense mutations. HPP may result from either an autosomal dominant or autosomal recessive pattern; penetrance is variable resulting in a wide range of clinical expressivity.<sup>1</sup> Autosomal recessive inheritance generally results in more severe HPP, whereas less severe presentation of

### Overview of TNSALP Function and Dysfunction

TNSALP is an enzyme bound to cell surfaces through glycosylphosphatidylinositol anchoring.<sup>8</sup> TNSALP is a Zn<sup>2+</sup> metalloenzyme that is physiologically active in its dimeric form and that cleaves several phosphocompounds, including PPi, PLP, and PEA. Mg<sup>2+</sup> is a required cofactor. When TNSALP activity is deficient, these substrates accumulate in the body, leading to impaired mineralization of bone and teeth and other systemic complications.<sup>12</sup>

#### Inorganic Pyrophosphate (PPi)

Mineral deposition during endochondral bone formation involves 2 basic phases<sup>1,13</sup>: 1) accumulation of calcium and phosphate with initiation of hydroxyapatite (HA) crystal formation in membrane-bound osteoblast organelles called membrane vesicles (MVs), and 2) rupture of MVs with release of their content onto bone matrix where additional HA formation and growth occurs. In HPP, MV HA formation does not appear to be impaired. However, when present extracellularly in high concentrations, PPi reportedly accumulates around MVs where it adsorbs both to amorphous calcium phosphate (preventing new HA formation) and to existing HA crystals (preventing growth and deposition of HA).

The discovery that PPi levels are increased in the plasma and urine of patients with HPP as a direct result of deficient TNSALP activity represented a major breakthrough in explaining the defective skeletal mineralization observed in HPP patients.<sup>12</sup>

PPi is not available as a routine clinical assay; it is used presently in research studies.

#### Pyridoxal 5'-Phosphate (PLP)

PLP is the major circulating form of vitamin B6 and is essential for many enzymatic processes within cells. Following digestion, numerous dietary forms of vitamin B6 (eg, pyridoxine, pyridoxal, pyridoxamine) undergo absorption from the gastrointestinal tract and are converted in the liver to PLP which is secreted into the circulation.<sup>1,8</sup> PLP must be dephosphorylated to PL by ALP in order to enter tissues and cross the blood-brain barrier where it is rephosphorylated to PLP or converted to pyridoxamine-5-phosphate, both of which act as cofactors in a variety of intracellular enzymatic reactions, including glutamate decarboxylase activity and the synthesis of the neurotransmitters serotonin and gamma-aminobutyric acid (GABA).

Vitamin B6-responsive seizures observed in the most severe HPP patients are likely the result of deficiency of PLP in the central nervous system. The fact that all but the most severe HPP patients have normal or somewhat elevated plasma PL levels, unremarkable vitamin B6 status, and few seizures suggests that another mechanism may exist to dephosphorylate PLP extracellularly.<sup>1,14,15</sup>

PLP is available as a routine clinical assay, most often reported as Vitamin B6.

#### Phosphoethanolamine (PEA)

Elevated PEA in the plasma and urine is a common finding in HPP.<sup>14</sup> PEA is a component of the phosphatidylinositol-glycan anchor that tethers proteins to the cell surface. As such, an accumulation of PEA may result from the degradation of these anchors. Alternatively, excess PEA may originate from the liver, which normally metabolizes PEA in a reaction controlled by

O-phosphorylethanolamine phosphatase that requires PLP as a cofactor.<sup>1,12</sup> PEA is available as a commercial assay, generally at specialty laboratories.

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HPP can result from either autosomal dominant or autosomal recessive inheritance. In most cases, autosomal recessive inheritance involves compound heterozygote inheritance of 2 different *TNSALP* alleles.<sup>11</sup>

The physiologic role of TNSALP in the liver and kidney is not well defined; in contrast, TNSALP is known to have a critical role in regulating the mineralization of bones and teeth, and vitamin B6 metabolism. Low TNSALP activity results in increased levels of 3 known phosphocompound substrates: inorganic pyrophosphate (PPi), pyridoxal 5'-phosphate (PLP; the major circulating form of vitamin B6), and phosphoethanolamine (PEA).<sup>1</sup> Inorganic pyrophosphate inhibits skeletal mineralization, causing rickets or osteomalacia despite normal or elevated circulating levels of calcium and inorganic phosphate. (See *Overview of TNSALP Function and Dysfunction* for additional details.)

