13 Alkaline Phosphatase Replacement Therapy for Hypophosphatasia in Development and Practice

S. A. Bowden and B. L. Foster

Abstract

Hypophosphatasia (HPP) is an inherited disorder that affects bone and tooth mineralization characterized by low serum alkaline phosphatase. HPP is caused by loss-of-function mutations in the *ALPL* gene encoding the protein, tissue-nonspecific alkaline phosphatase (TNSALP). TNSALP is expressed by mineralizing cells of the skeleton and dentition and is associated with the mineralization process. Generalized reduction of activity of the TNSALP leads to accumulation of its substrates, including inorganic pyrophosphate (PP_i) that inhibits physiological mineralization. This leads to defective skeletal mineralization, with manifestations including rickets, osteomalacia, fractures, and bone pain, all of which can result in multi-systemic complications with significant morbidity, as well as mortality in severe cases. Dental manifestations are nearly universal among affected individuals and feature most prominently premature loss of deciduous teeth. Management of HPP has been limited to supportive care until the introduction of a TNSALP enzyme replacement therapy (ERT), asfotase alfa (AA). AA ERT has proven to be transformative, improving survival in severely affected infants and increasing overall quality of life in children and adults with HPP. This chapter provides an overview of TNSALP expression and functions, summarizes HPP clinical types and pathologies, discusses early attempts at therapies for HPP, summarizes development of HPP mouse models, reviews design and validation of AA ERT, and provides up-to-date accounts of AA ERT efficacy in clinical trials and case reports, including therapeutic response, adverse effects, limitations, and potential future directions in therapy.

Keywords

Hypophosphatasia · Alkaline phosphatase · Asfotase alfa · Bone mineralization · Rickets · Osteomalacia · Teeth

Abbreviations

HPP	Hypophosphatasia
ALPL	Alkaline phosphatase gene
TNSALP	Tissue-nonspecific isoenzyme of alkaline phosphatase

S. A. Bowden (✉)
Division of Endocrinology, Department of Pediatrics, Nationwide Children's Hospital/The Ohio State University College of Medicine, Columbus, OH, USA
e-mail: Sasigarn.Bowden@nationwidechildrens.org

B. L. Foster
Division of Biosciences, College of Dentistry, The Ohio State University, Columbus, OH, USA

N. Labrou (ed.), *Therapeutic Enzymes: Function and Clinical Implications*, Advances in Experimental Medicine and Biology 1148, https://doi.org/10.1007/978-981-13-7709-9_13

ALP	Alkaline phosphatase enzyme activity, circulating
IAP	Intestinal alkaline phosphatase protein
ALPI	Intestinal alkaline phosphatase gene
PLAP	Placental alkaline phosphatase protein
ALPP	Placental alkaline phosphatase gene
GCAP	Germ cell alkaline phosphatase protein
ALPPL2	Germ cell alkaline phosphatase gene
AA	Asfotase alfa
ERT	Enzyme replacement therapy
P_i	Inorganic phosphate
PP_i	Inorganic pyrophosphate
PLP	Pyridoxal 5′-phosphate
PEA	Phosphoethanolamine
GPI	Glycosylphosphatidylinositol
HAP	Hydroxyapatite
CHO	Chinese hamster ovary cells
BSP	Bone sialoprotein
OPN	Osteopontin
DSP	Dentin sialoprotein
PHEX	Phosphate-regulating endopeptidase
XLH	X-linked hypophosphatemia
PDB	Paget's disease of the bone
PDL	Periodontal ligament
OMIM	Online Mendelian Inheritance in Man compendium
ANK/ANKH	Progressive ankylosis protein
ENPP1	Ectonucleotide pyrophosphatase phosphodiesterase 1 protein
PTH	Parathyroid hormone
TPTD	Teriparatide
BMT	Bone marrow transplantation
SCB	Stromal cell boost
IV	Intravenous
SC	Subcutaneous
IP	Intraperitoneal

13.1 Introduction

Hypophosphatasia (HPP) is an inherited disorder (OMIM #146300, #241500, #241510) characterized by low serum alkaline phosphatase. HPP is caused by loss-of-function mutations in the *ALPL* gene encoding the protein, tissue-nonspecific alkaline phosphatase (TNSALP). TNSALP is expressed by mineralizing cells of the skeleton and dentition and is associated with the mineralization process. Generalized reduction of activity of the TNSALP leads to accumulation of substrates of TNSALP including inorganic pyrophosphate (PP_i), a potent inhibitor of mineralization. This leads to defective skeletal and dental mineralization, resulting in multisystemic complications with significant morbidity and mortality in the most severe cases. Management of HPP has been limited to supportive care until the introduction of a TNSALP enzyme replacement therapy (ERT), asfotase alfa (AA). AA ERT has proven to be transformative, improving survival in severely affected infants and increasing overall quality of life in children and adults with HPP. This chapter provides an overview of TNSALP expression and functions, summarizes HPP clinical types and pathologies, discusses early attempts at therapies for HPP, summarizes development of HPP mouse models, reviews design and validation of AA ERT, and provides up-to-date accounts of AA ERT efficacy in clinical trials and case reports, including therapeutic recommendations and response, adverse effects, and limitations.

13.2 Tissue Non-specific Alkaline Phosphatase: An Enzyme Essential for Mineralization

The enzyme we now know as tissue-nonspecific alkaline phosphatase (TNSALP, TNALP, or TNAP) was first reported by Dr. Robert Robison in 1923 (Robison 1923 and reviewed in Siller and Whyte 2018). Robison hypothesized that the

enzymatic activity he detected in extracts from rat and rabbit bones that liberated inorganic phosphate (P_i) from hexosemonophosphoric acid was involved in calcium phosphate deposition in the skeleton. Robison explored properties of the enzyme further in subsequent studies and terminology regarding the enzyme shifted from phosphoric esterase to bone enzyme, phosphatase, and bone phosphatase, finally being referred to as alkaline phosphatase based on non-physiologic alkaline conditions used to assay the enzyme activity in vitro, as well as to differentiate it from an acidic prostate phosphatase discovered shortly afterwards (Goodwin and Robison 1924; Gutman and Gutman 1938; Kay and Robison 1924; Martland and Robison 1924, 1926, 1927, 1929; Robison et al. 1930; Robison and Soames 1924, 1925; Siller and Whyte 2018).

TNSALP is encoded by the *ALPL* gene on chromosome 1 in humans (NM_000478.5). Three additional alkaline phosphatase isozymes exist in humans: intestinal (IAP encoded by *ALPI* on chromosome 2; NM_001631.4), placental (PLAP encoded by *ALPP* on chromosome 2; NM_001632.4), and germ cell (GCAP or ALPG encoded by *ALPPL2* on chromosome 2; NM_031313.2) (Millán 2006; Millan and Whyte 2016). In humans, the *ALPL* gene encodes 12 exons and 11 introns, adding up to a total of 69,034 base pairs, though exons Ia and Ib are noncoding and separated from the ATG initiation site in exon II. Human *ALPL* mRNA is translated into the TNSALP protein comprising 524 amino acids (Fig. 13.1a).

TNSALP includes a number of important conserved amino acid sites, motifs, and domains that can be delineated in the two-dimensional sequence (Fig. 13.1b), though these function in the context of the three-dimensional protein structure, that includes a dimeric structure composed of two TNSALP monomers (Fig. 13.1c, d). TNSALP is bound to cell plasma membrane surfaces by a glycosylphosphatidylinositol (GPI) anchor that can be cleaved to release the enzyme into circulation, where circulating alkaline phosphatase activity (ALP) can be detected in plasma. The enzyme active site is located in the extracellular domain making TNSALP an ectoenzyme. Additional important structural and functional motifs in the TNSALP amino acid sequence include a hydrophobic domain involved in monomer-monomer interactions to assemble the functional enzyme homodimer, three metal cation (Zn^{2+} and Mg^{2+}) binding sites critical for enzyme activity, a flexible crown domain involved in interactions with collagen matrix and inhibitors, an N-terminal α-helix that (along with the crown domain) contributes to stabilization of the dimeric structure, and N-linked glycosylation sites that affect catalytic activity and kinetic properties of the enzyme. Across nearly its entire amino acid sequence, TNSALP is extremely highly evolutionarily conserved (Fig. 13.1e), suggesting functional importance for the majority of the protein structure.

While a broad substrate specificity has been demonstrated in vitro, natural substrates indicated by TNSALP loss-of-function include inorganic pyrophosphate (PP_i), phosphoethanolamine (PEA), and pyridoxal 5′-phosphate (PLP), described in more detail in the next section. TNSALP is highly expressed in bones, teeth, liver, and kidney (and at lower levels in fibroblasts, endothelial cells, and nervous system), thus its nomenclature as a "non-specific" enzyme. In the skeleton, bone forming and mineralizing osteoblasts are the primary cells expressing TNSALP, while in teeth and their supporting tissues, ameloblasts, odontoblasts, cementoblasts, and other cells of the PDL all express TNSALP (Fig. 13.2a–l).

TNSALP is likely the most critical enzyme for mineralization of bones and teeth. Upon its discovery, Robison presciently hypothesized that TNSALP was associated with skeletal mineralization, possibly by locally increasing P_i through dephosphorylation of substrates (Robison 1923; Siller and Whyte 2018). The ability for TNSALP to hydrolyze and thus inactivate PP_i came to be understood as a possibly more important function of TNSALP in mineralization. PP_i is a potent inhibitor of calcium phosphate (hydroxyapatite;

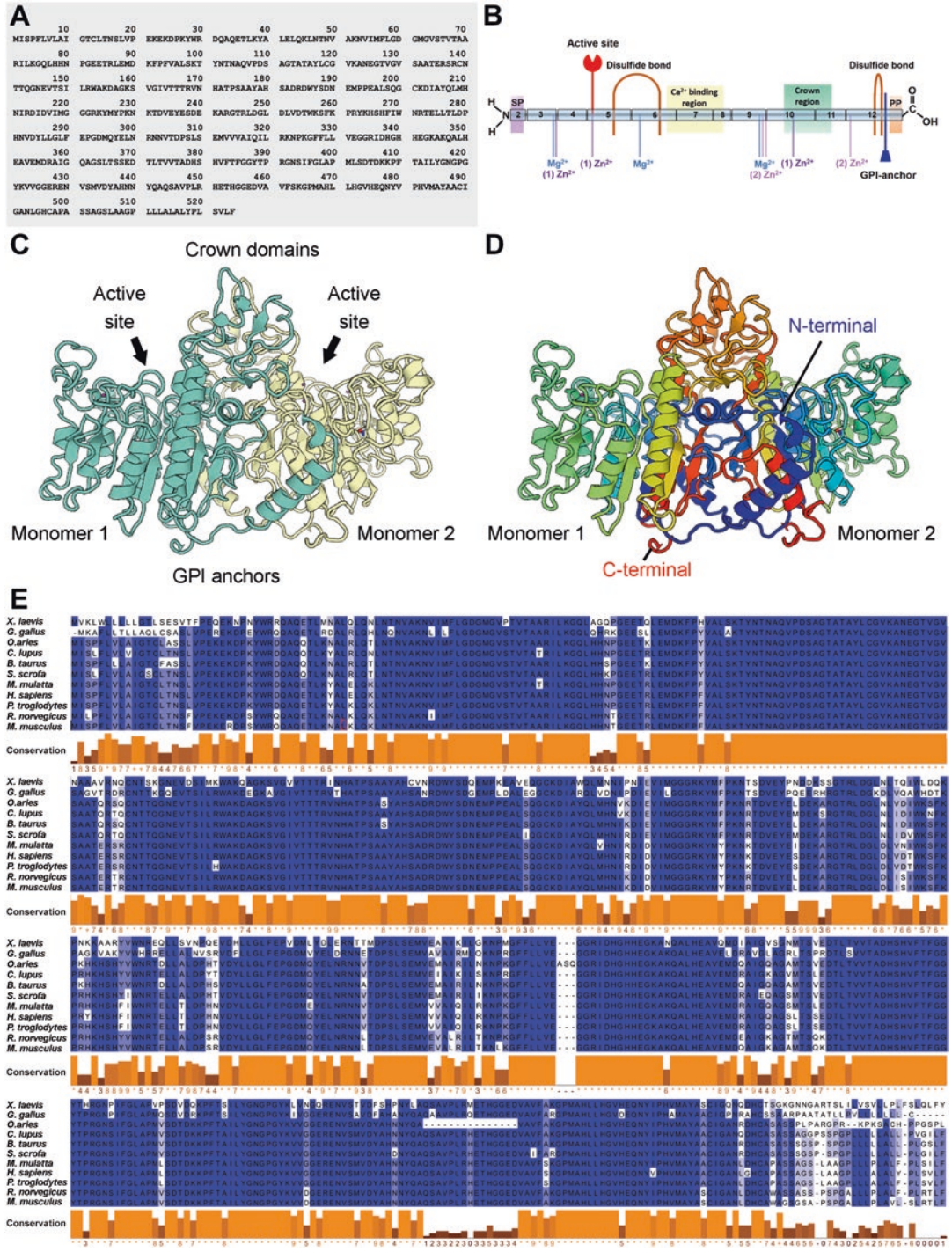

Fig. 13.1 Human TNSALP sequence and structure. (**a**) Human TNSALP is comprised of 524 amino acids, shown here by their one-letter codes. (**b**) Human TNSALP 2D protein structure superimposed over the 12 exons and demarcating conserved functional sites and domains. (**c**) Human TNSALP 3D structure showing the functional dimer with monomer 1 (green, on the left) and monomer 2 (yellow, on the right). Functional sites and domains are indicated for both monomers. 3D images are based on crystalized structure of PLAP and were imaged through Swiss-Model (www.swissmodel.expasy.org) and UniProt (www.uniprot.org). (**d**) Human TNSALP 3D structure

HAP) crystal growth that constitutes the inorganic mineralized component of bones and teeth (Bisaz et al. 1968; Fleisch and Bisaz 1962a, b; Fleisch et al. 1965, 1966; Meyer 1984; Meyer and Fleisch 1984). While TNSALP acts as a promineralization enzyme decreasing local levels of PP_i, counter-regulatory proteins, including progressive ankylosis protein (ANK/ANKH) and ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1), increase PP_i production, altogether comprising a complex feedback system of regulators that control the location and extent of mineralization in the body (Fig. 13.2m) (Foster et al. 2012; Gurley et al. 2006; Harmey et al. 2004; Ho et al. 2000; Johnson et al. 2000; Millan 2013; Murshed et al. 2005; Nociti et al. 2002; Rutsch et al. 2001, 2003; Terkeltaub 2001; Zweifler et al. 2015).

13.3 Hypophosphatasia

HPP is characterized by reduced ALP activity and extracellular accumulation of PP_i, resulting in defective mineralization of bones and teeth. HPP is caused by loss-of-function mutations in the *ALPL* gene that encodes TNSALP. At this writing, 377 such mutations have been reported (http://www.sesep.uvsq.fr/03_hypo_mutations.php), with the majority (~70%) being missense mutations, but also including nonsense mutations, deletions, and alterations in *ALPL* regulatory regions. The mode of inheritance can be either autosomal recessive or autosomal dominant (Mornet 2017; Thakker et al. 2017). The prevalence is highest among Mennonites in Manitoba, Canada, where approximately 1 in 2500 neonates manifests lethal form of HPP (Greenberg et al. 1993). The prevalence of severe and moderate HPP in Europe has been estimated to be 1 in 300,000 and 1 in 6370, respectively (Mornet et al. 2011). A critical function of TNSALP in skeletal tissues is to hydrolyze and thus reduce levels of PP_i, allowing physiological mineralization to proceed. Deficiency of TNSALP in HPP leads to increased PP_i levels that inhibit HAP crystal nucleation and growth in the extracellular matrix, thereby impairing skeletal and dental mineralization. This condition also secondarily leads to disturbances of calcium and phosphorus homeostasis. Elevation of serum calcium or phosphorus levels sometimes occurs and is thought to be the result of a combination of normal gut absorption of these ions and the inability to effectively incorporate them into bone HAP. In addition to increased PP_i, HPP also leads to increased extracellular accumulation of two other known physiological substrates of TNSALP, PLP and PEA.

The clinical spectrum of HPP is broad and highly variable, even within families. Clinical manifestations range from perinatal death to severe bone deformities in early childhood, to primary tooth loss with little or no other clinically detectable systemic or skeletal manifestations. The severity typically correlates with earlier disease onset. HPP is classified into six different clinical forms, based on the age onset of clinical symptoms: perinatal, benign prenatal, infantile, childhood, adult, and odontohypophosphatasia (odonto-HPP) (Table 13.1). This classification delineates disease severity and may correlate with prognosis and ALP levels, although there is considerably overlap in clinical phenotypes and biochemical hallmarks in HPP (Whyte et al. 2018).

Fig. 13.1 (continued) showing both monomers in rainbow colors to indicate N-terminal (blue) to C-terminal (red) sequence. N- and C-termini are indicated for monomer 1 (left side). (**e**) TNSALP multiple sequence comparisons between human (*Homo sapiens*; UniProt record P05186), chimpanzee (*Pan troglodytes*; K7B4Y6), Rhesus macaque monkey (*Macaca mulatta*; A0A1D5R5B1), rat (*Rattus norvegicus*; P08289) mouse (*Mus musculus*; P09242); dog (*Canis lupus familiaris*; F1PF95), cow (*Bos taurus*; P09487), sheep (*Ovis aries*; W5PFB8), pig (*Sus scrofa*; A0A287BSC3), chicken (*Gallus gallus*; Q92058), and African clawed frog (*Xenopus laevis*; Q7ZYJ4). Regions well conserved across species are indicated by darker blue coloration of the amino acids in the sequence rows. Relative conservation is also indicated by a histogram below the sequence comparisons, with numerical scores from 0 to 9, where higher scores indicate better conservation (also shown by lighter orange color) and ∗ indicating perfect conservation across all species. Sequence comparison performed by Clustal Omega (www.clustal.org/omega) and viewed by Jalview (www.jalview.org)

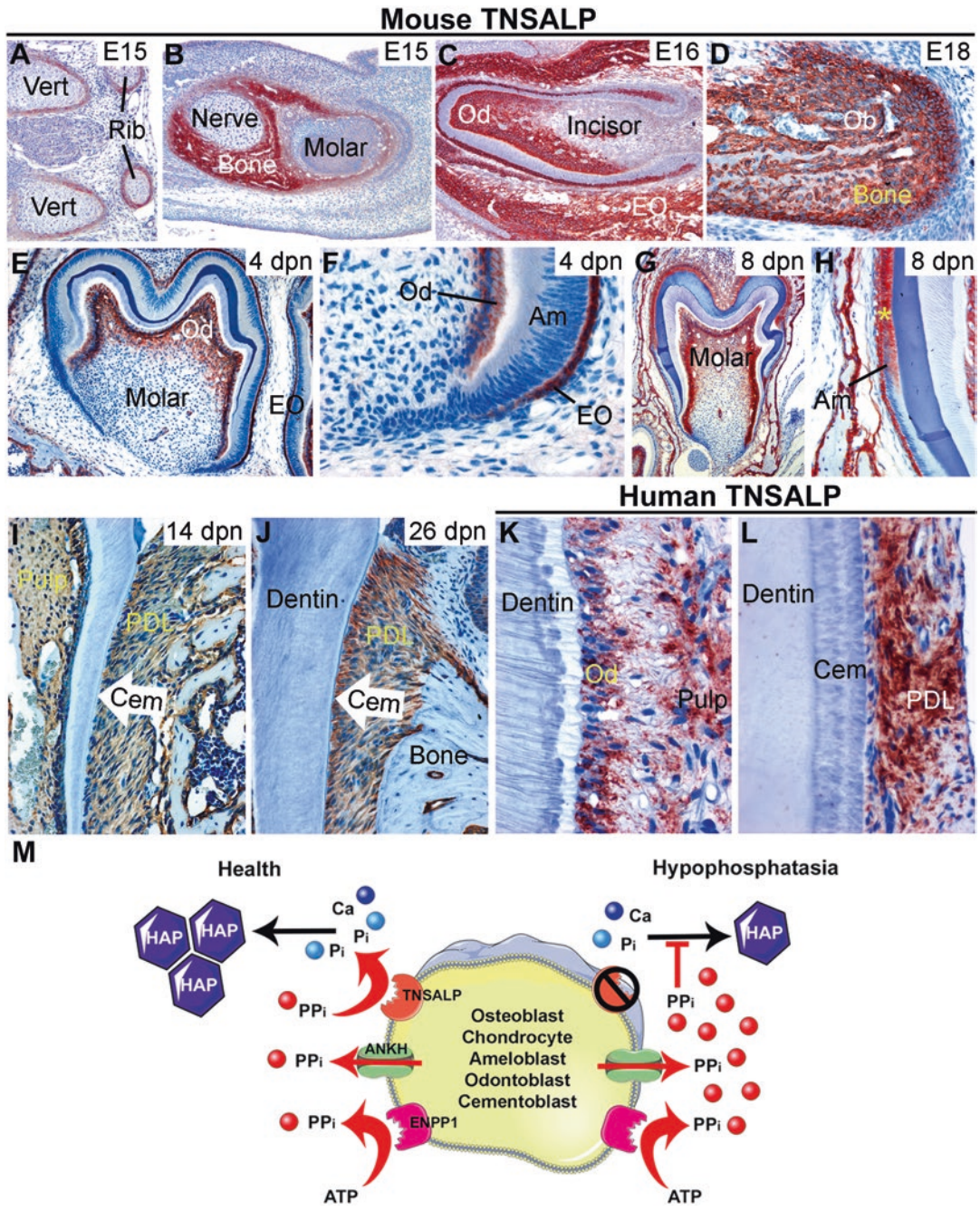

Fig. 13.2 Mineralizing cells express TNSALP. Immunohistochemistry in mouse (**a**–**j**) and human (**k** and **l**) tissues shows the expression of TNSALP (red color). TNSALP is expressed around (**a**) forming vertebrae (Vert) and ribs at mouse embryonic day 15 (E15), and is strongly expressed in the mineralizing bone of the mandible, (**b**) though is not yet expressed in the molar tooth that is undergoing morphogenesis but not yet mineralizing. (**c**) The incisor tooth is mineralizing by E16, and TNSALP is found in the enamel organ (EO) and in odontoblasts (Od) forming dentin. (**d**) Osteoblasts (Ob) of the mouse jaw bone at E18 strongly express TNSALP. (**e** and **f**) At 4 days postnatal (dpn), Od and EO of the molar strongly express TNSALP, though ameloblasts (Am) do not. (**g** and **h**) By 8 dpn, Am entering the maturation stage (∗) of enamel mineralization begin expressing TNSALP. (**i** and **j**) At later ages of 14 and 26 dpn, after periodontal tissues have formed, the entire periodontal ligament (PDL) between

13.3.1 Clinical Classification of HPP and the Clinical/Radiologic Findings

13.3.1.1 Perinatal HPP

Perinatal HPP is the most severe form of HPP and is typically lethal as a result of an almost complete absence of skeletal mineralization. Severely affected infants often die shortly after birth due to respiratory complications arising from hypoplastic lungs and skeletal deformities of the thorax. The key radiographic and sonographic features that are characteristic of and unique to severe lethal perinatal HPP are absent ossification of whole bones at or after 11 weeks' gestation (Offiah et al. 2018). Other prenatal imaging findings, characteristic of perinatal HPP and not unique to HPP, include shortening, bowing and angulation of the long bones, mid-diaphyseal ("Bowdler") spurs, slender and poorly ossified thin ribs, metaphyseal lucencies, and deficient ossification in the skull observed as wide sutures and fontanelles (Fig. 13.3a–c) (Offiah et al. 2018; Zankl et al. 2008). These features also arise from other skeletal dysplasias (e.g. osteogenesis imperfecta, cleidocranial dysplasia, campomelic dysplasia, and achondrogenesis subtypes), which should be in the differential diagnosis. HPP can be distinguished and confirmed by prenatal ALP measurements.

13.3.1.2 Benign Prenatal HPP

Benign prenatal HPP manifests in utero, with abnormal imaging findings similar to the perinatal HPP. Affected fetuses exhibit skeletal deformities including poorly mineralized bone or short, severely bowed legs, which can sometimes be diagnosed as the perinatal lethal form of HPP (described above) (Fig. 13.3d, e). However, in cases of the benign prenatal form of HPP, the skeletal phenotype can be less severe and spontaneous improvement is observed beginning in the third trimester of pregnancy and continuing after birth (Offiah et al. 2018; Wenkert et al. 2011). Postnatal clinical outcomes range in severity from infantile to odonto-HPP phenotypes, therefore, abnormal prenatal ultrasound findings before the third trimester are not predictive of perinatal lethal HPP.

13.3.1.3 Infantile HPP

Infantile HPP presents before 6 months of age and is associated with approximately 50% mortality due to respiratory failure due to severe hypomineralization and mechanical weakness of the chest wall. Affected infants can appear normal after birth until emergence of poor feeding, failure to thrive, and hypotonia with delayed motor milestones develop. Radiologic findings include generalized hypomineralization with severe skeletal deformities, including rachitic defects of the long bones and chest (Fig. 13.3f). Infants may also have muscle pain and weakness from a static myopathy, possibly related to accumulation of TNSALP substrate, PP_i (Seshia et al. 1990). Craniosynostosis and other skull abnormalities occur in about 40% of infants with infantile HPP (Fig. 13.3g, h), and these complications may require neurosurgical intervention due to intracranial hypertension (Collmann et al. 2009). Proptosis, mild hypertelorism, and brachycephaly can develop. Unlike other forms of hereditary

Fig. 13.2 (continued) the root surface cementum (Cem) and bone becomes strongly positive for TNSALP. (**k** and **l**) Human TNSALP dental expression patterns parallel those in mice, with strong expression in Od and in the PDL. (**m**) Model of TNSALP function in mineralizing cells. Levels of the mineralization inhibitor, PP_i, are controlled by activities of ENPP1, ANKH/ANK, and TNSALP, all expressed by mineralizing cells. ENPP1 enzymatically cleaves nucleotide triphosphates (e.g. adenosine triphosphate, ATP) to generate PP_i, while ANKH/ANK directs PP_i transport to the extracellular space, both increasing pericellular PP_i levels. TNSALP hydrolyzes PP_i to allow inorganic phosphate (P_i) and calcium (Ca) to precipitate as hydroxyapatite (HAP), the inorganic component of bones and teeth. When TNSALP activity is lost in HPP, excess PP_i inhibits HAP crystal initiation and growth, causing mineralization defects in the skeleton and dentition. Figure designed with images from Servier Medical Art (https://smart.servier.com/) under a Creative Commons Attribution 3.0 Unported License. (Images in panels **e**, **f** reused with permission from McKee et al. J Dent Res 92(8): 721–727, 2013. Images in panels **g**, **h** reused with permission from Yadav et al. 2012. Images in panels **i–l** reused with permission from Zweifler et al. 2015. Image in panel **m** reused with permission from Bowden and Foster. Drug Des Devel Ther 12: 3147–3161, 2018)

Table 13.1 Clinical forms of HPP and their characteristics

Form	Onset	Clinical features	Imaging findings
Perinatal lethal	*In utero*/at birth	Profound hypomineralization Short and deformed extremities Polyhydraminos Respiratory insufficiency at birth to the first week of life Pyridoxine-responsive seizures Stillbirth or death within days/weeks after birth	Absence of mineralization of the roof of the skull and bones Thin ribs Fractures
Benign prenatal	*In utero*/at birth	Severe hypomineralization with skeletal deformities Spontaneous improvement in the skeletal disease after birth	Same as perinatal lethal form
Infantile	First 6 months of life	Severe hypomineralization with severe skeletal deformities (rachitic ribs, *genu varum*) Craniosynostosis Pyridoxine-responsive seizures Failure to thrive and delayed developmental milestones Hypercalcemia and hypercalciuria Premature loss of primary teeth	Absent bones or severe mineralization defects; Gracile bones; "Tongues" radiolucency in metaphyses; Patchy areas of osteosclerosis; Copper beaten appearance of skull radiograph; Wedging of the lower thoracic and upper lumbar vertebrae; Nephrocalcinosis on renal ultrasound.
Childhood	After 6 months of life	Rickets (bowed legs with bony enlargement near joints due to widened metaphyses) Chronic skeletal pain Recurrent fractures Short stature Abnormal ambulation or gait Premature loss of primary teeth	Same as infantile form
Adult	After 18 years	Fragility fractures Stress fractures of metatarsals, tibias Delayed fracture healing Osteomalacia Chondrocalcinosis Osteoarthropathy	Recurrent metatarsal fractures; Vertebral crush fractures; Femoral pseudofractures.
Odonto-HPP	Before 4–5 years of age	Premature loss of primary teeth prior to age 5 years Loss of permanent teeth Abnormal dentin Thin roots with wide pulp chambers Delayed eruption	Alveolar bone loss; Lack of other skeletal or radiologic manifestations.

and nutritional rickets, serum calcium levels are generally high at diagnosis. Hypercalciuria and nephrocalcinosis can also occur as consequences of hypercalcemia. Irritability and vomiting are also common, arising from hypercalcemia or increased intracranial pressure with papilledema secondary to craniosynostosis.

13.3.1.4 Childhood HPP

Childhood HPP presents after age 6 months, with wide ranging clinical manifestations that were suggested to be subdivided into "mild" and "severe" types in 2015 (Whyte et al. 2015a, 2018). Individuals with mild childhood HPP can maintain good physical function with minimal

symptoms or skeletal changes. Severe childhood-onset HPP can feature cranial hypomineralization or craniosynostosis (Fig. 13.3i), however skeletal rickets is typically the main feature (Whyte et al. 2015a), manifesting as bowed legs and bony enlargement near joints due to widened metaphyses, and radiographic findings including characteristic 'tongues' of radiolucency projecting from growth plates into metaphyses (Fig. 13.3j, k). Chronic skeletal pain, recurrent fractures, short stature, muscle weakness, abnormal ambulation or gait, and premature loss of deciduous teeth are common signs or symptoms (Fig. 13.3l). Rarely, childhood HPP can present with chronic multifocal non-bacterial osteomyelitis mimicking malignancy thought to be due to marrow edema secondary to PP_i crystal deposition (Girschick et al. 2007; Whyte et al. 2009). Craniosynostosis can cause chronic increased intracranial pressure, papilledema and impaired visual acuity (Libby Kosnik-Infinger et al. 2015). Individuals exhibiting more severe manifestations of childhood HPP may present more obvious dental defects, including enamel hypoplasia and discoloration (Fig. 13.3m).

13.3.1.5 Adult HPP

Adult HPP is typically diagnosed in middle age based on presentation of chondrocalcinosis, osteoarthropathy, and/or recurrent stress fractures that are often poorly healing (Fig. 13.3n). Femoral pseudofractures and atypical subtrochanteric femoral fractures have been reported in adult HPP; the latter can occur without or following exposure to bisphosphonates (Berkseth et al. 2013; Genest and Seefried 2018; Lawrence et al. 2017; Sutton et al. 2012). Individuals with lower ALP levels and higher PLP and PEA levels tend to exhibit more fractures. There is a diverse spectrum of clinical manifestations within adult HPP, ranging from minimal symptoms such as dental abnormalities with no pain and normal bone mineralization on bone biopsy**,** to significant pain and fractures, with osteomalacia on bone biopsy (Fig. 13.3o, p). Asymptomatic or minimally symptomatic adults with HPP are identified based on low ALP levels or family history of HPP, and may report a history of 'childhood rickets' or fractures (Berkseth et al. 2013). Some affected adults recall a history of premature loss of their deciduous teeth, and may also have early loss of the adult dentition (Whyte 2016). Bone mineral density is low in adult HPP with severe skeletal manifestations, but is normal in asymptomatic adult HPP or those with mild manifestations. However, despite the absence of clinical skeletal abnormalities or decreased BMD, low bone turnover or low bone remodeling has been described in individuals with mild adult HPP, consistent with persistent hypophosphatasemia (Lopez-Delgado et al. 2018). Adult HPP can become debilitating, with severe disability secondary to recurrent fractures, muscle weakness, musculoskeletal pain and restricted range of motion from chondrocalcinosis (Berkseth et al. 2013; Lawrence et al. 2017; Weber et al. 2016).

13.3.1.6 Odonto-HPP

Odonto-HPP features dental defects as the primary manifestations, with biochemical characteristics of HPP and mild skeletal abnormalities or lack of clinically apparent skeletal changes. Dental defects are nearly universal among individuals affected by HPP regardless of the clinical form (Bloch-Zupan 2016; Feeney et al. 2018; Reibel et al. 2009). The most common dental sign is premature loss of primary/deciduous teeth prior to age 5 years, an important diagnostic criterion for HPP (Fig. 13.3q). In a large cohort of pediatric patients, about 98% exhibited premature loss of primary teeth (Whyte et al. 2015b). Anterior teeth (maxillary and mandibular incisors and canines) are the most frequently lost, typically require no or very mild trauma to extrude them from the sockets, and have an unusual and characteristic appearance of being fully rooted, rather than featuring partially resorbed roots typical of physiologically exfoliated primary teeth (Fig. 13.3r). In cases of odonto-HPP or mild childhood HPP, premature tooth loss is often the first recognized sign that something is amiss, and is thus a critical diagnostic criterion that places the general or pediatric dentist in a position to refer the patient to an endocrinologist. Tooth loss is the direct result of elevated PP_i inhibiting formation and mineraliza-

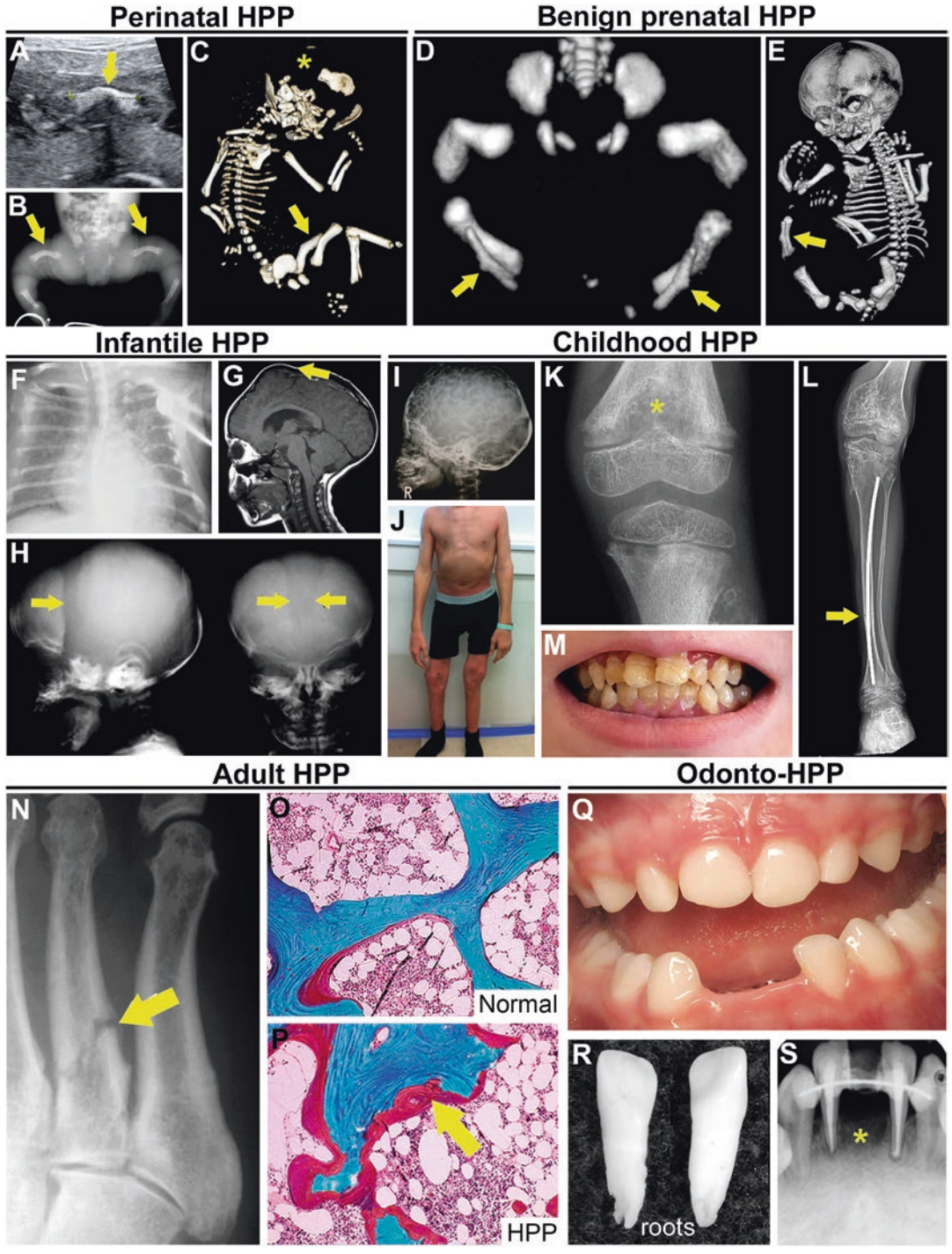

Fig. 13.3 Skeletal and dental defects associated with clinical forms of HPP. Overview of characteristic features of perinatal (**a**–**c**), benign prenatal (**d**, **e**), infantile (**f**–**h**), childhood (**i**–**m**), adult (**n**–**p**), and odonto-HPP (**q**–**s**) forms. (**a**) A fetus with perinatal HPP at 18 weeks-of-gestation displays a short and angulated femur (yellow arrow) by ultrasound. (**b**) Postmortem radiograph of a fetus with perinatal HPP at 38 weeks of gestation reveals bowed femora (yellow arrows) and metaphyseal tongues of radiolucency. (**c**) CT image of the same fetus

tion of tooth root cementum (Bruckner et al. 1962; van den Bos et al. 2005). Additional dental abnormalities reported in association with HPP include loss of secondary/permanent teeth, tooth mobility, abnormal or thin dentin, large pulp space, abnormal tooth root shapes, periodontal disease or alveolar bone loss, malocclusion, and enamel defects (Fig. 13.3s). However, there is presently not a consensus on how common these manifestations are, or how they relate to genotype, biochemical findings, or musculoskeletal effects of HPP, or why a small percentage of affected individuals do not lose their teeth prematurely. It is important to note that a subset of patients diagnosed with odonto-HPP have been observed to develop mild to moderate skeletal manifestations such as fractures and bone pain later in life, therefore, long-term follow-up is recommended (Mori et al. 2016).