**Presentation and Burden of Disease:** Phenotypic expression of HPP is quite variable and does not clearly correlate with genotype, even within a given family of patients. Traditional characterization of HPP has relied on the observation that the severity of disease is generally related to patient age at onset of disease, resulting in description of HPP as perinatal, infantile, childhood, or adult forms of HPP (Table 1).<sup>2</sup> It is now recognized that such distinct categories, although helpful in describing the disease, are arbitrary. Variability in presentation—including within these defined clinical categories—underlies the observation that HPP may span the greatest range of severity among all inherited diseases.<sup>1</sup>

Perinatal HPP manifests *in utero* with marked skeletal hypomineralization and is the most severe form of HPP.<sup>1</sup> Perinatal HPP can typically be detected *in utero*

with prenatal ultrasound during the second trimester of pregnancy but may not be differentially diagnosed from other skeletal dysplasias, such as osteogenesis imperfecta, at this point.<sup>16</sup> Radiographic findings are typically very distinguishable. The bones may appear completely unmineralized; if skeletal mineralization is present, marked rachitic abnormalities are often evident. Poorly ossified epiphyses, fractures, osteochondral ("Bowdler") spurs protruding laterally midshaft from the long bones (ulna, fibula) and sometimes piercing the skin, and poorly formed teeth may be evident. At birth, the characteristic clinical signs include caput membranaceum, shortened limbs, and Bowdler spurs. Vitamin B6-responsive seizures secondary to PLP accumulation may also present in these patients and portend a poor outcome.<sup>1,17</sup> The majority of patients with perinatal HPP die at or soon after birth, primarily as a result of respiratory insufficiency due to hypoplastic lungs and functional defects secondary to rachitic deformity of the chest.

Patients defined as having infantile HPP develop symptoms after birth but within the first 6 months of life. Development may initially seem normal but then becomes compromised by failure to thrive (including poor feeding and poor weight gain), rickets, muscle hypotonia, pulmonary insufficiency, and seizures.<sup>1,16,17</sup> Hypercalcemia and hypercalciuria commonly occur, with high calcium levels leading to irritability, poor feeding, abdominal pain, vomiting, and risk of nephrocalcinosis.<sup>1</sup> Wide separation between cranial sutures may be felt initially due to diminished ossification of the skull; however,

**Table 1**

## Clinical Features of the Major Forms of HPP<sup>2,a</sup>

Feature	Perinatal Onset	Infantile Onset	Childhood Onset	Adult Onset
Age at onset	<i>In utero</i> , at birth	< 6 months of age	6 months to 18 years of age	≥ 18 years of age
Clinical signs/symptoms	<ul style="list-style-type: none"> <li>• Apnea</li> <li>• Fractures</li> <li>• Hypomineralization</li> <li>• Long bone deformity</li> <li>• Osteochondral spurs</li> <li>• Poorly ossified epiphyses</li> <li>• Radiolucencies into metaphyses</li> <li>• Severe chest deformity</li> <li>• Stillbirth</li> <li>• Vitamin B6-responsive seizures</li> </ul>	<ul style="list-style-type: none"> <li>• Craniosynostosis</li> <li>• Failure to thrive</li> <li>• Fractures</li> <li>• Hypercalcemia/ hypercalciuria</li> <li>• Hypomineralization</li> <li>• Hypotonia</li> <li>• Nephrocalcinosis</li> <li>• Poor feeding</li> <li>• Poor weight gain</li> <li>• Premature deciduous tooth loss</li> <li>• Pulmonary insufficiency</li> <li>• Rickets</li> <li>• Vitamin B6-responsive seizures</li> </ul>	<ul style="list-style-type: none"> <li>• Chronic muscle/bone pain</li> <li>• Hypomineralization</li> <li>• Low bone mineral density</li> <li>• Missed motor milestones</li> <li>• Muscle weakness</li> <li>• Poorly healing or recurrent fractures</li> <li>• Premature tooth loss</li> <li>• Rachitic disease</li> <li>• Short stature</li> <li>• Skeletal deformity</li> </ul>	<ul style="list-style-type: none"> <li>• Abnormal dentition</li> <li>• Adult tooth loss</li> <li>• Chondrocalcinosis</li> <li>• Chronic muscle/bone pain</li> <li>• Hypomineralization</li> <li>• Osteoarthritis</li> <li>• Osteomalacia</li> <li>• Pseudofractures/fractures</li> <li>• Pseudogout</li> </ul>

<sup>a</sup>Presented here are classically defined categories based on patient age at onset of disease. It is now recognized that such distinct categories, although helpful in describing the disease, are arbitrary,<sup>1</sup> and that there is significant variability in presentation and severity of disease both across and within these categories. Representative clinical signs and symptoms are listed alphabetically; see text for additional details.