13.3.2 Diagnosis of HPP

Diagnosis of HPP can be made with confidence when the clinical history, and physical and radiographic skeletal findings (as described in detail above) are consistent with this diagnosis, and when serum ALP is below the normal range for the patient's age. Patients with perinatal or infantile onset HPP may be misdiagnosed with severe form of osteogenesis imperfecta, however, HPP can be distinguished by low serum ALP. Circulating ALP is elevated in osteogenesis imperfecta and other forms of rickets. Other pediatric skeletal disorders with low serum ALP levels that can mimic HPP and should be considered as a differential diagnosis of HPP include a rare lethal form of osteogenesis imperfecta (Royce et al. 1988), neonatal lethal osteochondrodysplasia (Wyckoff et al. 2005), and severe cleidocranial dysplasia (El-Gharbawy et al. 2010; Unger et al. 2002). Evaluation for high substrates

Fig. 13.3 (continued) with perinatal HPP at 25 weeks-of-gestation shows bowed femurs (yellow arrow), deficient rib ossification, and widened cranial sutures (yellow ∗). (**d**) CT image of a fetus with benign prenatal HPP shows bowed long bones with normal metaphyses, in addition to mid-diaphyseal spurs on the fibulas (yellow arrows). (**e**) CT image of the same infant with benign prenatal HPP shows bowed leg bones and normal cranial ossification. (**f**) Radiograph of a 23-week-old child with infantile HPP shows gracile, deformed, and fractured ribs. (**g**) MRI of the skull of a 6-year-old child with infantile HPP exhibiting craniosynostosis and the resulting bregmatic bump (yellow arrow). (**h**) Lateral and anterior radiograph of a 14-week-old child with infantile HPP showing hypomineralization of the calvarium, giving the appearance of widened sutures (yellow arrows). (**i**) Radiograph of a 4-year-old individual with childhood HPP reveals hypomineralization of the cranial vault as seen by the "copper beaten" appearance of the skull. (**j**) Deformities of lower extremities with joint widening at knees and elbows in a 15-year-old boy with severe childhood hypophosphatasia. (**k**) Radiograph of the knee of a child with severe childhood HPP reveals tongues of radiolucency (yellow ∗) extending from the distal metaphysis of the femur. (**l**) Knee radiograph of the same individual from (panel **j**) reveals hypomineralized bone, coarsened trabeculae, and an intramedullary rod in the tibia. A fracture line is seen in the diaphysis of the tibia (yellow arrow). (**m**) Oral photograph of a boy with severe childhood HPP showing enamel discoloration and hypoplasia, manifested as horizontal bands and irregular crown appearance and texture. (**n**) Radiograph of an individual with adult HPP showing right fourth metatarsal fracture (yellow arrow). (**o**) Goldner trichrome stain of normal iliac crest biopsy compared to (**p**) the same from an individual with adult HPP, showing accumulation of excessive osteoid (red layer indicated by yellow arrow) on the surface of the mineralized bone (green). (**q**) Oral photograph of a 2.5-year-old child with HPP exhibiting premature loss of primary lower incisors. (**r**) Primary incisors that spontaneously exfoliated from a child with HPP. (**s**) Oral radiograph of a 20-year-old individual diagnosed with odonto-HPP showing loss of secondary incisor, endodontic treatment after fracture, splinting to try and stabilize remaining anterior teeth, and generalized alveolar bone loss (yellow ∗). (Images in panels **a**–**e** reproduced from Offiah et al. Pediatr Radiol 1–20, 2018, and used under the terms of the Creative Commons CC BY license. Images in panel **f**, **h** reproduced from Millán and Whyte 2016, and used under the terms of the Creative Commons CC BY license. Images in panels **g**, **i** reproduced with permission from Collmann et al. 2009. Images in panels **j**, **l** reproduced with permission from Bowden and Foster, Drug Des Devel Ther 12: 3147–3161, 2018. Image in panel **k** reproduced with permission from Whyte 2017. Image in panel **n** reproduced with permission from Whyte et al. 2007. Images in panels **o**, **p** reproduced with permission from Berkseth et al. 2013. Image in panel **q** reproduced from Reibel et al. 2009, in accordance with BMC's open access policy. Image in panel **r** reproduced with permission from Whyte 2017. Image in panel **s** reproduced with permission from Rodrigues et al. 2012)

(PP_i, PLP, and PEA) or molecular genetic testing can be important to make a correct diagnosis.

Serum ALP levels in parents, as a noninvasive diagnostic tool, may aid in prenatal differential diagnosis when bone dysplasia is detected by fetal imaging; when ALP is low, a high index of suspicion for perinatal HPP is raised (Castells et al. 2018).

For less severe forms of HPP, diagnosis can be challenging, and requires further laboratory evaluation to assess TNSALP substrate levels, as described below in next section. Low serum ALP can be found in several clinical situations (Table 13.2) where a distinct cause may be apparent. For example, a boy with Duchene muscular dystrophy on chronic corticosteroid treatment would feature low serum ALP due to the long-term suppression of bone turnover resulting from chronic corticosteroid treatment. In this clinical setting, further laboratory workup to rule out HPP is not required. In some clinical situations, where the reason for low ALP level cannot be ascertained, clinicians may need to obtain additional laboratory tests including serum zinc, magnesium, thyroid function test, complete blood count, parathyroid hormone (PTH), vitamin B_{12}, vitamin C, vitamin D, celiac antibodies, serum ceruloplasmin, and renal and liver function tests, in order to rule out conditions outlined in Table 13.2 (Saraff et al. 2016).

Table 13.2 Differential diagnosis of hypophosphatasia (HPP) from low serum alkaline phosphatase (ALP)

Conditions with low ALP
Hypophosphatasia (HPP)
Hypothyroidism
Cushing syndrome or chronic corticosteroid treatment
Profound anemia
Wilson's disease
Celiac disease
Starvation
Milk-alkali syndrome
Cardiac-bypass surgery
Zinc or magnesium deficiency
Vitamin C deficiency
Vitamin D intoxication
Radioactive heavy metals
Improper collection of blood specimen (e.g., EDTA, oxalate)
Inappropriate reference range
Conditions with low serum ALP that can mimic HPP
Osteogenesis imperfecta (OI) type II
Cleidocranial dysplasia

13.3.3 Laboratory Workup for HPP

The key to correct diagnosis of HPP is a low ALP level. A critical caveat is that ALP levels must be interpreted within the context of age- and gender-appropriate reference ranges. ALP levels are considerably much higher in healthy children compared to adults. Serum ALP is especially high during the growth spurt of adolescence, which occurs earlier in girls than in boys. Some clinical laboratories still report values only for adults. Therefore, a child's serum ALP level may be incorrectly interpreted to be normal if using an adult reference range, when it is actually remarkably low if using the correct and much higher pediatric reference range.

Patients with persistently low ALP levels require further diagnostic evaluation for HPP, even in the absence of other clinical symptoms (Saraff et al. 2016). To confirm diagnosis of HPP, clinicians should document elevation of TNSALP substrates resulting from markedly reduced ALP activity. Three substrate markers elevated in HPP are plasma/serum PLP and PP_i, and urine PEA. Serum PLP typically is ordered as "vitamin B_6". Ingestion of vitamin supplements containing vitamin B_6 can result in false positive values, therefore, vitamin supplements must be avoided for 1 week before laboratory testing. Serum PLP concentrations are associated with phenotype severity (Akiyama et al. 2018), and the occurrence of fractures and multiple symptoms in adult HPP (Schmidt et al. 2017). A medical laboratory test for serum PP_i is not commercially available and is performed only in research laboratory settings. Elevated urine PEA supports a diagnosis of HPP, but is not pathognomonic. When low serum ALP levels are associated with elevated PLP and/or PEA concentrations, radiographs of wrist and knee joints should be obtained. Antero-posterior and lateral view of skull may also need to be evaluated. Patients with suspected HPP should be referred to endocrinologists or bone specialists.

13.3.4 Genetic Testing

Mutational analysis of the *ALPL* gene can be performed to establish the diagnosis of HPP, although it may not be necessary in straightforward cases when clinical, radiographic and biochemical laboratory findings are consistent with HPP. *ALPL* gene analysis in affected individuals and family members can provide genetic information to help understand inheritance pattern and recurrence risks for genetic counselling to the family. In some cases, *ALPL* mutations are difficult to identify through the most common sequencing methods.

13.4 Attempted Treatments for Severe Hypophosphatasia Prior to Enzyme Replacement Therapy

Prior to the development and approval of AA ERT described below, management of HPP was limited to supportive care that included pain relief, orthopedic surgeries for fractures, treatment of hypercalcemia with hydration and low-calcium diet, or ventilator support for severely affected infants with respiratory insufficiency. While this range of support remains the standard of care for the majority of affected individuals, novel therapeutic interventions have been attempted in cases of severely affected infants and children. Despite the lack of long-term success of these attempted therapies, they provided invaluable insights into the etiopathology of HPP.

13.4.1 Blood Transfusion

Recognition that HPP caused low circulating ALP levels prompted interventions to attempt to ameliorate effects of severe HPP. Paget's disease of bone (PDB; OMIM# 167250, 239000, 602080, 616833), a metabolic disease of increased and disorganized bone remodeling that typically strikes in adulthood, causes dramatically increased ALP, likely due to increased numbers and activity of osteoblasts (Vallet and Ralston 2016). In a novel and clever approach to treat a metabolic disorder, Whyte and colleagues collected ALP-rich plasma from adult subjects with PDB and administered this by intravenous (IV) infusion to four subjects with severe infantile HPP (Whyte et al. 1982, 1984). Weekly treatment over the course of five or more weeks elevated ALP into the normal range for all subjects. Hypercalcemia was better controlled in subjects, one subject showed radiographic stabilization of rickets, and another exhibited histological improvement of skeletal mineralization. However, urinary PP_i and PEA remained elevated, and hypomineralization and skeletal deformities persisted and worsened over time. After failure of PDB plasma to improve the manifestations of HPP, one subject was additionally serially administered parathyroid hormone (PTH), prednisone, and then infused with normal plasma over several months [mimicking another report indicating improvement after infusing an HPP subject with normal plasma (Albeggiani and Cataldo 1982). These combined interventions failed to make a substantial improvement and all four patients died from pneumonia secondary to HPP developmental defects (Whyte et al. 1986). Based on these disappointing outcomes, the authors speculated that perhaps ALP in circulation is not physiologically active or is not accessing the tissues where its activity is required, an observation supported by previous in vivo studies (Clubb et al. 1965; Jung et al. 1970).

13.4.2 Bone Marrow Transplantation

In 2003, Whyte and colleagues took a different tack, administering bone marrow cell transplantation (BMT) to an 18-month-old female subject with severe infantile HPP (Whyte et al. 2003). With this intervention, T-cell depleted marrow from a healthy sister was successfully engrafted

and provided substantial benefit to the subject, notably reversing rickets and improving skeletal mineralization at 3 months post-BMT. Improvements proved transient when host hematopoiesis returned, accompanied by worsening rickets, scoliosis, and fractures by 6 months post-BMT. This reversal prompted a follow-up "stromal cell boost" (SCB) treatment. This second round again appeared to promote skeletal improvements, though severe disabilities remained and biochemical features of HPP were not corrected. This therapy was also accompanied by use of cyclosporine, glucocorticoids, and calcitonin, all of which can affect skeletal remodeling, though engraftment of donor mesenchymal cells likely contributed the largest improvement. In a modified approach, Whyte and colleagues treated a 9.5-month-old female with severe infantile HPP with BMT using her father's marrow, followed by implantation of bone fragments (also from the father) subcutaneous (SC) and intraperitoneally (IP), and IV administration of primary osteoblasts harvested and expanded ex vivo from additional bone fragments (Cahill et al. 2007). Though minimal engraftment was detectable over time, the subject showed decreased severity of rickets and scoliosis and improvement in skeletal mineralization, described by the authors as a shift from infantile to a milder phenotype more consistent with childhood HPP. Intriguingly, the subject was reported to retain her deciduous teeth after BMT and bone transfer.

13.4.3 Other Interventions

Shortcomings of these attempted therapies have been hypothesized to arise from relatively short treatment times, insufficiently elevated steady state ALP in circulation, and/or inability for soluble TNSALP to access or be retained at sites of skeletal mineralization, i.e. the mineralization front. Other than attempts to directly or indirectly supply functional ALP, few treatments have been attempted and none have been very successful. Bisphosphonates, a group of drugs that prevent bone loss by inhibiting osteoclast function, have been given to some individuals with HPP, usually due to misunderstanding of pathological mechanisms of HPP or without proper diagnosis (Cundy et al. 2015; Deeb et al. 2000; Doshi et al. 2009; Righetti et al. 2018; Sutton et al. 2012). As bisphosphonates do not promote bone formation, and the first generation of the drugs functioned much like synthetic PP_i to disrupt mineralization, these drugs typically worsen the hypomineralization caused by HPP and are strongly contraindicated for use in HPP patients.

Teriparatide (TPTD), a recombinant peptide based on the N-terminal portion of human parathyroid hormone (PTH), is an anabolic agent used to treat osteoporosis. Administration of TPTD to an individual with adult HPP and several stress fractures was able to increase ALP levels to within normal range (albeit at the lower edge), reduce bone pain, and was associated with good fracture healing (Whyte et al. 2007). Additional case reports on TPTD use in subjects with HPP have indicated potentially positive effects on reducing osteomalacia or accelerating bone healing (Cundy et al. 2015; Doshi et al. 2009; Righetti et al. 2018). Relative success of TPTD treatment may depend on the phenotype and biochemistry of each individual HPP patient as the increased numbers and activity of osteoblasts will still produce defective TNSALP enzyme.

13.5 Animal Models of Hypophosphatasia

13.5.1 *Alpl* Knock-Out Mice

The experimental interventions described above were performed with consent on patients with severe HPP and a high likelihood of lethality. While these therapeutic attempts were mostly unsuccessful at promoting long-term improvement in individuals with severe HPP, they revealed important clues about the pathology of HPP and what a successful therapeutic intervention would require. However, an animal model of HPP was necessary to systematically test the safety and efficacy of new therapies prior to use in human patients. With the discovery that loss-of-function mutations in the gene *ALPL* caused

HPP (Weiss et al. 1988, 1989a, b), researchers now had a target for developing clinical interventions. Based on breakthroughs in mouse genetics in the 1980s (Doetschman et al. 1987; Evans and Kaufman 1981; Mak 2007; Skoultchi et al. 1987; Thomas and Capecchi 1987), the ability to genetically inactivate specifically targeted genes in mice emerged in the 1990s as a novel and powerful strategy for understanding the functional importance of the encoded proteins (Hall et al. 2009). As they would for many other genes and diseases, these advances paved the way for development of mouse models to study HPP.

In the first reported mouse model of HPP from the laboratory of Dr. Grant MacGregor (sometimes referred to as the EM strain, for their creation at Emory University), exons 2–6 of mouse *Alpl* were targeted by homologous recombination in embryonic stem (ES) cells, reducing serum ALP by more than 90% (Waymire et al. 1995). Interestingly, authors reported that homozygous *Alpl* knockout (*Alpl*$^{-/-}$) mice at early postnatal ages did not display the expected skeletal defects. However, by 2 weeks of age, *Alpl*$^{-/-}$ mice developed spontaneous seizures, leading to early lethality. *Alpl*$^{-/-}$ mice featured increases in proposed TNSALP substrates, PP_i, PEA, and PLP. Increased PLP associated with decreased γ-aminobutyric acid (GABA) in the brain was found to be responsible for seizures, and pyridoxal injections combined with soft diet (chewable even with hypomineralized teeth and jaws) rescued some mice from seizures and early lethality, establishing the importance of TNSALP in vitamin B6 metabolism. In knockout mice where lifespan was extended by rescue, authors identified apparent defects in incisors consistent with dental enamel phenotypes reported in some human subjects with HPP. The second mouse model of HPP was reported shortly thereafter from the laboratory group of Dr. Jose Luis Millán (the so-called LJ strain, for creation at the Burnham Institute in La Jolla), targeting *Alpl* exons 5–8 through homologous recombination in ES cells (Narisawa et al. 1997). Like the previous model, these *Alpl*$^{-/-}$ mice displayed severe seizures and died before weaning. While body sizes and skeletons of *Alpl*$^{-/-}$ mice appeared similar to controls at early ages, analysis of bones at 8 days postnatal (dpn) or later revealed numerous examples of hypomineralization and fractures, as well as long bone growth plate abnormalities and hypomineralized and abnormal vertebrae (Fig. 13.4a–c). A follow-up report directly comparing the EM and LJ mouse strains determined that by 10 dpn, both models featured substrate accumulation and prominent bone hypomineralization, followed by progressive rachitic changes in long bones, accumulation of osteoid, and occurrence of bone fractures (Fedde et al. 1999). While minor differences in HPP severity were documented (possibly due to genetic background), both models replicated clinical manifestations of severe infantile HPP. The Millán lab employed the LJ HPP mouse model extensively over the next two decades, exploring the role of TNSALP in bone mineralization, vitamin B metabolism, and other organ systems, as well as elucidating interactions of TNSALP with other mineralization regulators including ANK and ENPP1 (Anderson et al. 2004, 2005; Cruz et al. 2017; Harmey et al. 2004, 2006; Hessle et al. 2002; Johnson et al. 2000; Narisawa et al. 2001, 2003; Sebastian-Serrano et al. 2016; Shao et al. 2000; Street et al. 2013; Wennberg et al. 2000).

While an earlier publication confirmed that *Alpl*$^{-/-}$ mice featured cementum hypoplasia consistent with descriptions in the HPP case report literature (Beertsen et al. 1999), a series of additional reports focused in greater detail on other developmental dental and craniofacial defects in *Alpl*$^{-/-}$ mice, finding disturbed enamel mineralization, dentin hypomineralization, and defective cranial base mineralization, abnormal cranial shape, and craniosynostosis (Fig. 13.4d–g) (Durussel et al. 2016; Foster et al. 2013; Liu et al. 2014; Nam et al. 2017; Yadav et al. 2012). In particular, it became clear through studies in HPP (and other mouse) models and that the acellular cementum critical for tooth attachment was especially sensitive to disturbances in local PP_i metabolism (Fig. 13.4h) (Foster et al. 2012; Nociti et al. 2002; Rodrigues et al. 2011; Zweifler et al. 2015), an insight made possible through the initial clues provided by the natural experiment of *ALPL* loss-of-function mutations.

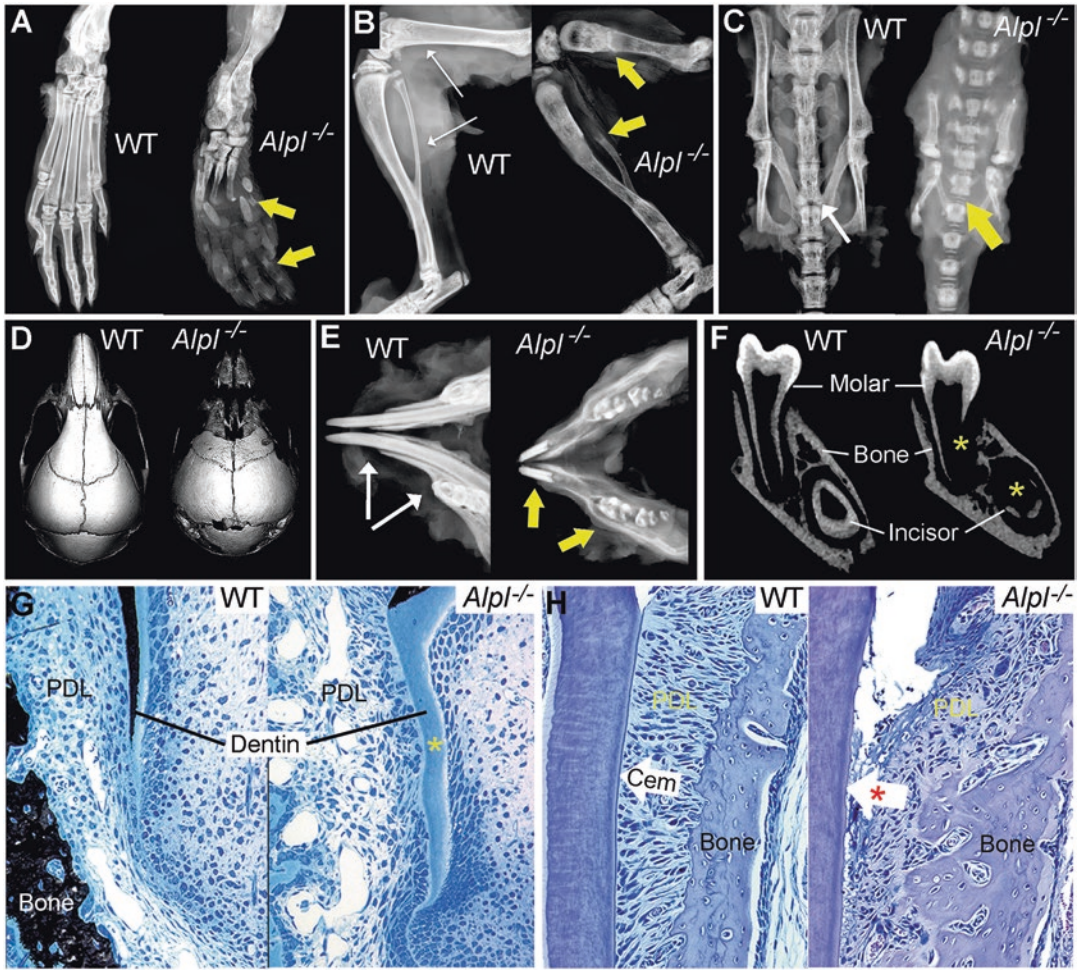

Fig. 13.4 The *Alpl* knockout mouse model of severe infantile HPP. (**a**) Radiographs of hind paws of wild type (WT) and *Alpl*$^{-/-}$ mice at 22 dpn. *Alpl*$^{-/-}$ mouse phalanges and metatarsals exhibit hypomineralization and deformities (yellow arrows). (**b**) Radiographs of hind limbs of WT and *Alpl*$^{-/-}$ mice at 22 dpn. *Alpl*$^{-/-}$ mouse femurs, tibias, and fibulas (white arrows) exhibit reduced mineralization, bowing, fracturing, and growth plate defects (yellow arrows). (**c**) Radiographs of caudal vertebrae (white arrow) of WT and *Alpl*$^{-/-}$ mice at 22 dpn. *Alpl*$^{-/-}$ mice show enlarged spaces between hypomineralized vertebrae (yellow arrow). (**d**) Radiographs of skulls of WT and *Alpl*$^{-/-}$ mice at 15 dpn. *Alpl*$^{-/-}$ mouse cranial bones feature severe hypomineralization and altered craniofacial shape. (**e**) Radiographs of mandibles with molars and incisors of WT (white arrows) and *Alpl*$^{-/-}$ mice at 22 dpn. *Alpl*$^{-/-}$ mouse mandibles show reduced radiopacity in molars and incisors (yellow arrows). (**f**) Micro-CT of WT and *Alpl*$^{-/-}$ mouse mandibles of at 14 dpn. *Alpl*$^{-/-}$ mouse molars, incisors, and alveolar bone show radiolucency (yellow $*$) indicative of severe hypomineralization. (**e**) Von Kossa stained undecalcified tissue sections of WT and *Alpl*$^{-/-}$ mouse mandibles at 12 dpn. Compared to well mineralized molar dentin in WT (indicated by black stain), *Alpl*$^{-/-}$ mouse molar roots featured hypomineralized dentin matrix (lack of black stain). (**f**) Hematoxylin and eosin (H&E) tissue sections of WT and *Alpl*$^{-/-}$ mouse mandibles at 22 dpn. Compared to the organized and functional periodontal complex in WT, *Alpl*$^{-/-}$ mouse molars lack acellular cementum (Cem) (red $*$), causing detachment of the periodontal ligament (PDL) and disorganized PDL and alveolar bone. (Images in panels **a**–**c**, and **e** reproduced with permission from Yadav et al. 2011. Images in panel **d** reproduced with permission from Liu et al. 2014. Images in panel **g** reproduced with permission from Foster et al. 2013. Images in **h** reproduced with permission from Bowden and Foster, Drug Des Devel Ther 12:3147–3161, 2018)

13.5.2 *Alpl* Knock-In Mice

Alpl$^{-/-}$ mice have been invaluable for providing insights into HPP pathology and treatment (as detailed in the following sections), however, the severity of the disease and resulting early lethality have been limitations for understanding the less severe end of the HPP spectrum experienced by many patients, and have additionally prevented long-term studies on therapeutic interventions at later ages. Therefore, attempts have been made to develop novel and less severe HPP mouse models. One approach was to genetically knock into mice an autosomal dominant mutation from a well-characterized kindred of HPP subjects reporting primarily dental defects and identified as falling within the odonto-HPP clinical type (Hu et al. 2000). Heterozygous *Alpl*$^{+/A116T}$ mice featured 50% decreased ALP and no apparent developmental, structural, or mechanical long bone phenotype by 120 dpn, considered to be adulthood in mice (Foster et al. 2015). Alterations were described in parietal bones of the skull, as well as alveolar bone of the jaw, where accumulation of osteoid and increased bone resorption were noted. Based on this phenotype, *Alpl*$^{+/A116T}$ mice were described as a mouse model of odonto-HPP, though prominent defects in cementum, dentin, and enamel were not found, making it difficult to use these mice in therapeutic rescue experiments.

13.5.3 *Alpl* Conditional Knock-Out Mice

A second approach at creating a less severe manifestation of HPP in mice employed conditional ablation of the *Alpl* gene through the Cre/lox genetic system. In this strategy, two short loxP nucleotide sequences were inserted into mouse *Alpl* allele introns surrounding exons 3 and 4. Conditional deletion of *Alpl* was achieved by crossing floxed *Alpl* (*Alpl*$^{fl/fl}$) mice with mouse lines carrying a *Cre* recombinase transgene under either the *Col1a1* promoter (to delete *Alpl* in osteoblasts and dental cells) or *Prx1* promoter (to delete *Alpl* in limb buds, chondrocytes, osteoblasts, and craniofacial mesenchyme) (Foster et al. 2017). Both conditional knockout lines lacked seizures and early lethality prominent in *Alpl*$^{-/-}$ mice, but displayed 75% reduced ALP and profound skeletal defects including rachitic changes, osteomalacia, deformations, and signs of multiple fractures at the advanced age of 180 dpn. Key aspects of HPP-associated dental defects were recapitulated, and these conditional *Alpl* knockouts were the first to demonstrate periodontal breakdown and alveolar bone loss, likely in part due to their longer lifespan allowing sufficient time for this manifestation. *Alpl*$^{fl/fl}$ mice may be crossed with any number of other Cre recombinase-carrying mouse lines, allowing targeted *Alpl* ablation in tissue- or time-specific manner that makes this a powerful approach for understanding pathological mechanisms and investigating potential therapies.

13.5.4 *Alpl* Knock-In Sheep

In 2018, the first large animal model of HPP was established. Gaddy, Suva, and colleagues knocked into sheep the same A116T *ALPL* exon 10 mutation used to engineer *Alpl*$^{+/A116T}$ mice, as described above. *Alpl*$^{+/A116T}$ heterozygous sheep exhibited approximately 30% decrease in ALP activity, decreased vertebrae size, metaphyseal flaring, altered gait, primary incisors with thin and short roots, reduced alveolar bone levels, and abnormal muscle histology (Williams et al. 2018). This model may provide novel insights into pathology and therapies because unlike in mice, sheep bone organization and remodeling is highly analogous to humans and sheep are diphiodont with primary and secondary dentitions.

13.6 Development of Recombinant Mineral-Targeting TNSALP for ERT

13.6.1 Origins of the Mineral-Targeted ERT Concept

Based on unsuccessful attempts to treat HPP with transfusions of high TNSALP blood and cell transplants, a new goal was set to develop a

recombinant TNSALP enzyme that could be effectively targeted to where PP_iase activity was required, i.e. the mineralization front of developing bones and teeth. Dr. Philippe Crine had begun exploring this concept in the context of another enzyme, PHEX, and its associated hereditary metabolic disorder, X-linked hypophosphatemia (XLH; OMIM# 307800) (Boileau et al. 2001; Campos et al. 2003). Along with scientists at Enobia Pharma (Montreal, Canada) Dr. Crine turned his attention to HPP.

As a first step to engineer a soluble secreted TNSALP that could be expressed in vitro, the hydrophilic GPI-anchor was removed from the C-terminus, and the Fc region of human IgG antibody was added to allow column chromatography purification (Millan et al. 2008). In order to enhance delivery of recombinant TNSALP to bone, a highly negatively charged sequence of ten sequential aspartic acid residues, the so-called deca-aspartate or D_{10} extension, was added to the C-terminus of the protein (Fig. 13.5a). This type of acidic amino acid "tail" had been previously demonstrated to significantly improve in vivo delivery and retention of TNSALP to bones in mice (Nishioka et al. 2006). The high negative charge density of the D_{10} tail mimicks naturally occurring bone-associated proteins with acidic amino acid motifs, e.g. members of the Small Integrin-Binding Ligand N-Linked Glycoprotein (SIBLING) protein family including osteopontin (OPN), dentin phosphoprotein (DPP), and bone sialoprotein (BSP) (Fisher and Fedarko 2003; Staines et al. 2012). Recombinant TNSALP-D_{10} expressed in vitro in Chinese hamster ovary (CHO) cells was purified and molecular mass was confirmed by Western blotting to be consistent with homodimer formation (Millan et al. 2008). Importantly, TNSALP-D_{10} bound to HAP surfaces 32-fold more efficiently than the unmodified TNSALP control, and exhibited enzymatic catalytic activity in the bound fraction.

As a first in vivo test for TNSALP-D_{10}, pharmacokinetics and tissue distribution were investigated in adult and newborn mice. A single intravenous (IV) bolus of 5 mg/kg in adult mice provided proof-of-concept when prolonged retention of radiolabeled enzyme was detected in bone, but sustained accumulation was not found in other tissues (Millan et al. 2008). Repeated daily subcutaneous injection of 10 mg/kg enzyme in newborns reproducibly elevated circulating ALP levels to about 50-fold higher than normal levels, also increasing enzyme catalytic activity in bone.

13.6.2 Preclinical Studies of Recombinant TNSALP Enzyme Replacement Therapy

Efficacy of asfotase alfa was investigated using the LJ *Alpl*$^{-/-}$ mouse line, a model of severe infantile HPP developed in the lab of Dr. Jose Luis Millán (described in detail above). Based on daily subcutaneous injections to newborns over the course of 15 days, 2 mg/kg was determined to be the minimal efficacious dose, as indicated by normalized growth rate, increased vertebral bone mineral density (BMD), and positive changes in cortical and trabecular bone (Millan et al. 2008). The higher dose of 8.2 mg/kg injected subcutaneously to newborns increased lifespans and prevented skeletal defects and fractures in *Alpl*$^{-/-}$ mice over both short and long term experiments (Fig. 13.5b, c). Untreated knockout mice died by a median 18.5 days, whereas 75% of treated *Alpl*$^{-/-}$ mice lived to 52 dpn (the preset conclusion of the experiment), displaying normal physical activity and dramatic improvement in long bone length, appearance of secondary ossification centers, and lack of fractures. Further dose-response experiments established that the dose preventing 80% of bone defects in mice (ED_{80}) was 3 mg/kg for skeletal sites including feet, lower limbs, ribs, and jaws (Yadav et al. 2011). High dose AA ERT prevented craniosynostosis and largely normalized cranial shape and mineralization of craniofacial bones (Fig. 13.5d) (Durussel et al. 2016; Liu et al. 2015; Nam et al. 2017). AA treatment significantly improved dental mineralization and function in *Alpl*$^{-/-}$ mice, preventing enamel defects, significantly improving dentin mineralization, and allowing formation of functional acellular cementum to normalize tooth attachment (Fig. 13.5e) (Foster et al. 2013; McKee et al. 2011; Millan et al. 2008; Yadav et al. 2012).

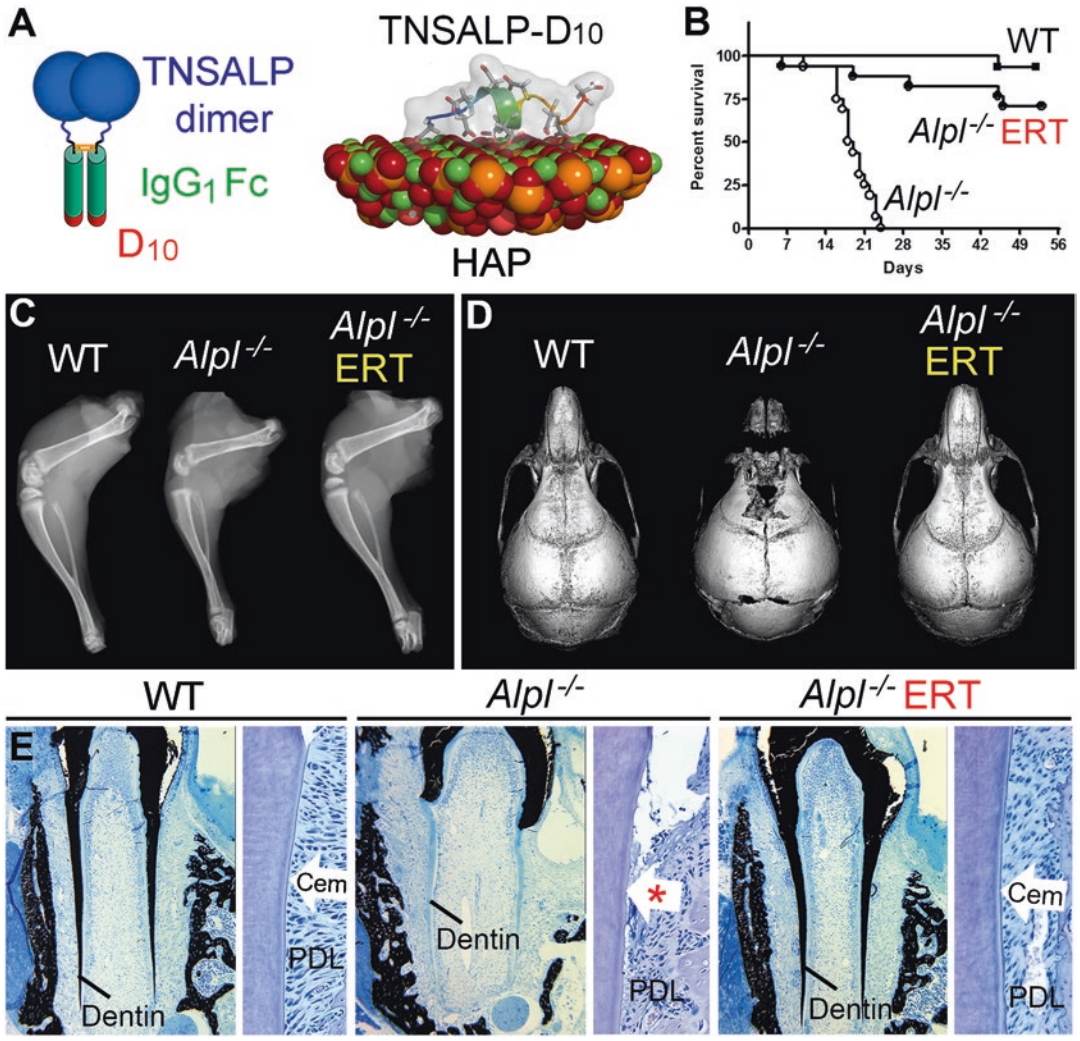

Fig. 13.5 TNSALP enzyme replacement therapy in a mouse model of HPP. (**a**) Model of recombinant asfotase alfa enzyme showing the TNSALP dimer (blue), human IgG_1 Fc domain (green), and D_{10} deca-aspartate tail (red). On the right, a simulated model shows predicted interaction of the highly negatively charged D_{10} tail with the positively charged calcium plane in the hydroxyapatite (HAP) crystal. (**b**) Percent survival of wild type (WT), untreated *Alpl*$^{-/-}$, and *Alpl*$^{-/-}$ mice receiving 8.2 mg/kg TNSALP ERT over the course of the study. (**c**) Radiographs of hind limbs at 16 dpn shows improvements in long bone lengths, shape, and appearance of secondary ossification centers in *Alpl*$^{-/-}$ mice receiving ERT. (**d**) Micro-CT of skulls at 15 dpn shows that ERT produces improvements in size, shape, and mineralization of craniofacial bones in *Alpl*$^{-/-}$ mice. (**e**) Von Kossa stained undecalcified tissue sections (left panels for each group) indicate that ERT rescues root dentin mineralization in *Alpl*$^{-/-}$ mice, as indicated by black stained appearance of mineralized tissues. Hematoxylin and eosin (H&E) stained sections (right panels for each group) reveal that ERT prevents acellular cementum (AC) hypoplasia (∗) in *Alpl*$^{-/-}$ mouse molars, allowing periodontal ligament (PDL) attachment and normal periodontal architecture. (Images in panel **a** reproduced with permission (via republication of material within the agreed-upon thresholds between STM Permissions Guidelines signatories) from McKee et al. 2011. Graph in panel **b**, images in panel **c**, and von Kossa images in panel **e** reproduced with permission from Millán et al. 2008. Images in panel **d** reproduced with permission from Liu et al. 2015. Images in panel **e** reproduced with permission from Bowden and Foster, Drug Des Devel Ther 12:3147–3161, 2018)

The importance of the mineral-targeting aspect of TNSALP-D_{10} was tested by another group that attempted to use recombinant TNSALP lacking the C-terminal GPI anchor, effectively making this a soluble form of the enzyme (Oikawa et al. 2014). Purified enzyme produced by CHO cells was administered to 1 dpn *Alpl*$^{-/-}$ mice by IV infusion of 10 U/g TNSALP, followed by SC administration of a larger dose of 20 U/g from 3 to 28 dpn and finally with 10 U/g delivered IV every 3 days until mice were 6 month old. The lifespans of treated *Alpl*$^{-/-}$ mice were extended, however, decreased body weight compared to WT control mice persisted from about 30 dpn until the end of the study. Treated *Alpl*$^{-/-}$ mice exhibited shorter body length, reduced bone length, hypomineralization, and incisor malocclusion indicating cementum defects. Pharmacokinetic studies indicated rapid clearance and development of antibodies against recombinant TNSALP over time, factors potentially diminishing efficacy. Thus, despite improving survival and eliminating seizures, soluble TNSALP therapy proved much less effective than mineral-targeted TNSALP-D_{10} at correcting skeletal and dental disorders in this study.

These initial *Alpl*$^{-/-}$ mouse studies indicated tremendous potential for enzyme replacement therapy in treating HPP. However, one important limitation in the study designs and their interpretation was that enzyme replacement was initiated prior to onset of the majority of HPP skeletal and dental manifestations, as mouse pups were injected starting at early postnatal ages. Therefore, AA was demonstrated to prevent HPP-associated pathology in mice, but not necessarily reverse or ameliorate already existing pathology. Because the skeleton is in a constant state of remodeling through the actions of osteoblasts, osteocytes, and osteoclasts, introduction of functional TNSALP-D_{10} to mice or humans with HPP would be expected to promote mineralization of osteoid, resulting in replacement of poor quality bone with much improved bone tissue with superior mechanical properties. However, enamel, dentin, and cementum of teeth do not remodel, and dentin and cementum have limited ability for repair, therefore timing of therapeutic intervention is likely critical to correct formation and improve function of the teeth. This lesson on early intervention of treatment has been learned through other endocrine disorders and metabolic disorders affecting teeth, such as nutritional vitamin D deficiency and X-linked hypophosphatemia (XLH; OMIM 307800) (Biosse Duplan et al. 2017; Davit-Beal et al. 2014; Foster and Hujoel 2018).

13.7 Therapeutic Efficacy of TNSALP ERT in Clinical Trials and Case Reports

Following the successful preclinical studies in *Alpl*$^{-/-}$ mice described in the preceding section, in 2008, the first clinical trials for TNSALP-D_{10} in infants and young children with perinatal or infantile HPP began (NCT00744042 phases 1 and 2 interventional study in infants). Additional clinical trials that followed included: NCT00739505 phase 1 interventional study in adults with HPP, NCT01205152 phase 2 interventional study in infants and children in 2009, NCT00952484 phase 2 interventional study in juveniles in 2009, NCT01203826 phase 2 interventional extension study in children in 2010, NCT01163149 phase 2 interventional study in adolescents and adults in 2010, NCT01176266 phases 2 and 3 open label interventional study in infants and young children in 2010, and NCT02797821 phase 2 interventional study in adults with pediatric-onset HPP in 2016, all of which now completed in the US, Canada, Europe, and Australia. In Japan, clinical trials completed include NCT02456038 phase 2 interventional study in children and adults in 2014, and NCT02531867 phase 4 interventional study in children and adults in 2015. Ongoing and recruiting clinical trials as of this writing include: NCT02496689 expanded access trial for children and adults with HPP (in the U.S. and France); NCT03418389 observational trial in adults with pediatric-onset HPP treated with AA (Germany); NCT02306720 prospective long-term observational trial in children and adults who have received AA (multiple countries); and NCT02751801 observational retrospective trial to evaluate the personal and economic burden of HPP to determine whether a clinical trial for less

severe clinical forms is warranted (United Kingdom). TNSALP-D_{10} was renamed as asfotase alfa (AA) (under Alexion Pharmaceuticals, Inc., New Haven, CT, USA; also known as ALXN1215 or previously ENB-0040 under Enobia Pharma, Inc.)

In 2015, AA (Strensiq) was approved by regulatory agencies in Japan, then Canada, the European Union, and the United States for pediatric-onset HPP. Treatment outcomes of AA ERT from clinical trials and case reports published to date are summarized in Table 13.3.