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neonates that survive beyond infancy may experience premature bony fusion of sutures (craniosynostosis) with the illusion of an open fontanel from calvaria hypomineralization.<sup>1</sup> Radiographic series may reveal gradual, generalized skeletal demineralization, together with persistent defective skeletal mineralization (ie, rickets). Bone deformities worsen over time and may be accompanied by fractures.<sup>1</sup> The initial prognosis of infantile HPP is unpredictable. While approximately 50% of affected infants die, most classically with respiratory compromise and infection due to progressive skeletal disease of the chest, others, for reasons unknown, spontaneously recover, and are referred to as having benign perinatal HPP.<sup>1,16</sup>

Childhood onset of HPP is defined by onset after the first 6 months of life but prior to 18 years old. Patients may present with skeletal bowing, short stature, fractures, bone pain, nonprogressive myopathy, and muscle pain and stiffness.<sup>1,18,19</sup> In addition to bowed legs, rachitic deformities may include beading of costochondral junctions; widened metaphyses leading to enlarged wrists, knees, and ankles; and brachycephalic skull.<sup>1</sup> Premature (ie, prior to age 5) loss of deciduous teeth which painlessly slide out from the tooth sockets with an intact root in the absence of bleeding, is an early and often unrecognized symptom of HPP<sup>1,19</sup> and results from aplasia of the root cementum consequent to deficient TNSALP activity.<sup>19,20</sup> Radiographs of the long bones typically show defects of the cartilage that extend from the growth plates into the metaphyses, which are described as radiolucent “tongues.” If present, craniosynostosis may cause the skull to take on a “beaten copper” appearance on a radiograph.<sup>1</sup> Individuals with childhood onset of HPP suffer from significant morbidity in childhood in the form of deformities and delays in motor skill development. Walking can be delayed by rickets, and involve a waddling gait due to muscle weakness.<sup>1,18</sup> Symptoms may improve in young adulthood following fusion of the growth plates, although physical complications (eg, ongoing difficulty climbing stairs) may persist.<sup>1,18</sup>

HPP that presents in adulthood typically manifests during middle age despite relatively good health in early adulthood. Patients may recall remitting rachitic deformities and premature loss of deciduous teeth during childhood. Patients may present with pain in the feet and hips/thighs owing to recurrent metatarsal stress fractures and subtrochanteric femoral pseudofractures, respectively.<sup>1,21,22</sup> Additional signs and symptoms of the adult form of HPP include osteopenia; osteoporosis; myopathy; musculoskeletal pain; early loss or extraction of adult teeth;

PPi arthropathy, sometimes resulting in attacks of pseudogout; calcific peri arthritis, commonly in ligaments of the spine; and, more rarely, primary hyperparathyroidism.<sup>1,22</sup>

Common radiographic findings in adult patients with HPP include pseudofractures (Looser’s zones, Milkman fractures) indicative of osteomalacia, along with generalized osteopenia and chondrocalcinosis.<sup>1,21,22</sup> Notably, femoral pseudofractures tend to occur in the lateral, rather than the medial, part of the cortex.<sup>21</sup> Pseudofractures that are not appropriately managed with surgical intervention usually progress to complete fractures with only very minor trauma.<sup>21</sup> Low bone mineral density can be detected in the femur and lumbar spine by dual-energy x-ray absorptiometry (DXA).<sup>23</sup> Bone disease may worsen gradually with time. As disease progresses, the incidence of pain and fractures increases<sup>1,21</sup> and significant disability may result from widespread, nonhealing fractures.<sup>1</sup> Of patients who report bone and joint pain, nearly all note that the pain limits their daily activities; roughly half of these patients require assistive devices (eg, wheelchair, walking device), and many need to modify their home environment.<sup>24</sup>