Table 13.3 Treatment outcomes of asfotase alfa in clinical trials and case reports

Reference and clinical trial number	Study type, patient number (N)	Age at treatment	Dosage and duration of therapy	Main clinical outcomes (skeletal, respiratory, and survival)	Comments
Perinatal and infantile HPP (treatment initiation before 3 years of age)					
Whyte et al. (2012); NCT00744042	Open-label, multinational clinical trial; N = 10 (4 perinatal, 6 infantile)	Mean age: 13.1 months (Range 0.6–36 months)	Single IV infusion at 2 mg/kg, followed by SC 1 mg/kg 3 times/ week; Dose could be increased up to 3 mg/kg if no improvement; Duration: 1 year	Healing of rickets and improved mineralization at 6 months. Most patients were off ventilator by 48 weeks of treatment. Improvement in motor function. Improved survival rate	Progressive craniosynostosis requiring neurosurgery in 2 patients. Nephrocalcinosis did not progress after 6 months of treatment and even improved in some patients. No hypocalcemia, no ectopic calcification detected. One patient died from sepsis at age 7.5 months.
Whyte et al. (2016b) NCT00744042 NCT01176266 NCT01205152 NCT01419028	Open-label, multinational, multicenter, phase 2 clinical trial; N = 37 vs. 48 historical controls	Mean age: 23 months (Range 0–71 months)	SC 1 mg/kg 6 times/week or 2 mg/kg 3 times/ week; Duration: 2.7 years	Improved skeletal mineralization. 75% weaned off ventilator support. Improved survival rate in treated patients vs. historical controls: 95% vs. 42% at age 1 year and 84% vs. 27% at age 5 years.	2 deaths: 1 from pneumonia and 1 from neurological complications of craniosynostosis
Kitaoka et al. (2017) NCT02456038	Open-label, multicenter clinical trial; N = 10	Mean age: 3 months (Range birth – 7.6 months)	SC 2 mg/kg 3 times/week; Duration: 1 year (Range 0.1–2.4 years)	Improved mineralization of bones including ribs. Two patients weaned off respiratory support during the first month of AA treatment. 100% survival rate	Hypocalcemia with seizure in 1 patient. No progression of nephrocalcinosis in 3 patients.

(continued)

Table 13.3 (continued)

Reference and clinical trial number	Study type, patient number (N)	Age at treatment	Dosage and duration of therapy	Main clinical outcomes (skeletal, respiratory, and survival)	Comments
Rodrigues et al. (2012)	Case report; Perinatal lethal HPP	3 weeks old	Single IV 2 mg/kg infusion, then SC 1 mg/kg 3 times/ week for 8 weeks, then 2 mg/kg 3 times/week; Duration: 31 weeks	Improved bone mineralization. Decreased oxygen and pressure on mechanical ventilation at 10 weeks. Discharged home with a portable ventilator without oxygen at 32 weeks. The patient died at 34 weeks of sepsis and lung infections.	The patient was born at 33 weeks gestational age and developed bronchopulmonary dysplasia
Okazaki et al. (2016)	Case report; Perinatal lethal HPP	1 day old	SC 2 mg/kg 3 times per week; Duration: 1.5 years (at time of report)	Visible improvement of bone mineralization by 3 weeks of age. Weaned off ventilation at 7 months of age. Discharged from hospital at 10 months of age and still on tracheostomy and oxygen at 18 months of age.	No craniosynostosis. Hypocalcemia and convulsion after AA treatment, requiring calcium supplement for 3 months. Hearing loss improved.
Oyachi et al. (2018)	Case report; Perinatal lethal HPP	6 days old	SC 2 mg/kg 3 times/week; Duration: 0.5 year (at time of report)	Rickets disappeared by 2 months of age. Discharged home at 6 months of age.	Pyridoxine treatment for seizures was discontinued after AA treatment. Transient hypocalcemia within 1 week of AA treatment, not requiring calcium supplement.

(continued)

Table 13.3 (continued)

Reference and clinical trial number	Study type, patient number (N)	Age at treatment	Dosage and duration of therapy	Main clinical outcomes (skeletal, respiratory, and survival)	Comments
Costain et al. (2018)	Case report; Perinatal lethal HPP	13 days old	SC 2 mg/kg 3 times/week; Dose increased to a maximum of 9 mg/kg/dose on day 67 due to lack of clinical improvement	Improved bone mineralization but no improvement in respiratory function and persistently small chest size; suspected pulmonary hypoplasia. Patient died on day 100 after AA treatment and respiratory support were withdrawn.	Hypocalcemia requiring calcium supplementation
Hacihamdioglu et al. (2018)	Case report; Perinatal lethal HPP	21 days old	SC 2 mg/kg 3 times/week; Duration: 1 year (at time of report)	Discharged home at 7 months of age. Intubated at birth, though after 12 months of treatment, ventilation via tracheostomy was needed only during sleep.	No hypocalcemia during AA treatment. No sign of craniosynostosis at 12 months.
Rougier et al. (2018)	Case report; Perinatal lethal HPP	22 days old	SC 2 mg/kg 3 times/week for 1 month, then 3 mg/kg 3 times per week, then back to 2 mg/kg 3 times per week at 34 weeks of age; Duration: 3 years	Improvement in mineralization of bones and ribs. Intubated at birth, though taken off ventilation at 41 weeks of age. Improved muscle tone.	Neurosurgery for craniosynostosis at age 34 weeks. Severe nephrocalcinosis discovered at age 5 months.
Ucakturk et al. (2018)	Case report; Perinatal lethal HPP	40 days old	SC 2 mg/kg 3 times/week; Duration: 8 weeks	Minimal improvement in bone mineralization. Intubated at birth, and no significant improvement in respiratory function. The patient died at 97 days due to ventilator-associated pneumonia and sepsis.	AA treatment had not been long enough to detect improvement in bone mineralization and respiratory function.

(continued)

Table 13.3 (continued)

Reference and clinical trial number	Study type, patient number (N)	Age at treatment	Dosage and duration of therapy	Main clinical outcomes (skeletal, respiratory, and survival)	Comments
Children with HPP					
Whyte et al. (2016a); NCT00952484 NCT01203826	Open-label study in infantile or childhood HPP; N = 13 (5 infantile, 8 childhood)	Mean age 8.8 years (Range 6–12 years)	Initially randomized to SC 2 or 3 mg/kg 3 times/week for 6 months, then 6 mg/kg/week; Duration: 5 years	Significant healing of the skeletal manifestations. Significant improvements in growth, strength, motor function, agility, and quality of life. Pain and disability resolved.	Mild to moderate injection-site reactions. Localized lipohypertrophy persisted in 6 of 8 patients after 3 years of treatment.
Adolescents and adults with HPP					
Kishnani et al. (2017a, b)	Phase II, randomized, dose-ranging, open-label, multi-center study; N = 19	6 children aged 13–18 years, and 13 adults aged 18–65years.	0.3 (n = 7) or 0.5 mg/kg/day (n = 6) asfotase alfa for 6 months, then all received 0.5 mg/kg/day, increased 6–12 months later to 1 mg/kg 6 times/ week; Duration: 5 years	Improved physical function as assessed by 6 min walk test and Bruininks-Oseretsky Test of Motor Proficiency.	3 patients withdrew: 2 for injection site reactions at 2 years and 3.5 years, 1 for poor compliance at 2.5 years.
Bowden and Adler (2018a)	Case report; Severe childhood HPP	15 years old	SC 2 mg/kg 3 times/week; Duration: 1 year	Improved growth and physical function. Chronic pain resolved.	Worsening scoliosis from 60 to 110 degrees.
Remde et al. (2017)	Case report; Adult with childhood-onset HPP	57 years old	SC 80 mg 3 times/ week (given after hemodialysis for chronic renal failure); Duration: 13 months	Reduced bone pain; Able to walk independently for 3 m; Improved bone mineralization; No new fractures.	Prior to AA, patient received bisphosphonate therapy for a year and teriparatide for 4 months.
Klidaras et al. (2018)	Case report; Adult with infantile-onset HPP	41 years old	SC 1 mg/kg 6 times/week; Duration: 14 months at time of report	Healing of femoral pseudofracture of 17 years.	
	Case report; Adult with HPP	61 years old	AA treatment for 16 months at time of report	Complete healing of nonhealing femoral fracture of 8 years. Significantly less pain with ambulation. Improvement in gait.	Prior to AA, patient was treated with teriparatide for 6 months to assist with fracture healing; discontinued due to side effect and no response in fracture healing.

(continued)

Table 13.3 (continued)

Reference and clinical trial number	Study type, patient number (N)	Age at treatment	Dosage and duration of therapy	Main clinical outcomes (skeletal, respiratory, and survival)	Comments
Freitas et al. (2018)	Case report; Adult with childhood-onset HPP	36 years old	SC 2 mg/kg 3 times/week; Duration: 12 months at time of report	Able to walk without assistive devices. Improved bone mineral density. Improved bone microarchitecture detected by high-resolution peripheral quantitative computed tomography (HR-pQCT).	Also had stage III chronic kidney disease. Prior to AA, patient was treated with alendronate 70 mg weekly for 8 years until 1 year before AA treatment.

13.7.1 Treatment of Perinatal and Infantile HPP

AA ERT substantially improved bone mineralization, leading to improvement in respiratory function and survival in neonates and infants with HPP (Table 13.3) (Costain et al. 2018; Hacihamdioglu et al. 2018; Kitaoka et al. 2017; Oyachi et al. 2018; Rougier et al. 2018; Ucakturk et al. 2018; Whyte et al. 2012, 2016b). Improvements in skeletal radiographs were apparent as early as week 3 (Okazaki et al. 2016) and were dramatic by 24 weeks of treatment. Specifically, radiographs indicated improved mineralization, healing of rickets, resolution of radiolucencies and sclerosis, fracture healing, and reduced deformity (Fig. 13.6a–f). Patients with gross motor delay were bearing weight or walking by 48 weeks of treatment. The survival rate in 37 infants and young children (≤5 years) with perinatal or infantile HPP treated with AA had significantly improved compared to the historical control group (95% vs. 42% at age 1 year and 84% vs. 27% at age 5 years, respectively) (Whyte et al. 2016b). Another study in Japan reported 100% survival rate in perinatal and infantile HPP treated with AA (Kitaoka et al. 2017), which might have been associated with early treatment with AA (mean age at start of treatment 3 months vs. 13–23 months in cohorts described by Whyte et al). AA therapy started on day one of life resulted in survival in an infant with lethal perinatal HPP as well as dramatic improvement in skeletal mineralization (Okazaki et al. 2016). The window of survival with AA treatment maybe narrow in lethal perinatal HPP, as one neonate with this severe life-threatening form died on day 4 of life without AA treatment (Castells et al. 2018). Prompt diagnosis and expeditious initiation of AA therapy is crucial to survival as it will likely improve ventilation and minimize intensive care.

13.7.2 Treatment of Children (6–12 Years) with HPP

In 2016, Whyte and colleagues reported results of 5 years of AA ERT in a cohort of 11 children aged 6–12 years (Table 13.3) (Whyte et al. 2016a). Similar to the cohort with the more severe form of infantile HPP, substantial radiographic improvements were noted by 6 months, and persisted through 5 years of the study. Significant increase in weight Z scores was noted at 6 weeks of ERT, followed by significant increase in height Z scores (from mean −1.26 at

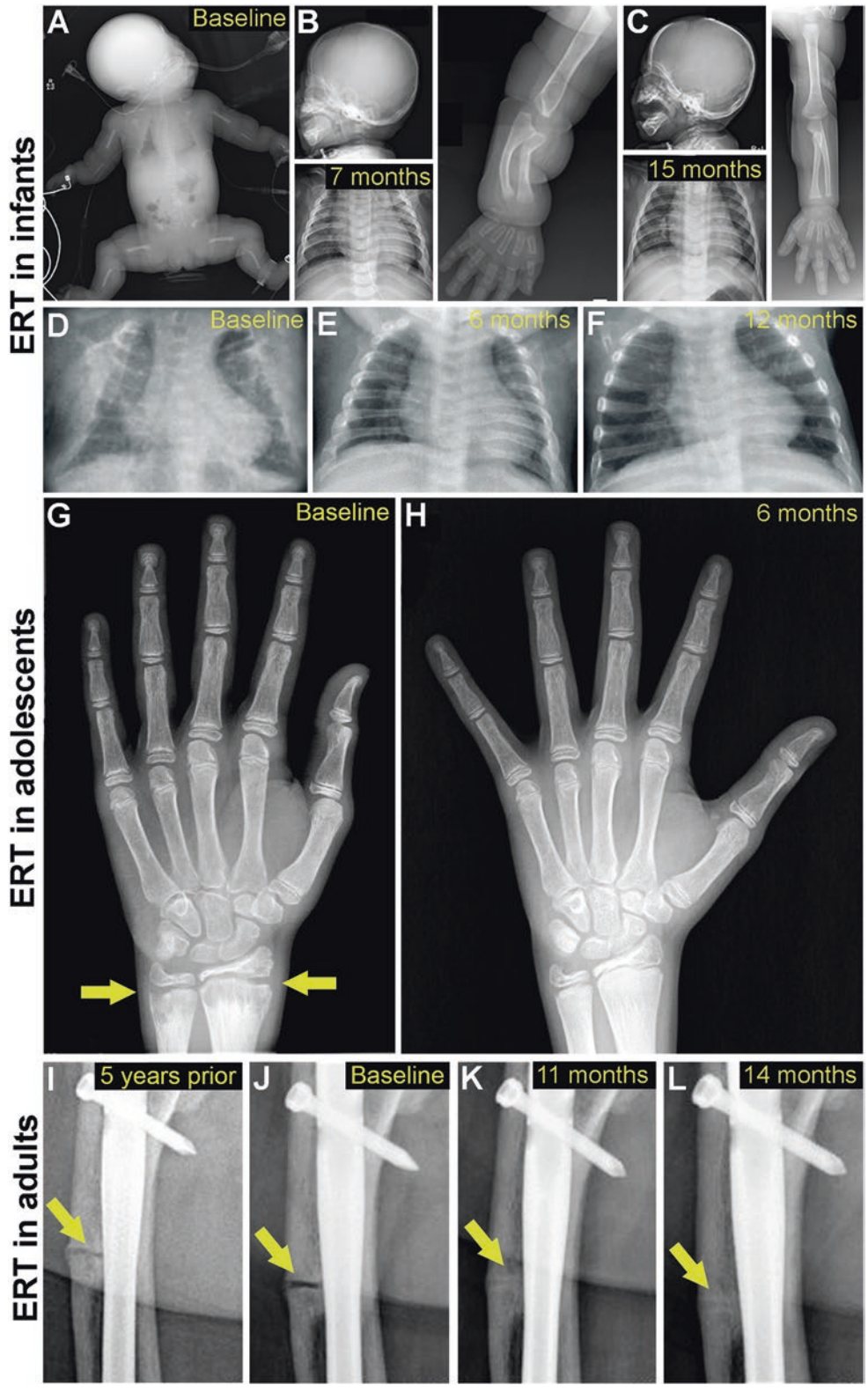

Fig. 13.6 TNSALP enzyme replacement therapy in human subjects. (**a–c**) Whole body, cranial, rib, and arm radiographs of an infant with perinatal HPP at baseline and 7 and 15 months after AA ERT. In addition to allowing survival, ERT was associated with substantial improvements in mineralization and reduction of rachitic

baseline to −0.8) after 1.5 years of AA treatment. Most children receiving ERT displayed increased muscle strength and motor skills on par with healthy children, had normal ambulation and reduced disability, and reported reduced pain. Case reports of children administered AA ERT reported similarly dramatic skeletal improvements (Kitaoka et al. 2017).

13.7.3 Treatment of Adolescents and Adults with HPP

Similar to the study in children, after 5 years of AA treatment, adolescents and adults with HPP had significant improvement in physical function with increases in 6-min walking test, and improvement in health-related quality of life (HRQOL) as measured by the Childhood Health Assessment Questionnaire Disability Index and the Pediatric Outcomes Data Collection Instrument global function (Table 13.3) (Kishnani et al. 2016, 2017a; Tomazos et al. 2017). ERT resulted in improved mobility without use of the previously used walking assistive device was achieved in an adolescent after 6 months of AA treatment (Bowden and Adler 2018a), when striking improvements in bone radiographs was observed (Fig. 13.6g, h). In a 59 year-old adult with childhood-onset HPP, treatment with HPP significantly increased quality of life in terms of increased mobility, reduction in pain medication, and improved bone mineralization with healing of non-union fractures and no occurrence of new fractures (Remde et al. 2017). In two adult HPP patients with longstanding non-healing fractures, AA ERT dramatically improved healing and resolution of the fractures (Fig. 13.6i–l) (Klidaras et al. 2018).

13.7.4 Effects of ERT on Specific Clinical Features of HPP

As diverse and multi-systemic as HPP clinical manifestations are, so are therapeutic outcomes of AA ERT, and these deserve further attention and investigation. While AA ERT has produced dramatic improvements in skeletal manifestations of HPP, as described above, effects on other manifestations are not entirely uniform. Effects of ERT on biochemistry and various clinical aspects, including respiratory function, bone health, physical function, mobility, nephrocalcinosis, craniosynostosis, neurological manifestations, chronic pain, scoliosis, and dental tissues are outlined below.

13.7.4.1 Biochemistry

Low serum ALP levels with elevated serum PLP are diagnostic for HPP and these levels change dramatically after AA treatment and can be used to monitor the effect of, and patient compliance with ERT. As a result of three or six times weekly AA, levels of ALP have been reported as high as ~24,000 IU/L within 4 weeks of initiating ERT (Kitaoka et al. 2017), remaining at elevated levels of 3000–6000 IU/L even after 5 years of therapy (Whyte et al. 2016a). To date, these markedly elevated ALP levels do not appear to have harmful effects. In contrast, when AA therapy is discontinued completely, serum ALP levels may decrease to undetectable levels (similar to pre-treatment level), as demonstrated in an adolescent boy who voluntarily discontinued AA ERT due to non-adherence (Bowden and Adler 2018b). This indicates that serum ALP level is a good marker for treatment adherence monitoring. Elevated serum PP_i and serum PLP decrease dra-

Fig. 13.6 (continued) skeletal deformities. (**d–f**) Chest radiographs of an infant with HPP at baseline and 6 and 12 months after AA ERT. Dramatic improvements in rib mineralization, chest structure, and thoracic volume were noted. (**g** and **h**) Hand radiographs of a 15-year-old patient with severe childhood HPP show marked metaphyseal fraying and characteristic "tongues" of radiolucency in the distal radius and ulna (yellow arrow) that are substantially resolved after 6 months of AA ERT. (**i–l**) Series of radiographs of a nonhealing subtrochanteric fracture in a 61-year-old male HPP patient showing lack of resolution 12 years (**i**) and 17 years (**j**) after fracture, with marked improvements after 11 months (**k**) and 14 months (**l**) of AA ERT. (Images in **a–c** reproduced with permission from Okazaki et al. 2016. Images in **d–f** reproduced with permission from Whyte et al. 2016b. Images in **g**, **h** reproduced with permission from Bowden and Adler 2018a, b. Images in **i–l** reproduced from Klidaras et al. 2018 and used under the terms of the Creative Commons CC BY license)

matically in response to treatment (Akiyama et al. 2018; Bowden and Adler 2018a; Kitaoka et al. 2017; Whyte et al. 2012, 2016a). Additional PLP-related metabolites, pyridoxal (a product of the enzymatic reaction of ALP) and 4-pyridoxic acid (a metabolite of pyridoxal) have been investigated as a diagnostic marker of HPP and an indicator of the AA treatment effect (Akiyama et al. 2018). The PLP-to-pyridoxal ratio (PLP/PL) is considered to reflect the activity of ALP (converting PLP to PL) and has been shown to be useful to evaluate the early treatment effect of AA ERT before the skeletal improvement occurs (Akiyama et al. 2018).

While calcium abnormalities are rare in adults with HPP, hypercalcemia is common in infants and children and often identified at the time of diagnosis. Some infants have continued to present hypercalcemia and hyperphosphatemia after AA ERT, requiring continued use of low-calcium and/or low phosphorus formula (Kitaoka et al. 2017). Hypocalcemic seizures were described in a neonate with perinatal HPP and serum calcium levels of 4.7 mg/dL, following 3 weeks of ERT initiated on the first day of life (Kitaoka et al. 2017). The seizures and hypocalcemia were resolved by increasing calcium supplementation. Hypocalcemia after AA treatment suggests "hungry bone" syndrome, or rapid formation and mineralization of previously deficient and hypomineralized bone that requires a significant influx of calcium. Dietary calcium restriction in children with HPP should be liberalized after AA ERT is initiated and hypercalcemia is no longer present, or when serum PTH levels increase, to prevent hypocalcemia and "hungry bones" (Whyte et al. 2012). Serum PTH increased during treatment, coinciding with the skeletal remineralization as evidenced by the radiographic improvements.

Falsely low serum testosterone level obtained by competitive radioimmunoassay has been reported in a patient taking asfotase alfa ERT, thought to be due to assay interference by exogenous TNSALP (Sofronescu et al. 2018).

13.7.4.2 Respiratory Function

In perinatal or infantile HPP, respiratory failure is often the cause of mortality. The etiology of respiratory insufficiency in HPP is multi-factorial and complex, and usually stems from thoracic deformity and fractures, as a result of the skeletal manifestations. Respiratory failure can also be due to pulmonary hypoplasia, muscle weakness, tracheomalacia, central nervous system dysfunction associated with craniosynostosis, episodic seizures, and increased susceptibility to infection due to low TNSALP levels in leukocytes (Whyte 2012). However, once skeletal mineralization improves, with stabilization of the chest wall and/or improved muscle strength, patients have improved respiratory function and can discontinue mechanical ventilation (Whyte et al. 2016b). Crying vital capacity has been utilized to evaluate respiratory status and is reported to be a good indicator for weaning mechanical ventilation (Shimada et al. 1979). Crying vital capacity of 15 ml/kg was used as a criterion for extubation and greater than 20 ml/kg as a criterion for discharge from the hospital in a patient with perinatal HPP who was discharged home at 6 months of life (Oyachi et al. 2018). Some children with perinatal or infantile HPP may not be fully weaned off the mechanical ventilation and may require tracheostomy with oxygen (Okazaki et al. 2016), or ventilation via tracheostomy only during sleep (8 h a day) (Hacihamdioglu et al. 2018).

13.7.4.3 Bone Health, Physical Function, and Mobility

As AA ERT is designed to bind to HAP in mineralized bone and tooth tissues, the effects on skeletal mineralization are dramatic and precede improved respiratory and motor function. At 6 months of AA treatment, improvements in skeletal health include diffusely increased bone mineralization, corrected or improved endochondral and membranous bone formation, reduced deformity, healing of fractures, and extensive modeling and remodeling of bone, with resolution of sclerosis (Whyte et al. 2012). While skeletal radiographs showing remarkable improvement after AA ERT have been consistently reported in clinical trials and case reports, reports on bone mineral density using dual-energy X-ray absorptiometry (DXA) after AA ERT have been limited.

In a 15 year-old boy with severe childhood HPP, the lumbar spine bone mineral density Z score was markedly low at −5.9 (height-adjusted Z score of −2.7). After 12 months of AA therapy, the absolute values of his lumbar spine BMD and total body bone mineral content and increased by 19% and 23%, respectively, but his height-adjusted Z scores for lumbar BMD decreased from baseline of −2.7 to −3.1 (Bowden and Adler 2018a). In an adult with late diagnosis of severe childhood HPP who suffered from multiple fractures and impaired mobility, treatment with AA for 12 months increased lumbar spine bone mineral density by 10% and improved mobility (Freitas et al. 2018). This patient also had improvement in bone microarchitecture parameters assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT) (the first reported use of such imaging for bone assessment after AA ERT) in the distal tibia, with stabilization of bone parameters at the distal radius. Increased proteinaceous components of hypomineralized bones may affect bone density readings by DXA (Kishnani et al. 2017b). Further research is warranted to evaluate and characterize overall bone health and the therapeutic outcome of AA on bone density and bone microarchitecture by different bone imaging tools such as DXA or HR-pQCT.

Along with skeletal improvement, profound delays in growth and gross motor function in pediatric patients with severe HPP improved substantially from AA ERT, across all studies described above. Adolescents and adults patients with years of ambulatory disability, or even complete immobility, began to walk independently after 6–12 months of AA therapy (Bowden and Adler 2018a; Freitas et al. 2018; Klidaras et al. 2018; Remde et al. 2017), with one adult patient improving walking distance up to 4 miles (Klidaras et al. 2018). It is important to inform patients that continuation of AA therapy without interruption is critical to maintain optimal ALP and mineralization activity, and to prevent recurrence of clinical deterioration. Reappearance of hypomineralization of metaphyses, similar to the pre-treatment appearance, has been reported in an adolescent with severe childhood HPP who stopped AA therapy for 1 year due to non-adherence (Bowden and Adler 2018b).

Improvement in physical function, mobility and musculoskeletal function after AA therapy has been documented in clinical trials (Table 13.3) using objective, validated, and age-appropriate assessment tools: the Alberta Infant Motor Scale and Gross Motor Function Measure, the Bayley Scales of Infant and Toddler Development, and the Peabody Developmental Motor Scales in young children; Bruininks-Oseretsky Test of Motor Proficiency, Second Edition [BOT-2] (for age 4–21 years); and the 6-min walk test (6MWT) (for ambulatory children aged ≥5 years and adults). Pain (see pain section below) and disability assessment obtained by the parent-reported Child Health Assessment Questionnaire (CHAQ) and Pediatric Outcomes Data Collection Instrument (PODCI) also showed substantial improvement from AA ERT (Whyte et al. 2016a). The 6MWT can be administered in the clinical setting and may be recorded on video to allow comparison of gait and mobility over time during AA therapy. Modified Performance-Oriented Mobility Assessment-Gait (mPOMA-G) (observational gait analysis from video footage during 6MWT) has been validated specifically in children with HPP and is strongly correlated with CHAQ, PODCI, and 6MWT scores (Phillips et al. 2018).

13.7.4.4 Nephrocalcinosis and Nephrolithiasis

Nephrocalcinosis is sometimes identified at the time of diagnosis in infants and children with HPP, and is thought to be secondary to hypercalcemia with hypercalciuria. During clinical trials to date, ERT seemed to cause no progression of nephrocalcinosis in children with infantile HPP (Kitaoka et al. 2017; Whyte et al. 2012), with some improvement even noted in some patients (Whyte et al. 2012). Monitoring of nephrocalcinosis by renal ultrasound is recommended at baseline and every 3 months in cases of perinatal and infantile HPP, and at baseline, 6 months, and then annually in childhood and adult HPP (Kishnani et al. 2017b).

In addition to nephrocalcinosis, nephrolithiasis with parietal calcifications can occur in adults with HPP (Freitas et al. 2018), likely from long-standing HPP disease with hypercalciuria, and/or long-term therapy with calcium and vitamin D for an 'osteoporosis' diagnosis. Nephrocalcinosis and nephrolithiasis can result in impaired renal function or chronic kidney failure in adults. AA ERT was safe and efficacious in an adult dialysis patient with HPP and stage 4 chronic kidney disease (Remde et al. 2017). AA was given immediately after hemodialysis three times a week at the recommended dose without the need for dose adjustment.

13.7.4.5 Craniosynostosis

Premature fusion of cranial sutures occurs in perinatal, infantile, and childhood forms of HPP. Clinical evidence of the abnormal skull shape (scaphocephaly or oxycephaly) secondary to the loss of one or several sutures, and/or the absence of head circumference growth, are clues to the diagnosis of craniosynostosis. Craniosynostosis is sometimes detected before HPP is diagnosed, and should alert clinicians to evaluate serum ALP. The underlying mechanism for craniosynostosis remains poorly understood. Clinical sequelae of craniosynostosis include papilledema, optic nerve atrophy, increased intracranial pressure with a copper-beaten skull on a skull x-ray (Poryo et al. 2016), and secondary ectopia of the cerebellar tonsils that can lead to hydrosyringomyelia (Collmann et al. 2009). To date, AA ERT has not prevented craniosynostosis, and the condition progressed during the treatment, requiring neurosurgical intervention (Whyte et al. 2012). The timing of ERT initiation may be critical for prevention of craniosynostosis, as a neonate with perinatal HPP receiving AA treatment from day 1 did not develop suture fusion (Okazaki et al. 2016), and similarly, early postnatal treatment in the mouse model of infantile HPP was also shown to prevent craniosynostosis (Liu et al. 2015).

13.7.4.6 Neurological Manifestations

In severe perinatal HPP, seizures occur as a result of defective PLP metabolism, and are an indicator of HPP severity and lethal prognosis, associated with 100% mortality rate (Whyte et al. 2016b). PLP is the active form of vitamin B6 that is unable to cross the cell's plasma membrane or the blood brain barrier. TNSALP dephosphorylates PLP into the pyridoxal form of vitamin B_6, able to cross the cell membrane and blood brain barrier and that is subsequently phosphorylated back into PLP, serving as a key co-factor for many enzymes. PLP in the brain is required for biosynthesis of many neurotransmitters in the brain (e.g. dopamine, norepinephrine, gamma-aminobutyric acid [GABA]). Reduced TNSALP enzymatic capability in HPP therefore results in elevated serum PLP and reduction of PLP in the brain, making severely affected neonates and infants with HPP prone to seizures. Provision of high doses of pyridoxine hydrochloride (vitamin B_6) can temporarily correct this deficiency and prevent seizures. AA ERT resulted in markedly improved survival rate of 77% in infants experiencing seizures (Whyte et al. 2016b) and prevented further seizures so that pyridoxine hydrochloride was discontinued (Oyachi et al. 2018). Pyridoxine-responsive seizures have been reported as the first symptom of infantile HPP in a neonate without bony abnormalities (Baumgartner-Sigl et al. 2007), further illustrating clinical heterogeneity in HPP. HPP was not diagnosed in this patient until age 7 months when clinical HPP became apparent with skeletal manifestations. Unfortunately, this child developed rib fractures and died from respiratory failure at age 9 months. Assessment of any neonate with pyridoxine-responsive seizures should include measurement of serum ALP to facilitate early detection of HPP, which can be life-saving due to the availability of ERT.

While seizures are not observed in older children or adults with HPP, other neurological symptoms such as fatigue, headaches, sleep disturbances, vertigo, depression, anxiety, neuropathy, and hearing loss occurred commonly (33–66%) in individuals with HPP, at a greater prevalence than the US general population (Colazo et al. 2018). Use of psychiatric medications for mental illness was observed in 65% of patients and preceded the diagnosis of HPP. Interestingly, patients with ves-

tibulocochlear symptoms (hearing loss, tinnitus, and vertigo) had lower ALP levels than those without. The mental health issues and neurological symptoms described above should be included in the assessment and evaluation of HPP in clinical practice. Resolution of any of these symptoms after AA treatment should be documented to provide insights into this under-reported and understudied aspect of HPP, and future prospective studies should be conducted to evaluate the effects of AA treatment on these neurological symptoms.

13.7.4.7 Chronic Pain

Musculoskeletal pain is a significant feature of HPP, experienced by many affected individuals across their lifespan. Infants and young children with HPP may not be able to communicate pain sensation, therefore, signs of irritability through vocalization, facial expression, or color changes, along with validated and age-appropriate pain scales such as the Neonatal Pain, Agitation and Sedation Scale (N-PASS), or the Face, Leg, Activity, Cry, Consolability Scale (FLACC) should be used to document pain and response to ERT (Phillips et al. 2016). In older children or adults, Wong-Baker FACES pain rating scale can be used. Pain is thought to be due to chronic inflammation with elevated prostaglandin levels (Girschick et al. 2006) caused by calcium pyrophosphate dihydrate (CPPD) crystal depositions (Beck et al. 2009). This can result in incapacitating bone and joint pain. Resolution of chronic musculoskeletal pain following AA therapy has been reported in all clinical trials and case reports. One adolescent male with severe childhood HPP discontinued daily analgesic use within 3 months of AA ERT (Bowden and Adler 2018a).

13.7.4.8 Scoliosis

Scoliosis has been described in four children with HPP in the literature (Arun et al. 2005; Bowden and Adler 2018a; Whyte et al. 2003). A child with infantile HPP had a normal spinal radiograph at age 2.5 months, but developed scoliosis by age 7 months (Whyte et al. 2003). Another two patients also exhibited early onset of severe scoliosis (Arun et al. 2005). The first child with severe infantile HPP was noted to have scoliosis of 62° at age 3 years, which progressed to 94° by age 7 years. The second child had odonto-HPP with no skeletal demineralization or deformity except for early onset of scoliosis of 74° at age 3.5 years, which progressed to 90° by age 10 years (Arun et al. 2005). These two patients subsequently had corrective spinal surgeries with good outcomes. The fourth reported individual with severe childhood HPP developed scoliosis during childhood and began AA ERT therapy at age 15 years when scoliosis was noted to be 60°. The patient had improved growth during first 9 months of AA therapy. However, after 12 months of AA treatment, scoliosis progressed to 110°, with a decrease in height, necessitating spinal fusion surgery (Bowden and Adler 2018a). It is important to screen for and monitor scoliosis during AA therapy in HPP patients, as untreated progressive scoliosis associated with metabolic bone disease can lead to pulmonary compromise and death (Collins 2006).

13.7.4.9 Dental Defects

To date, effects of AA ERT on dental manifestations of HPP have been limited to reports on three young children with lethal perinatal HPP. One child who started AA ERT at age 7.5 months showed better mineralization of teeth on lateral radiographs of the skull after 1 year of AA ERT; the other starting AA at 36 months showed improved stability of loose teeth (Whyte et al. 2012). Another child in Japan who started AA on the first day of life exhibited no tooth loss at age 3 years 5 months, but featured dentin and enamel defects and delayed formation of permanent teeth (Okawa et al. 2017b). These three patients represent the extent of dental findings reported for severe lethal HPP, while the potential for AA ERT to make improvements in dentoalveolar tissues in other forms of HPP remains unknown. Clinical trials to date have not incorporated dental examinations into data collection plans, to provide information on how the timing of intervention affects development and retention of primary and secondary teeth, and to determine whether AA is equally effective at improving cementum, dentin, enamel, and alveolar bone or periodontal manifestations of HPP. The opportunity to collect quantitative and qualitative dental

findings in treated HPP subjects remains feasible, is a high priority for clinicians and researchers concerned with dental effects of HPP, and could prove important in justifying early administration of AA to prevent lifelong oral health problems and improve quality of life for individuals with severe dental manifestations of HPP. A registry has been initiated to collect qualitative and quantitative data on dental effects of HPP, allowing pre- and post-ERT comparisons to begin to determine efficacy of AA on ameliorating dental defects (http://u.osu.edu/hppstudy/).

13.7.5 Adverse Effects and Outcomes

Clinical trial results to date show very good safety profiles for AA ERT (Kitaoka et al. 2017; Whyte et al. 2012, 2016a). The most common adverse effects in patients treated with AA are injection-site reactions, occurring in about 75% of individuals treated (Kishnani et al. 2017b). Injection site reactions may include mild, localized, transient erythema, pain, induration, and lipohypertrophy or lipohypotrophy at injection sites. Localized lipohypertrophy persisted for more than 3 years in 6 out of 12 patients in the childhood HPP trial (Whyte et al. 2016a). In a patient undergoing AA ERT and not yet described in the literature, lipohypertrophy remained the same size with no regression even after discontinuing using the site for more than a year (personal observation) (Fig. 13.7). A rarer but more severe adverse effect reported has included anaphylactoid reaction (Kishnani et al. 2016). All patients enrolled in clinical trials developed anti-AA antibodies, but no tachyphylaxis has been reported to date (Kishnani et al. 2017b; Whyte et al. 2012).

Benefits of AA therapy are clearly evident in infants, children, and adults with HPP in clinical trials and case reports described above. Infants with life-threatening HPP survived; those who died were from sepsis, not attributed to AA therapy (Vidmar et al. 2017; Whyte et al. 2012), or from neurologic complications of craniosynostosis that occurred early in the treatment, possibly reflecting natural history and complications (Whyte et al. 2016b). Excellent clinical outcomes

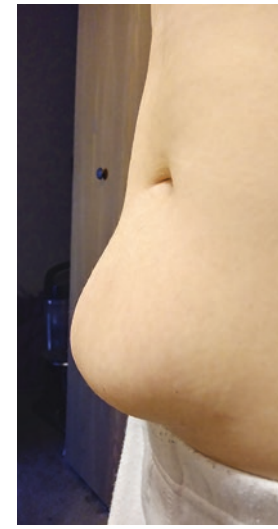

Fig. 13.7 Lipohypertrophy resulting from asfotase alfa injection. In an adolescent undergoing AA ERT, lipohypertrophy at an abdominal injection site remained the same size with no regression, even after discontinuing using the site for more than a year

in all areas including physical function and quality of life continue after 5 years or more follow-up in those children and adults (Kishnani et al. 2017a). There has been one case of a term infant with perinatal HPP with unsuccessful treatment outcome due to irreversible pulmonary hypoplasia, despite improvement in rib mineralization after AA dose increased to 9 mg/kg/day (Costain et al. 2018). The poor outcome in this case may not be directly related to treatment failure, but possibly due to underlying genetic factor associated with failure of postnatal alveolar development.

13.7.6 Dosage and Administration of AA

AA is indicated for patients with pediatric-onset HPP. The recommended dosage of AA in perinatal- or infantile-onset HPP is 2 mg/kg three times weekly or 1 mg/kg six times weekly. The dose can be increased for lack of efficacy (e.g., no improvement in respiratory status, growth, or radiographic findings) up to 3 mg/kg three times weekly (maximum: 9 mg/kg per week). The dos-

age for childhood-onset HPP is also 2 mg/kg three times weekly or 1 mg/kg six times weekly. AA is administered subcutaneously only in the abdominal area, thigh, or deltoid. Rotation of the injection sites should be emphasized to reduce the risk of lipodystrophy (as described above and in Fig. 13.7). Patients or caregivers of pediatric patients should be instructed to administer AA within 1 h upon removal from refrigerator and to not administer injections in areas that are reddened, inflamed, or swollen. The 80 mg/0.8 mL concentration vial should not be used in pediatric patients. AA solution is clear, slightly opalescent or opalescent, colorless to slightly yellow; few small translucent or white particles may be present. Vial(s) not consistent with this appearance should be discarded. The maximum injection should not exceed 1 ml, using a 1 mL syringe with 1/2 inch needle (25–29 gauge). If more than 1 mL is required, the total volume should be split equally between two syringes, and two injections are administered at the same time using separate injection sites.

13.8 Future Directions for HPP Therapy

The preclinical studies described above in Sect. 5 employed repeated daily injections to achieve steady state concentrations of ALP activity with a recombinant protein that had a short half-life of 34 h in plasma. Novel therapeutic approaches are necessary to reduce adverse effects, improve efficacy, or serve as a secondary approach for those who do not respond, have adverse outcomes, or develop immune reactions to AA. Several of these concepts have been tested at the preclinical stage and these studies are summarized below.

13.8.1 Gene Therapy Approaches

Several gene therapy approaches employing single injections of TNSALP-D_{10} expressing viral vectors have been reported to show positive effects in correcting the HPP phenotype in *Alpl*$^{-/-}$ mice. In the first study, a lentiviral (LV) vector expressing TNSALP-D_{10} was injected IV into newborn pups at 1–3 dpn (Yamamoto et al. 2011). A high copy number of integrated vector was detected in liver (with lower copy numbers in other organs) and by 10 dpn, ALP was measured at 25-fold higher than untreated *Alpl*$^{-/-}$ mice and tenfold higher than WT control mice. While ALP in WT mice declined with age, treated *Alpl*$^{-/-}$ mice posted more than 70-fold increased ALP over WT by 60 dpn, indicating stable and even increasing enzyme production. LV expressed TNSALP-D_{10} normalized body size and eliminated seizures in *Alpl*$^{-/-}$ mice, extending lifespans of six of seven mice until 160 dpn, the termination of the study. Radiological and histological evaluation of long bones of treated *Alpl*$^{-/-}$ mice indicated dramatic improvements.

A second approach for viral vector mediated TNSALP-D_{10} gene therapy used adeno-associated virus (AAV) injected IV into newborn pups. This recombinant AAV expressed TNSALP-D_{10} under the tissue-nonspecific CAG promoter, a hybrid of the actin promoter and CMV-IE enhancer (Matsumoto et al. 2011). Recombinant AAV-mediated delivery also promoted phenotypic correction of *Alpl*$^{-/-}$ mice as indicated by radically increased ALP, lack of seizures, extended lifespan, and improved bone mineralization. As perinatal and infantile forms of HPP are the most severe and the most critical cases can sometimes be detected in utero, AAV-expressed TNSALP-D_{10} administration was also tested in utero by transuterine IP injection in a pregnant dame carrying *Alpl*$^{-/-}$ mouse fetuses on embryonic day 15 (E15), about 3–4 days before birth (Sugano et al. 2012). In total, seven of nine treated *Alpl*$^{-/-}$ mice treated in utero showed normalized weight and growth rate and absence of seizures during the study period of 2 months. Fetal gene therapy effectively transduced tissues including bone, muscle, heart, and liver, stably increasing ALP levels to tenfold greater than WT control mice. Elevated ALP activity was visualized at bone surfaces and in chondrocytes in treated mice, and skeletal phenotype was largely corrected.