An additional form of HPP, odontohypophosphatasia, considered the least severe form of HPP, affects only the teeth; there is no evidence of skeletal disease.<sup>1</sup> This form of HPP is characterized by spontaneous loss of fully rooted deciduous teeth with an intact root and/or severe caries, along with enlarged pulp chambers and root canals.<sup>25,26</sup> Odontohypophosphatasia, like premature loss of deciduous teeth in childhood HPP, results from failure of the cementum to form on tooth roots.<sup>1,20</sup> It has been demonstrated that cementum is sensitive to changes in PPi level.<sup>20</sup> The anterior teeth, specifically the incisors, are most likely to be affected.<sup>26</sup>

**Diagnosis:** Low serum ALP is the distinguishing feature of HPP (Table 2).<sup>1</sup> Generally, HPP can be diagnosed with confidence based on low serum ALP activity together with physical and radiographic findings consistent with HPP.<sup>1</sup> When evaluating whether serum ALP is within or below the normal range, important consideration must be given to the age- and gender-specific reference ranges of the testing laboratory (see *Reference Ranges for ALP* for more information). Genetic testing, although it may provide additional insight as to the inheritance pattern of an individual patient, is not necessary to diagnose HPP.

## Laboratory Findings

Low serum ALP activity, elevated plasma PLP, elevated serum PPi, and elevated serum or urinary PEA are bio-

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Table 2

## Low ALP is the hallmark of HPP<sup>a</sup>

TNSALP Level	Associated Disease
Low	Hypophosphatasia
Normal	Osteogenesis imperfecta <sup>b</sup>
High	Nutritional rickets X-linked hypophosphatemic rickets Paget's disease Healing fracture

<sup>a</sup>In distinction to other types of rickets or osteomalacia, it is notable that elevated PLP is expected only for HPP, serum calcium is not low in HPP, and serum Pi is not low in HPP.

<sup>b</sup>Rarely, osteogenesis imperfecta may present with low ALP,<sup>1</sup> thus emphasizing the need for attention to laboratory-specific, age-adjusted, and gender-adjusted ALP reference ranges and, possibly, additional laboratory data (ie, PLP, PPi, PEA) for differential diagnosis.

chemical hallmarks of HPP. Notably, in distinction to other types of rickets or osteomalacia, elevated PLP is expected only for HPP.<sup>1</sup> Because of its specific association with HPP and its availability as a routine clinical assay (commonly reported as vitamin B6 level), elevated plasma PLP is most easily used, along with a low serum ALP level, to determine a laboratory-based diagnosis of HPP. Generally, the more severe the form of HPP, the lower the serum ALP level is and the higher the plasma PLP level is. Elevated serum or urine PEA can also support the diagnosis of HPP; however, PEA assays are not routinely performed outside of specialty laboratories and elevated PEA may occur with other disorders, including other metabolic bone disorders. Laboratory tests for PPi are not commercially available; this assay is used solely in research at this time.

In contrast to nearly all other types of rickets or osteomalacia, serum calcium and serum inorganic phosphate (Pi) levels are not low in HPP. Thus, these measures may be considered as part of differential diagnosis. In infantile HPP, hypercalcemia and hypercalciuria are frequent and commonly associated with nephrocalcinosis; serum parathyroid hormone (PTH) is suppressed and associated with hyperphosphatemia. With childhood HPP, hypercalciuria is relatively common and sometimes accompanied by low circulating PTH levels; serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels are typically unremarkable. Hypercalcemia is not common in childhood HPP, but when present, it is accompanied by low serum PTH and low 1,25-dihydroxyvitamin D levels. In childhood and adult HPP, serum Pi levels may be elevated relative to the mean value for age-matched controls; hyperphosphatemia is present in approximately 50% of these

## Reference Ranges for ALP

Careful attention must be paid to age- and gender-adjusted ALP reference ranges for accurate diagnosis of HPP. Serum ALP activity is considerably higher in healthy infants, children, and adolescents compared with adults owing to an abundance of TNSALP in developing bone.<sup>1</sup> Clinical laboratories often provide reference ranges for adults only, and sometimes report only an upper limit of normal. Inter-laboratory variability precludes use of reference ranges across laboratories. An example of laboratory-specific age- and gender-adjusted lower reference values for serum ALP (ARUP laboratories) is presented in this table.<sup>27</sup>

### Lowest Normal Total Serum or Plasma ALP Activity (U/L)<sup>27</sup>

Age	Male	Female
< 1 month	60	60
1-11 months	70	70
1-3 years	125	125
4-11 years	150	150
12-13 years	160	110
14-15 years	130	55
16-19 years	60	40
≥ 20 years	40	40

cases. Patients with childhood or adult forms of HPP are usually eucalcemic.