An additional study examined the potential to employ a muscle-directed AAV-based therapy by using a novel AAV8 construct that expressed

TNSALP-D_{10} under the muscle creatine kinase (MCK) promoter (Nakamura-Takahashi et al. 2016). Muscle provides a large, easily accessible, and vascular target for AAV transduction. As with the previous gene therapy experiments, mice were injected at early postnatal age, though here injections were made intra-muscular (IM) bilaterally into the quadriceps femoris muscles. Using a control vector that expressed enhanced green fluorescent protein (EGFP), investigators demonstrated that this approach promoted high expression in muscle and heart, but not in other organs. Muscle-driven production of TNSALP-D_{10} proved capable of increasing ALP levels in *Alpl*$^{-/-}$ mice by more than tenfold WT levels and extending the lifespans of nine out of ten treated mice to 90 dpn. As with previous gene therapy approaches, muscle expression of TNSALP-D_{10} was accompanied by normalization of mobility, increased body size, and improved mineralization. However, abnormal chondrocyte arrangement in treated mice was associated with altered cortical and trabecular bone parameters, hypomineralization, and reduced length of long bones, indicating incomplete rescue of the HPP phenotype in *Alpl*$^{-/-}$ mice. Of the dentoalveolar tissues of treated *Alpl*$^{-/-}$ mice, only the jawbones showed clear signs of improvement, whereas teeth exhibited thin and hypomineralized root dentin, and detachment at the root surface indicative of defective cementum (Ikeue et al. 2018).

While concerns about the safety of gene therapy remain, the frequency of administration (three or six times weekly, as described in more detail above), injection site reactions in many subjects, production of costly recombinant enzyme, and potentially lifetime requirement for AA, all support investigation into gene or cell therapies in human subjects. Concerns regarding gene therapy in human subjects include potential for germline gene transfer or immune response to viral vector or gene product. As an alternative gene therapy approach to address some of these issues, Shimada and colleagues attempted ex vivo LV expressed TNSALP-D_{10} transduction of bone marrow cells (BMC) (Iijima et al. 2015). BMC harvested from 8 to 12 week-old WT mice were transduced with TNSALP-D_{10} LV or EGFP expressing control LV. Neonatal *Alpl*$^{-/-}$ mouse pups were irradiated on day 2 after birth to ablate the recipient BMC component, and donor BMC were injected IV. Engraftment of donor BMC was measured at approximately 30% over the 90-day study. Untransduced and EGFP expressing control BMC were unable to extend the lifespans or provide phenotypic correction in *Alpl*$^{-/-}$ mice. In contrast, TNSALP-D_{10} transduced BMC prolonged survival, significantly improved but did not totally normalize growth curves, and boosted ALP activity tenfold higher than WT mice and 400-fold higher than untreated or mock treated *Alpl*$^{-/-}$ mice. By histology, ALP activity was found at bone surfaces of TNSALP-D_{10} treated *Alpl*$^{-/-}$ mice, supporting the concept that enzyme produced by engrafted BMC made its way to mineralizing sites as predicted. Numerous cortical and trabecular bone parameters were improved in treated mice, however growth plates and bone length were not completely rescued, as seen in previous gene therapy approaches described above. While dental tissues were examined in mice receiving transduced BMC, there was little evidence for improvement in dentin, cementum, or periodontal attachment and function (Okawa et al. 2017a). This is not surprising because even though intervention was early, dental precursors arise from cranial neural crest derived ectomesenchyme, which is an embryological tissue distinct from BMC precursors. Lack of improvement in dentoalveolar tissues reflected insufficient enzyme reaching these tissues during critical periods of their development and mineralization.

13.8.2 Soluble TNSALP Enzyme Replacement Therapy

All of the AAV and LV expressed enzyme replacement therapies summarized so far in this section focused on use of the mineral-targeted TNSALP-D_{10} construct. An alternative approach to the concept of targeted treatment is to attempt to achieve sustained high levels of soluble (non-targeted) TNSALP. As summarized above, a recombinant anchorless TNSALP extended the

lifespan of *Alpl*$^{-/-}$ mice, but was unable to correct skeletal and dental mineralization defects (Oikawa et al. 2014). AAV-mediated expression of soluble TNSALP showed far superior efficacy to injection of recombinant enzyme, with not only increased ALP levels and lifespan, but significant correction of bone mineralization by radiography (Matsumoto et al. 2011). Based on these successes with soluble forms of TNSALP, an additional strategy was attempted that employed a soluble intestinal-like chimeric alkaline phosphatase (ChimAP). ChimAP was engineered by substituting the crown domain of human intestinal phosphatase (IAP) with that of the placental isozyme (PLAP), making a chimeric form with IAP-like protein conformation, increased stability, Zn^{2+} binding in the enzyme active site, and narrowed substrate specificity (Kiffer-Moreira et al. 2014). Pharmacokinetic studies using bolus administration of 1, 8, or 16 mg/kg ChimAP indicated peak activity at 4 h and a half-life of 6 h, where residual ALP remained higher than untreated mice at 24 h after injection (Gasque et al. 2015). In a dose-response experimental design parallel to testing of TNSALP-D_{10} (Millan et al. 2008), ChimAP was administered 1, 8, or 16 mg/kg/day by daily SC injection. Median survival was increased to 44 dpn by 8 mg/kg/day, more than doubling lifespans of untreated or 1 mg/kg/day treated *Alpl*$^{-/-}$ mice, while mice treated with the highest dose survived to the termination of the experiment at 53 days. The highest ChimAP dose of 16 mg/kg/day normalized body weight and PP_i levels and significantly improved growth plate appearance, cortical bone parameters, and craniofacial shape. However, ChimAP was unable to completely normalize trabecular bone and all treated *Alpl*$^{-/-}$ mice featured increased osteoid accumulation compared to WT. While the high dose of ChimAP did seem to improve enamel and dentin in *Alpl*$^{-/-}$ mice, measurable dentin defects remained, substantial osteoid was observed on alveolar bone, and lack of cementum and periodontal breakdown was evident by histology, indicating a less robust response of dentoalveolar tissues. As of this writing, ChimAP (also known as recAP) has completed Phase I clinical trials and is being tested in Phase II trials (ClinicalTrials.gov identifier NCT02182440) in the U.S. and Europe sponsored by AM-Pharma (Bunnik, the Netherlands) for acute kidney injury (AKI), whereas ulcerative colitis (UC) and HPP studies remain in the preclinical stage. An oral formulation is in development for UC treatment. The rationale for application in AKI and UC lies in the potential for ALP to dephosphorylate lipopolysaccharide (LPS) that contributes to sepsis-associated AKI, and convert adenosine triphosphate (ATP) providing cell-protective and anti-inflammatory effects (Peters et al. 2013, 2014a, b, 2016a, b; Pickkers et al. 2012).

13.9 Application of Asfotase Alfa for Other Conditions

To date, AA has been studied almost entirely within the context of treating HPP. However, other metabolic conditions also feature altered or reduced ALP levels. Neurofibromatosis type 1 (NF1; OMIM# 162200) is caused by mutations in *NF1*, a cytoplasmic protein involved in a number of cell signaling cascades. NF1 clinical features include skin, ophthalmologic, and skeletal manifestations, with the latter reportedly including bone abnormalities, altered ALP and vitamin D levels, and osteopenia or osteoporosis in some affected individuals (Armstrong et al. 2013; Duman et al. 2008; Elefteriou et al. 2009; Lodish et al. 2012; Poyrazoglu et al. 2017; Rodari et al. 2018; Schnabel et al. 2013). A conditional knockout mouse model of NF1 featuring increased PP_i and reduced serum ALP exhibited skeletal hypomineralization (de la Croix Ndong et al. 2014; Wang et al. 2011). Treatment with TNSALP-D_{10} successfully increased bone growth and mineral density in these mice, providing preclinical data that use of ERT may improve skeletal effects of NF1 in affected individuals (de la Croix Ndong et al. 2014).

As TNSALP is perhaps the most critical enzyme for skeletal and dental mineralization, there is potential for AA to be applied to other hereditary mineral metabolism disorders, or even possibly used to promote more rapid fracture

healing in healthy individuals, however no preclinical studies have yet been performed to support this.

13.10 Summary and Conclusions

AA ERT has been transformative as the first treatment for HPP, the last form of rickets to receive a medical therapy (Whyte 2017). Based on the clinical trials conducted to date, AA is approved for use in individuals of any age with pediatric-onset HPP. Clinical trials and case reports evaluated to date have targeted individuals with a high disease burden caused by perinatal, infantile, childhood, or adult HPP, specifically those with significant skeletal manifestations of HPP. Improved skeletal mineralization in response to AA ERT in turn leads to improved respiratory status and increased survival in severely affected infants, revolutionizing the outcome of a once fatal form of the disease. Improvement of skeletal manifestations by AA ERT also alleviates additional complications stemming from bone abnormalities, improving growth, mobility, physical function, and quality of life. Currently, evidence-based therapeutic recommendations for children and adults with less symptomatic HPP are lacking and unclear. A long-term follow-up care with at least annual assessment for progression of disease is needed to monitor for late manifestations of HPP (Mori et al. 2016). Judicious use of this high-cost ERT is imperative; risk-benefit ratio, feasibility and safety of treatment need to be considered. In childhood HPP, five key manifestations including mobility, pain, rickets, growth, and fracture, have been proposed as guidance for decision to treat (Rush 2018). Conservative management should be the first line of management: physical therapy for patients with hypotonia, mobility limitations, or gait abnormalities; analgesics (acetaminophen or nonsteroidal anti-inflammatory drugs) for those with musculoskeletal pain. If symptoms improve with the conservative treatment, then ERT is not indicated. AA treatment should be considered in patients with childhood HPP who have limited mobility that impair quality of life or debilitating pain unresponsive to conservative treatment. In those patients with symptomatic and disabling HPP, with or without fractures, for whom AA is considered, clinicians should establish treatment goals in order to monitor the patient's response and determine what clinical outcomes are to be achieved (Kishnani et al. 2017b). Given the wide clinical variability in disease manifestations of adult HPP, recently Shapiro and Lewiecki (2017) have suggested that AA treatment should be considered in adult HPP if one or more of the following is present and attributable to HPP: musculoskeletal pain requiring prescription pain medications, disabling polyarthropathy, disabling functional impairment assessed by validated measures, low-trauma fracture, delayed or incomplete fracture healing, repeated orthopedic surgeries for HPP bone disease, low bone mineral density T-score ≤ -2.5 in postmenopausal women and men age 50 years and older, or Z-score ≤ -2.0 in younger adult women and men in patients with fractures, and nephrocalcinosis.

Ultimately, it remains to be seen how AA will be employed in the long-term management of severely and mildly affected HPP patients, whether dose titration is efficacious for different disease severity, or any additional beneficial outcomes or adverse effects are discovered, and whether alternative approaches for delivery or achieving ERT are successful and transform HPP therapy yet again.

Acknowledgments We thank the subjects and their families for their cooperation and assistance with this research. This research was supported by research grants from Soft Bones, Inc. to BLF and grant AR 066110 to BLF from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). We thank Dr. Jose Luis Millán for providing images for reproduction. We thank Mr. Michael B. Chavez for adaptation of the TNSALP 2D model in Fig. 13.2m. For more information on hypophosphatasia for affected individuals, researchers, or health care professionals, see the Soft Bones, Inc. website at https://www.softbones.org/.

Conflict of Interest BLF has served as a consultant and speaker for Alexion Pharmaceuticals, Inc., and received two research grants from Soft Bones, Inc., a nonprofit patient advocacy, sup-

port, and education group for families with hypophosphatsia. The authors report no other conflicts of interest in this work.

References

Akiyama T, Kubota T, Ozono K, Michigami T, Kobayashi D, Takeyari S, Sugiyama Y, Noda M, Harada D, Namba N et al (2018) Pyridoxal 5′-phosphate and related metabolites in hypophosphatasia: effects of enzyme replacement therapy. Mol Genet Metab 125(1–2):174–180

Albeggiani A, Cataldo F (1982) Infantile hypophosphatasia diagnosed at 4 months and surviving at 2 years. Helv Paediatr Acta 37(1):49–58

Anderson HC, Sipe JB, Hessle L, Dhanyamraju R, Atti E, Camacho NP, Millan JL, Dhamyamraju R (2004) Impaired calcification around matrix vesicles of growth plate and bone in alkaline phosphatase-deficient mice. Am J Pathol 164(3):841–847

Anderson HC, Harmey D, Camacho NP, Garimella R, Sipe JB, Tague S, Bi X, Johnson K, Terkeltaub R, Millan JL (2005) Sustained osteomalacia of long bones despite major improvement in other hypophosphatasia-related mineral deficits in tissue nonspecific alkaline phosphatase/nucleotide pyrophosphatase phosphodiesterase 1 double-deficient mice. Am J Pathol 166(6):1711–1720

Armstrong L, Jett K, Birch P, Kendler DL, McKay H, Tsang E, Stevenson DA, Hanley DA, Egeli D, Burrows M et al (2013) The generalized bone phenotype in children with neurofibromatosis 1: a sibling matched case-control study. Am J Med Genet A 161A(7):1654–1661

Arun R, Khazim R, Webb JK, Burn J (2005) Scoliosis in association with infantile hypophosphatasia: a case study in two siblings. Spine 30(16):E471–E476

Baumgartner-Sigl S, Haberlandt E, Mumm S, Scholl-Burgi S, Sergi C, Ryan L, Ericson KL, Whyte MP, Hogler W (2007) Pyridoxine-responsive seizures as the first symptom of infantile hypophosphatasia caused by two novel missense mutations (c.677t>c, p.M226t; c.1112c>t, p.T371i) of the tissue-nonspecific alkaline phosphatase gene. Bone 40(6):1655–1661

Beck C, Morbach H, Richl P, Stenzel M, Girschick H (2009) How can calcium pyrophosphate crystals induce inflammation in hypophosphatasia or chronic inflammatory joint diseases? Rheumatol Int 29(3):229–238

Beertsen W, VandenBos T, Everts V (1999) Root development in mice lacking functional tissue non-specific alkaline phosphatase gene: inhibition of acellular cementum formation. J Dent Res 78(6):1221–1229

Berkseth KE, Tebben PJ, Drake MT, Hefferan TE, Jewison DE, Wermers RA (2013) Clinical spectrum of hypophosphatasia diagnosed in adults. Bone 54(1):21–27

Biosse Duplan M, Coyac BR, Bardet C, Zadikian C, Rothenbuhler A, Kamenicky P, Briot K, Linglart A, Chaussain C (2017) Phosphate and vitamin d prevent periodontitis in x-linked hypophosphatemia. J Dent Res 96(4):388–395

Bisaz S, Russell RG, Fleisch H (1968) Isolation of inorganic pyrophosphate from bovine and human teeth. Arch Oral Biol 13(6):683–696

Bloch-Zupan A (2016) Hypophosphatasia: diagnosis and clinical signs – a dental surgeon perspective. Int J Paediatr Dent 26(6):426–438

Boileau G, Tenenhouse HS, Desgroseillers L, Crine P (2001) Characterization of phex endopeptidase catalytic activity: identification of parathyroid-hormone-related peptide107–139 as a substrate and osteocalcin, ppi and phosphate as inhibitors. Biochem J 355(Pt 3):707–713

Bowden SA, Adler BH (2018a) Asfotase alfa treatment for 1 year in a 16 year-old male with severe childhood hypophosphatasia. Osteoporos Int 29(2):511–515

Bowden SA, Adler BH (2018b) Reappearance of hypomineralized bone after discontinuation of asfotase alfa treatment for severe childhood hypophosphatasia. Osteoporos Int 29(9):2155–2156

Bruckner R, Rickles N, Porter D (1962) Hypophosphatasia with premature shedding of teeth and aplasia of cementum. Oral Surg Oral Med Oral Pathol 15:1351–1369

Cahill RA, Wenkert D, Perlman SA, Steele A, Coburn SP, McAlister WH, Mumm S, Whyte MP (2007) Infantile hypophosphatasia: transplantation therapy trial using bone fragments and cultured osteoblasts. J Clin Endocrinol Metab 92(8):2923–2930

Campos M, Couture C, Hirata IY, Juliano MA, Loisel TP, Crine P, Juliano L, Boileau G, Carmona AK (2003) Human recombinant endopeptidase phex has a strict s1' specificity for acidic residues and cleaves peptides derived from fibroblast growth factor-23 and matrix extracellular phosphoglycoprotein. Biochem J 373(Pt 1):271–279

Castells L, Cassanello P, Muñiz F, de Castro M-J, Couce ML (2018) Neonatal lethal hypophosphatasia: a case report and review of literature. Medicine 97(48):e13269

Clubb JS, Neale FC, Posen S (1965) The behavior of infused human placental alkaline phosphatase in human subjects. J Lab Clin Med 66(3):493–507

Colazo JM, Hu JR, Dahir KM, Simmons JH (2018) Correction to: neurological symptoms in hypophosphatasia. Osteoporos Int: J Established Result Cooperation Between Eur Found Osteoporos Natl Osteoporos Found USA 30:535

Collins MT (2006) Spectrum and natural history of fibrous dysplasia of bone. J Bone Miner Res 21(S2):P99–P104

Collmann H, Mornet E, Gattenlohner S, Beck C, Girschick H (2009) Neurosurgical aspects of childhood hypophosphatasia. Childs Nerv Syst 25(2):217–223

Costain G, Moore AM, Munroe L, Williams A, Shaul RZ, Rockman-Greenberg C, Offringa M, Kannu P (2018) Enzyme replacement therapy in perinatal hypophosphatasia: case report of a negative outcome and lessons for clinical practice. Mol Genet Metab Rep 14:22–26

Cruz T, Gleizes M, Balayssac S, Mornet E, Marsal G, Millan JL, Malet-Martino M, Nowak LG, Gilard V, Fonta C (2017) Identification of altered brain metabolites associated with tnap activity in a mouse model of hypophosphatasia using untargeted nmr-based metabolomics analysis. J Neurochem 140(6):919–940

Cundy T, Michigami T, Tachikawa K, Dray M, Collins JF, Paschalis EP, Gamsjaeger S, Roschger A, Fratzl-Zelman N, Roschger P et al (2015) Reversible deterioration in hypophosphatasia caused by renal failure with bisphosphonate treatment. J Bone Miner Res 30(9):1726–1737

Davit-Beal T, Gabay J, Antoniolli P, Masle-Farquhar J, Wolikow M (2014) Dental complications of rickets in early childhood: case report on 2 young girls. Pediatrics 133(4):e1077–e1081

de la Croix Ndong J, Makowski AJ, Uppuganti S, Vignaux G, Ono K, Perrien DS, Joubert S, Baglio SR, Granchi D, Stevenson DA et al (2014) Asfotase-alpha improves bone growth, mineralization and strength in mouse models of neurofibromatosis type-1. Nat Med 20(8):904–910

Deeb AA, Bruce SN, Morris AA, Cheetham TD (2000) Infantile hypophosphatasia: disappointing results of treatment. Acta Paediatr (Oslo, Norway: 1992) 89(6):730–733

Doetschman T, Gregg RG, Maeda N, Hooper ML, Melton DW, Thompson S, Smithies O (1987) Targetted correction of a mutant hprt gene in mouse embryonic stem cells. Nature 330(6148):576–578

Doshi KB, Hamrahian AH, Licata AA (2009) Teriparatide treatment in adult hypophosphatasia in a patient exposed to bisphosphonate: a case report. Clin Cases Miner Bone Metab 6(3):266–269

Duman O, Ozdem S, Turkkahraman D, Olgac ND, Gungor F, Haspolat S (2008) Bone metabolism markers and bone mineral density in children with neurofibromatosis type-1. Brain Dev 30(9):584–588