## Radiologic and Other Bone Findings

Radiographic analysis reveals pathognomonic skeletal abnormalities in infants and children with HPP (eg, rickets, impaired skeletal mineralization, Bowdler spurs, craniosynostosis), as described above. In adults, metatarsal stress fractures and femoral pseudofractures indicate adult-onset HPP.<sup>1</sup> Heterogeneous bone mineralization, patient deformity, or short stature may complicate the interpretation of DXA scans of children with HPP; algorithms that adjust for DXA bone mineral density in prepubertal children based on height and age are available.<sup>1,28</sup> Bone biopsy may show increased osteoid volume and osteoid surface consistent with pathologic enrichment of nonmineralized osteoid, even in patients without radiographic evidence of HPP, and thus may aid in diagnosis.<sup>29</sup>

## Genetic Testing

Testing for TNSALP mutations is not required for diagnosis of HPP; however TNSALP mutation analysis may be useful for establishing a diagnosis in patients with less severe HPP for whom laboratory findings are not definitive. Genetic testing may facilitate counseling for families of affected children who wish to be informed of possible inheritance patterns as they consider having additional children.<sup>1,30</sup>

**Clinical Management of HPP:** No established medical therapy currently exists for HPP.<sup>1</sup> Early at-



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tempts at enzyme replacement therapy sought to restore ALP in infants with life-threatening HPP. Separate studies evaluated infusion of the bone isoform of TNSALP taken from the plasma of patients with Paget's disease, and ALP extracted from healthy human placentas.<sup>31,32</sup> Although both attempts yielded some short-term benefits, mostly with regard to improved laboratory parameters, sustained clinical or radiographic improvement was not observed.<sup>1</sup> This may be due to the fact that ALP activity needs to be increased specifically within bone and not only in the circulation. Transplantation of bone fragments and cultured osteoblasts into a 9-month-old child with infantile HPP led to improved skeletal mineralization and long-term patient survival, although engraftment of donor cells was low.<sup>33</sup> Allogenic bone marrow and mesenchymal stem cell transplant in an 8-month-old patient with perinatal HPP improved and stabilized the patient's respiratory condition but did not improve skeletal abnormalities.<sup>34</sup> Other efforts to stimulate TNSALP biosynthesis or increase TNSALP activity have included cortisone administration, short-term treatment with  $Zn^{2+}$  or  $Mg^{2+}$ , and injection of PTH.<sup>1</sup>

In the absence of available therapy, management of patients with HPP consists of supportive care. In infancy, ventilator or other respiratory support measures may be required<sup>16,17</sup>; hypercalcemia may necessitate restriction of dietary calcium, fluid hydration, and administration of loop diuretics and/or glucocorticoids<sup>1</sup>; vitamin B6 may be administered if seizures are present<sup>14,17</sup>; and craniosynostosis may require surgical intervention. Standard management of fractures in children with HPP should lead to healing, albeit with prolonged need for stabilization<sup>1</sup>; in adults fixation with hardware may be necessary.<sup>21</sup> In all patients with HPP, expert dental care and follow-up is critical, particularly among children, as severe problems with dentition may impair speech and nutrition.<sup>1</sup>

Importantly, bisphosphonates are not recommended in HPP since these agents are analogs of PPi designed to reduce bone turnover and diminish ALP activity by binding  $Zn^{2+}$  and  $Mg^{2+}$ .<sup>1</sup> Vitamin D and mineral supplementation should also be avoided in patients with HPP unless frank deficiencies are observed, as these traditional treatments for rickets and osteomalacia may exacerbate the hypercalciuria and hypercalcemia common in severe forms of HPP.<sup>1</sup> However, there is no need to restrict vitamin D intake or sunshine exposure, as rickets caused by vitamin D deficiency may develop as a comorbidity with HPP.

**Emerging Therapies:** Asfotase alfa (Alexion Pharmaceuticals, Inc.) is currently in development as an enzyme replacement therapy for treatment of HPP. Asfotase alfa is a fusion protein consisting of the TNSALP ectodomain, the constant region of the human immunoglobulin G<sub>1</sub> F<sub>c</sub> domain, and a mineral-binding deca-aspartate motif that directs the recombinant protein to the surface of bone.<sup>35</sup>

In a preclinical study using a murine ALP double knock-out (Alpl<sup>-/-</sup>) model of severe infantile HPP,<sup>35</sup> mice treated with asfotase alfa (once-daily subcutaneous [SC] injection for up to 52 days) grew normally without skeletal defects, dental disease, and seizures. Plasma levels of calcium, PPi, and PLP remained within normal ranges. Untreated mice died at a median of 18.5 days with severe skeletal defects. In (Alpl<sup>-/-</sup>) mice neonates injected with lentiviral vector expressing a bone-targeted form of TNSALP, seizures were reduced, bone mineralization improved, and survival improved.<sup>36,37</sup>

Whyte et al<sup>38</sup> reported in 2012 that infants and children with severe HPP treated with asfotase alfa had improved bone mineralization, decreased radiographic evidence of rickets, reduced levels of TNSALP substrates (PPi and PLP), and improved physical function compared with baseline. Asfotase alfa treatment was well tolerated. This phase II open-label clinical trial (NCT00744042) enrolled patients up to 3 years old who first exhibited signs or symptoms of HPP prior to 6 months of age. Asfotase alfa was administered as a single intravenous loading dose (2 mg/kg), followed by SC injection of asfotase alfa (1 mg/kg, 3 times per week), with allowance to increase the dose based on physician discretion.<sup>38</sup>

Eleven patients (7 girls, 4 boys) ranging in age from 2 weeks old to 3 years old at baseline were enrolled. Disease was severe in all patients, typically characterized by failure to thrive, fractures, substantial motor delay, loss of erupted teeth, and nephrocalcinosis.<sup>38</sup> Ten patients completed at least 6 months of treatment. One patient died due to sepsis unrelated to treatment. Nine patients continue treatment in an ongoing extension study (NCT01205152).<sup>38</sup>

Marked and persistent radiographic improvement in skeletal abnormalities was observed from baseline, with skeletal healing noted as early as 3 weeks after the start of asfotase alfa therapy.<sup>38</sup> Radiographic response to treatment (ie,  $\geq 2$ -point improvement on the 7-point radiographic global impression of change [RGI-C] scale) was documented in 9 of 10 patients from baseline to week 24 and in 8 of 9 patients from baseline to week 48. Skeletal improvements included increased bone mineralization,

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improved fracture healing, reduced deformity, and resolution of radiolucencies and sclerosis. There was no apparent effect on craniosynostosis. Skeletal changes were accompanied by significantly better respiratory function and marked improvement in motor skills and cognitive development. Notably, whereas 10 of 11 patients required ventilator support at baseline, 6 of 9 patients were breathing ambient air without the need for mechanical ventilation by week 48. Children with apparent myopathy at baseline showed rapid improvement in muscle strength. PLP and PPi levels declined markedly during treatment, consistent with the mechanism of action of the drug. Noted improvements in skeletal health and general functioning continue to be observed in patients who continue asfotase alfa treatment in the extension study (**Figures 1 and 2**). Summarized long-term data will be the subject of a future publication.

There was no evidence of ectopic calcification, hypocalcemia, or progression of nephrocalcinosis during asfotase alfa treatment.<sup>38</sup> The most common treatment-related adverse event was mild and transient erythema at the injection site. Three serious adverse events (one case each of respiratory distress, craniosynostosis, and conductive hearing loss) were considered possibly related to asfotase alfa. No particular safety concerns were evident. Anti-asfotase alfa antibodies developed in 4 patients during the study; however, antibody titers were low and there was no evidence of resistance to therapy over the 48 weeks of treatment.

Preliminary interim results from an ongoing clinical trial (NCT01176266) are consistent with the above findings. That is, in infants and young children ≤5 years old in whom symptoms presented earlier than 6 months of age (n=15), there was significant improvement in skeletal mineralization from baseline to Week 24 of treatment, respiratory status improved, and asfotase alfa was well tolerated.<sup>39</sup>

An additional phase II clinical trial (NCT00952484) examined the effect of asfotase alfa in children between the ages of 5 and 12 with open growth plates at the time of enrollment (n = 13).<sup>40</sup> Asfotase alfa was

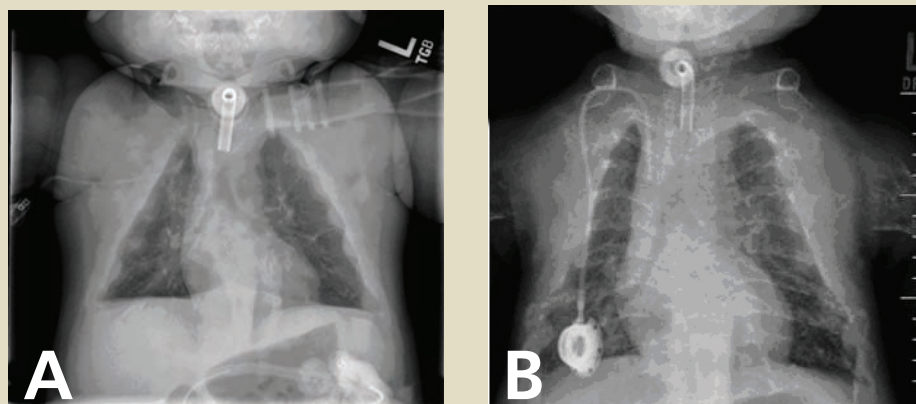
administered open-label as thrice-weekly SC injections (2 mg/kg or 3 mg/kg). Preliminary results indicate that, compared to equivalently aged historical control patients with HPP, patients treated with asfotase alfa had radiographic improvement in skeletal mineralization from baseline, functional improvement (significant improvement in the distance walked in 6 minutes; measures of gross motor function and muscle strength), and reduced pain. There were no serious adverse events or discontinuations related to treatment. Injection site reactions were common (occurred in 11 of 13 patients); erythema was the most common injection site reaction. There was no evidence of ectopic calcification or hypocalcemia. Patients continue treatment in an ongoing extension trial (NCT01203826).

In an additional Phase II clinical trial (NCT01163149), 18 patients (6 adolescents and 13 adults) with HPP were randomly assigned to receive daily SC injections of 0.3 mg/kg/day asfotase alfa (n = 7), 0.5 mg/kg/day asfotase alfa (n = 6), or no treatment (n = 6).<sup>41</sup> Mean patient age at baseline was 42 years old (range: 14-68 years). Levels of ALP substrates (PPi and PLP) were significantly decreased from baseline to Week 24 in patients treated with asfotase alfa compared with control patients. Improvement in 6-minute walking distance was observed in patients who received asfotase alfa, whereas control patients showed no improvement from baseline. Seven patients experienced injection site reactions; none leading to treatment discontinuation. There were no treatment-related serious adverse events.

**Figure 1**

## **Radiographic changes of the chest following asfotase alfa treatment.**

Baseline radiograph of the chest of this 3-year-old child (Panel A) shows pathognomonic changes of severe HPP, most notably lack of mineralization of the ribs and spine. Marked improvements in bone mineralization in ribs and clavicles are noted following 35 months of asfotase alfa therapy (Panel B). This patient was enrolled in study NCT00744042. To view additional radiographs, including from a patient who responded radiographically to asfotase alfa after 4 weeks of treatment, see the supplement to Whyte et al.<sup>38</sup>



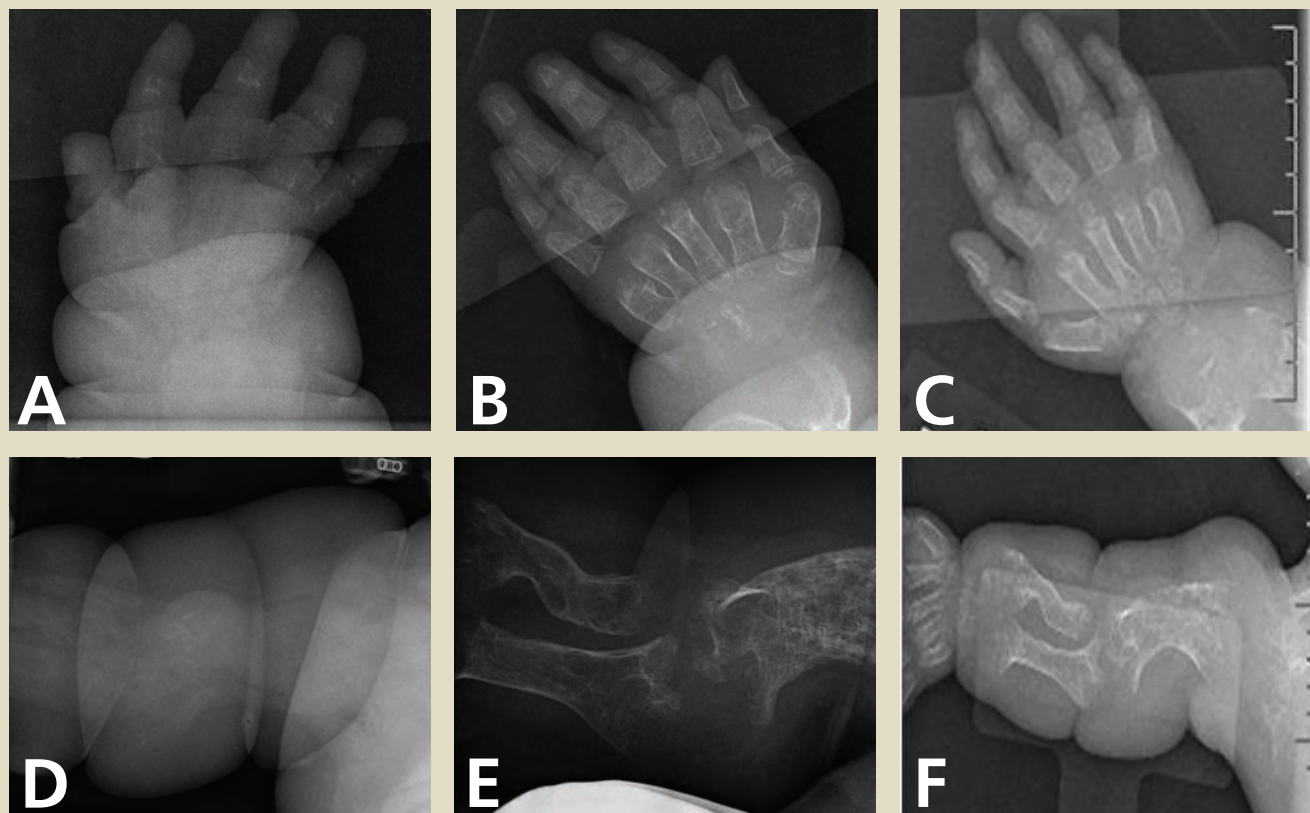
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Figure 2

## Radiographic changes of hand and arm following asfotase alfa treatment.

Radiographs obtained at baseline depict a near-complete lack of bone mineralization in the left hand and right arm (Panels A and D, respectively). Marked improvements in bone mineralization are noted following 24 months (Panels B and E) and 35 months (Panels C and F) of asfotase alfa therapy. Note mineralization in carpals, metacarpals, phalanges, humerus, radius, and ulna. This patient was enrolled in clinical trial NCT00744042. To view additional radiographs, including from a patient who responded radiographically to asfotase alfa after 4 weeks of treatment, see the supplement to Whyte et al.<sup>38</sup>



**Conclusions:** HPP is a rare, inborn error of metabolism caused by loss-of-function mutations in the gene that encodes the tissue nonspecific isoenzyme of ALP (TNSALP). The resulting deficiency in TNSALP enzyme activity causes extracellular accumulation of several phosphocompounds, most notably PPi, an inhibitor of hydroxyapatite crystal formation, and PLP, the major form of vitamin B6. Accumulation of PPi impairs skeletal mineralization, causing rickets or osteomalacia despite normal or above-normal levels of calcium and inorganic phosphate. Although PLP levels are elevated, PLP is not dephosphorylated to PL, and therefore does not cross the blood-brain barrier or enter cells where it is normally rephosphorylated to PLP or converted to pyridoxamine-5'-phosphate, to serve as cofactors in a variety of intracellular enzymatic reac-

tions. The descriptions of HPP used in this review were based on traditional classifications of HPP according to age at onset of disease. It is now recognized that there is a spectrum of disease severity both across these classifications (from lethal perinatal HPP to less severe adult HPP) and within these classifications (eg, from debilitating muscle and bone pain and frequent fractures to abnormal dentition in adults). Infants and young children with HPP who were treated with asfotase alfa, a bone-targeted recombinant TNSALP enzyme replacement therapy, had evidence of improved functional and physical outcomes, improved bone mineralization, and reduced TNSALP substrates. Preliminary analyses indicate positive results in additional trials enrolling adolescents and adults with HPP. Clinical evaluation of asfotase alfa continues via longer term extension trials.



# BEST PRACTICES IN: Recognizing and Diagnosing Hypophosphatasia

A supplement to CLINICAL ENDOCRINOLOGY NEWS®.

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