Durussel J, Liu J, Campbell C, Nam HK, Hatch NE (2016) Bone mineralization-dependent craniosynostosis and craniofacial shape abnormalities in the mouse model of infantile hypophosphatasia. Dev Dyn 245(2):175–182

Elefteriou F, Kolanczyk M, Schindeler A, Viskochil DH, Hock JM, Schorry EK, Crawford AH, Friedman JM, Little D, Peltonen J et al (2009) Skeletal abnormalities in neurofibromatosis type 1: approaches to therapeutic options. Am J Med Genet A 149A(10):2327–2338

El-Gharbawy AH, Peeden JN Jr, Lachman RS, Graham JM Jr, Moore SR, Rimoin DL (2010) Severe cleidocranial dysplasia and hypophosphatasia in a child with microdeletion of the c-terminal region of runx2. Am J Med Genet A 152a(1):169–174

Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292(5819):154–156

Fedde KN, Blair L, Silverstein J, Coburn SP, Ryan LM, Weinstein RS, Waymire K, Narisawa S, Millan JL, MacGregor GR et al (1999) Alkaline phosphatase knock-out mice recapitulate the metabolic and skeletal defects of infantile hypophosphatasia. J Bone Miner Res 14(12):2015–2026

Feeney C, Stanford N, Lee S, Barry S (2018) Hypophosphatasia and the importance of the general dental practitioner – a case series and discussion of upcoming treatments. Br Dent J 224(12):937–943

Fisher LW, Fedarko NS (2003) Six genes expressed in bones and teeth encode the current members of the sibling family of proteins. Connect Tissue Res 44(Suppl 1):33–40

Fleisch H, Bisaz S (1962a) Isolation from urine of pyrophosphate, a calcification inhibitor. Am J Physiol 203:671–675

Fleisch H, Bisaz S (1962b) Mechanism of calcification: inhibitory role of pyrophosphate. Nature 195:911

Fleisch H, Schibler D, Maerki J, Frossard I (1965) Inhibition of aortic calcification by means of pyrophosphate and polyphosphates. Nature 207(5003):1300–1301

Fleisch H, Russell RG, Straumann F (1966) Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature 212(5065):901–903

Foster BL, Hujoel PP (2018) Vitamin d in dentoalveolar and oral health. In: Feldman D, Pike JW, Bouillon R (eds) Vitamin d, 4th edn. Academic, London, pp 497–520

Foster BL, Nagatomo KJ, Nociti FH Jr, Fong H, Dunn D, Tran AB, Wang W, Narisawa S, Millan JL, Somerman MJ (2012) Central role of pyrophosphate in acellular cementum formation. PLoS One 7(6):e38393

Foster BL, Nagatomo KJ, Tso HW, Tran AB, Nociti FH Jr, Narisawa S, Yadav MC, McKee MD, Millan JI, Somerman MJ (2013) Tooth root dentin mineralization defects in a mouse model of hypophosphatasia. J Bone Miner Res 28(2):271–282

Foster BL, Sheen CR, Hatch NE, Liu J, Cory E, Narisawa S, Kiffer-Moreira T, Sah RL, Whyte MP, Somerman MJ et al (2015) Periodontal defects in the a116t knock-in murine model of odontohypophosphatasia. J Dent Res 94(5):706–714

Foster BL, Kuss P, Yadav MC, Kolli TN, Narisawa S, Lukashova L, Cory E, Sah RL, Somerman MJ, Millan JL (2017) Conditional alpl ablation phenocopies dental defects of hypophosphatasia. J Dent Res 96(1):81–91

Freitas TQ, Franco AS, Pereira RMR (2018) Improvement of bone microarchitecture parameters after 12 months of treatment with asfotase alfa in adult patient with hypophosphatasia: case report. Medicine 97(48):e13210

Gasque KC, Foster BL, Kuss P, Yadav MC, Liu J, Kiffer-Moreira T, van Elsas A, Hatch N, Somerman MJ, Millan JL (2015) Improvement of the skeletal and dental hypophosphatasia phenotype in alpl−/− mice by administration of soluble (non-targeted) chimeric alkaline phosphatase. Bone 72:137–147

Genest F, Seefried L (2018) Subtrochanteric and diaphyseal femoral fractures in hypophosphatasia—not atypical at all. Osteoporos Int 1–11

Girschick H, Schneider P, Haubitz I, Hiort O, Collmann H, Beer M, Shin Y, Seyberth H (2006) Effective nsaid

treatment indicates that hyperprostaglandinism is affecting the clinical severity of childhood hypophosphatasia. Orphanet J Rare Dis 1(1):24

Girschick HJ, Mornet E, Beer M, Warmuth-Metz M, Schneider P (2007) Chronic multifocal non-bacterial osteomyelitis in hypophosphatasia mimicking malignancy. BMC Pediatr 7(1):3

Goodwin HW, Robison R (1924) The possible significance of hexosephosphoric esters in ossification. Part iv. The phosphoric esters of the blood: preliminary communication. Biochem J 18(5):1161–1162

Greenberg CR, Taylor CL, Haworth JC, Seargeant LE, Philipps S, Triggs-Raine B, Chodirker BN (1993) A homoallelic gly317 – >asp mutation in alpl causes the perinatal (lethal) form of hypophosphatasia in Canadian mennonites. Genomics 17(1):215–217

Gurley K, Chen H, Guenther C, Nguyen E, Rountree R, Schoor M, Kingsley D (2006) Mineral formation in joints caused by complete or joint-specific loss of ank function. J Bone Miner Res 21(8):1238–1247

Gutman AB, Gutman EB (1938) An "acid" phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. J Clin Invest 17(4):473–478

Hacihamdioglu B, Ozgurhan G, Pereira C, Tepeli E, Acar G, Comert S (2018) Perinatal form hypophosphatasia caused by a novel large duplication of alpl gene and one year follow-up under enzyme replacement therapy; a case report. J Clin Res Pediatr Endocrinol 10:175–178

Hall B, Limaye A, Kulkarni AB (2009) Overview: generation of gene knockout mice. Curr Protoct Cell Biol. Chapter 19:Unit 19 12 19 12 11–17

Harmey D, Hessle L, Narisawa S, Johnson KA, Terkeltaub R, Millan JL (2004) Concerted regulation of inorganic pyrophosphate and osteopontin by akp2, enpp1, and ank: an integrated model of the pathogenesis of mineralization disorders. Am J Pathol 164(4):1199–1209

Harmey D, Johnson KA, Zelken J, Camacho NP, Hoylaerts MF, Noda M, Terkeltaub R, Millan JL (2006) Elevated skeletal osteopontin levels contribute to the hypophosphatasia phenotype in akp2(−/−) mice. J Bone Miner Res 21(9):1377–1386

Hessle L, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R, Millan JL (2002) Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. Proc Natl Acad Sci U S A 99(14):9445–9449

Ho A, Johnson M, Kingsley D (2000) Role of the mouse ank gene in control of tissue calcification and arthritis. Science 289(5477):265–270

Hu JC, Plaetke R, Mornet E, Zhang C, Sun X, Thomas HF, Simmer JP (2000) Characterization of a family with dominant hypophosphatasia. Eur J Oral Sci 108(3):189–194

Iijima O, Miyake K, Watanabe A, Miyake N, Igarashi T, Kanokoda C, Nakamura-Takahashi A, Kinoshita H, Noguchi T, Abe S et al (2015) Prevention of lethal murine hypophosphatasia by neonatal ex vivo gene therapy using lentivirally transduced bone marrow cells. Hum Gene Ther 26(12):801–812

Ikeue R, Nakamura-Takahashi A, Nitahara-Kasahara Y, Watanabe A, Muramatsu T, Sato T, Okada T (2018) Bone-targeted alkaline phosphatase treatment of mandibular bone and teeth in lethal hypophosphatasia via an scaav8 vector. Mol Ther Methods Clin Dev 10:361–370

Johnson KA, Hessle L, Vaingankar S, Wennberg C, Mauro S, Narisawa S, Goding JW, Sano K, Millan JL, Terkeltaub R (2000) Osteoblast tissue-nonspecific alkaline phosphatase antagonizes and regulates pc-1. Am J Physiol Regul Integr Comp Physiol 279(4):R1365–R1377

Jung A, Russel RG, Bisaz S, Morgan DB, Fleisch H (1970) Fate of intravenously injected pyrophosphate-32p in dogs. Am J Physiol 218(6):1757–1764

Kay HD, Robison R (1924) The possible significance of hexosephosphoric esters in ossification. Part iii: the action of the bone enzyme on the organic phosphorus compounds in blood. Biochem J 18(3–4):755–764

Kiffer-Moreira T, Sheen CR, Gasque KC, Bolean M, Ciancaglini P, van Elsas A, Hoylaerts MF, Millan JL (2014) Catalytic signature of a heat-stable, chimeric human alkaline phosphatase with therapeutic potential. PLoS One 9(2):e89374

Kishnani PS, Madson KL, Whyte MP, Gayron M, Fujita K, Rockman-Greenberg C (2016) Biochemical and physical function outcomes in adolescents and adults with hypophosphatasia treated with asfotase alfa for up to 4 years: interim results from a phase ii study. Metabolic and genetic bone disorders. Endocrine Society, pp OR26-23-OR26-23

Kishnani PS, Rockman-Greenberg C, Denker AE, Moseley S, Whyte MP (2017a) Biochemical and physical function outcomes after 5 years of treatment with asfotase alfa in adolescents and adults with hypophosphatasia: phase 2 study results. Paper presented at: International conference on Children's Bone Health (ICCBH). Wurzberg

Kishnani PS, Rush ET, Arundel P, Bishop N, Dahir K, Fraser W, Harmatz P, Linglart A, Munns CF, Nunes ME (2017b) Monitoring guidance for patients with hypophosphatasia treated with asfotase alfa. Mol Genet Metab 122(1):4–17

Kitaoka T, Tajima T, Nagasaki K, Kikuchi T, Yamamoto K, Michigami T, Okada S, Fujiwara I, Kokaji M, Mochizuki H et al (2017) Safety and efficacy of treatment with asfotase alfa in patients with hypophosphatasia: results from a japanese clinical trial. Clin Endocrinol 87(1):10–19

Klidaras P, Severt J, Aggers D, Payne J, Miller PD, Ing SW (2018) Fracture healing in two adult patients with hypophosphatasia after asfotase alfa therapy. JBMR Plus 2(5):304–307

Lawrence JE, Saeed D, Bartlett J, Carrothers AD (2017) Adult-onset hypophosphatasia diagnosed following

bilateral atypical femoral fractures in a 55-year-old woman. Clin Cases Miner Bone Metab 14(3):347–353
Libby Kosnik-Infinger M, Gendron C, Gordon CB (2015) Enzyme replacement therapy for congenital hypophosphatasia allows for surgical treatment of related complex craniosynostosis: a case series. Neurosurg Focus 38(5):E10
Liu J, Nam HK, Campbell C, Gasque KC, Millan JL, Hatch NE (2014) Tissue-nonspecific alkaline phosphatase deficiency causes abnormal craniofacial bone development in the alpl(−/−) mouse model of infantile hypophosphatasia. Bone 67:81–94
Liu J, Campbell C, Nam HK, Caron A, Yadav MC, Millan JL, Hatch NE (2015) Enzyme replacement for craniofacial skeletal defects and craniosynostosis in murine hypophosphatasia. Bone 78:203–211
Lodish MB, Dagalakis U, Sinaii N, Bornstein E, Kim A, Lokie KB, Baldwin AM, Reynolds JC, Dombi E, Stratakis CA et al (2012) Bone mineral density in children and young adults with neurofibromatosis type 1. Endocr Relat Cancer 19(6):817–825
Lopez-Delgado L, Riancho-Zarrabeitia L, Garcia-Unzueta MT, Tenorio JA, Garcia-Hoyos M, Lapunzina P, Valero C, Riancho JA (2018) Abnormal bone turnover in individuals with low serum alkaline phosphatase. Osteoporos Int: J Established Result Cooperation Between Eur Found Osteoporos Natl Osteoporos Found USA 29(9):2147–2150
Mak TW (2007) Gene targeting in embryonic stem cells scores a knockout in Stockholm. Cell 131(6):1027–1031
Martland M, Robison R (1924) The possible significance of hexosephosphoric esters in ossification: part v. the enzyme in the early stages of bone development. Biochem J 18(6):1354–1357
Martland M, Robison R (1926) Possible significance of hexosephosphoric esters in ossification: part vi. Phosphoric esters in blood-plasma. Biochem J 20(4):847–855
Martland M, Robison R (1927) The possible significance of hexosephosphoric esters in ossification: part vii. The bone phosphatase. Biochem J 21(3):665–674
Martland M, Robison R (1929) The preparation and use of the bone phosphatase. Biochem J 23(2):237–242
Matsumoto T, Miyake K, Yamamoto S, Orimo H, Miyake N, Odagaki Y, Adachi K, Iijima O, Narisawa S, Millan JL et al (2011) Rescue of severe infantile hypophosphatasia mice by aav-mediated sustained expression of soluble alkaline phosphatase. Hum Gene Ther 22(11):1355–1364
McKee MD, Nakano Y, Masica DL, Gray JJ, Lemire I, Heft R, Whyte MP, Crine P, Millan JL (2011) Enzyme replacement therapy prevents dental defects in a model of hypophosphatasia. J Dent Res 90(4):470–476
Meyer JL (1984) Can biological calcification occur in the presence of pyrophosphate? Arch Biochem Biophys 231(1):1–8
Meyer JL, Fleisch H (1984) Calcification inhibitors in rat and human serum and plasma. Biochim Biophys Acta 799(2):115–121
Millán JL (2006) Mammalian alkaline phosphatases: from biology to applications in medicine and biotechnology. Wiley-VCH, Weinheim
Millan JL (2013) The role of phosphatases in the initiation of skeletal mineralization. Calcif Tissue Int 93(4):299–306
Millan JL, Whyte MP (2016) Alkaline phosphatase and hypophosphatasia. Calcif Tissue Int 98(4):398–416
Millan JL, Narisawa S, Lemire I, Loisel TP, Boileau G, Leonard P, Gramatikova S, Terkeltaub R, Camacho NP, McKee MD et al (2008) Enzyme replacement therapy for murine hypophosphatasia. J Bone Miner Res 23(6):777–787
Mori M, DeArmey SL, Weber TJ, Kishnani PS (2016) Case series: odontohypophosphatasia or missed diagnosis of childhood/adult-onset hypophosphatasia?–call for a long-term follow-up of premature loss of primary teeth. Bone Rep 5:228–232
Mornet E (2017) Genetics of hypophosphatasia. Arch Pediatr 24(5S2):5S51–55S56
Mornet E, Yvard A, Taillandier A, Fauvert D, Simon-Bouy B (2011) A molecular-based estimation of the prevalence of hypophosphatasia in the european population. Ann Hum Genet 75(3):439–445
Murshed M, Harmey D, Millan JL, McKee MD, Karsenty G (2005) Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ecm mineralization to bone. Genes Dev 19(9):1093–1104
Nakamura-Takahashi A, Miyake K, Watanabe A, Hirai Y, Iijima O, Miyake N, Adachi K, Nitahara-Kasahara Y, Kinoshita H, Noguchi T et al (2016) Treatment of hypophosphatasia by muscle-directed expression of bone-targeted alkaline phosphatase via self-complementary aav8 vector. Mol Ther Methods Clin Dev 3:15059
Nam HK, Sharma M, Liu J, Hatch NE (2017) Tissue nonspecific alkaline phosphatase (tnap) regulates cranial base growth and synchondrosis maturation. Front Physiol 8:161
Narisawa S, Frohlander N, Millan JL (1997) Inactivation of two mouse alkaline phosphatase genes and establishment of a model of infantile hypophosphatasia. Dev Dyn 208(3):432–446
Narisawa S, Wennberg C, Millan JL (2001) Abnormal vitamin b6 metabolism in alkaline phosphatase knock-out mice causes multiple abnormalities, but not the impaired bone mineralization. J Pathol 193(1):125–133
Narisawa S, Huang L, Iwasaki A, Hasegawa H, Alpers D, Millán J (2003) Accelerated fat absorption in intestinal alkaline phosphatase knockout mice. Mol Cell Biol 23(21):7525–7530
Nishioka T, Tomatsu S, Gutierrez MA, Miyamoto K, Trandafirescu GG, Lopez PL, Grubb JH, Kanai R, Kobayashi H, Yamaguchi S et al (2006) Enhancement of drug delivery to bone: characterization of human tissue-nonspecific alkaline phosphatase tagged with an acidic oligopeptide. Mol Genet Metab 88(3): 244–255

Nociti FH Jr, Berry JE, Foster BL, Gurley KA, Kingsley DM, Takata T, Miyauchi M, Somerman MJ (2002) Cementum: a phosphate-sensitive tissue. J Dent Res 81(12):817–821

Offiah AC, Vockley J, Munns CF, Murotsuki J. 2018. Differential diagnosis of perinatal hypophosphatasia: radiologic perspectives. Pediatr Radiol

Oikawa H, Tomatsu S, Haupt B, Montano AM, Shimada T, Sly WS (2014) Enzyme replacement therapy on hypophosphatasia mouse model. J Inherit Metab Dis 37(2):309–317

Okawa R, Iijima O, Kishino M, Okawa H, Toyosawa S, Sugano-Tajima H, Shimada T, Okada T, Ozono K, Ooshima T et al (2017a) Gene therapy improves dental manifestations in hypophosphatasia model mice. J Periodontal Res 52(3):471–478

Okawa R, Kokomoto K, Yamamura-Miyazaki N, Michigami T, Nakano K (2017b) Oral findings in patient with lethal hypophosphatasia treated with enzyme replacement therapy. Pediatr Dent J 27(3):153–156

Okazaki Y, Kitajima H, Mochizuki N, Kitaoka T, Michigami T, Ozono K (2016) Lethal hypophosphatasia successfully treated with enzyme replacement from day 1 after birth. Eur J Pediatr 175(3):433–437

Oyachi M, Harada D, Sakamoto N, Ueyama K, Kondo K, Kishimoto K, Izui M, Nagamatsu Y, Kashiwagi H, Yamamuro M et al (2018) A case of perinatal hypophosphatasia with a novel mutation in the alpl gene: clinical course and review of the literature. Clin Pediatr Endocrinol 27(3):179–186

Peters E, van Elsas A, Heemskerk S, Jonk L, van der Hoeven J, Arend J, Masereeuw R, Pickkers P (2013) Alkaline phosphatase as a treatment of sepsis-associated acute kidney injury. J Pharmacol Exp Ther 344(1):2–7

Peters E, Heemskerk S, Masereeuw R, Pickkers P (2014a) Alkaline phosphatase: a possible treatment for sepsis-associated acute kidney injury in critically ill patients. Am J Kidney Dis 63(6):1038–1048

Peters E, Masereeuw R, Pickkers P (2014b) The potential of alkaline phosphatase as a treatment for sepsis-associated acute kidney injury. Nephron Clin Pract 127(1–4):144–148

Peters E, Ergin B, Kandil A, Gurel-Gurevin E, van Elsas A, Masereeuw R, Pickkers P, Ince C (2016a) Effects of a human recombinant alkaline phosphatase on renal hemodynamics, oxygenation and inflammation in two models of acute kidney injury. Toxicol Appl Pharmacol 313:88–96

Peters E, Mehta RL, Murray PT, Hummel J, Joannidis M, Kellum JA, Arend J, Pickkers P (2016b) Study protocol for a multicentre randomised controlled trial: safety, tolerability, efficacy and quality of life of a human recombinant alkaline phosphatase in patients with sepsis-associated acute kidney injury (stop-aki). BMJ Open 6(9):e012371

Phillips D, Case LE, Griffin D, Hamilton K, Lara SL, Leiro B, Monfreda J, Westlake E, Kishnani PS (2016) Physical therapy management of infants and children with hypophosphatasia. Mol Genet Metab 119(1):14–19

Phillips D, Griffin D, Przybylski T, Morrison E, Reeves AL, Vallee M, Fujita KP, Madson KL (2018) Development and validation of a modified performance-oriented mobility assessment tool for assessing mobility in children with hypophosphatasia. J Pediatr Rehabil Med 11(3):187–192

Pickkers P, Heemskerk S, Schouten J, Laterre PF, Vincent JL, Beishuizen A, Jorens PG, Spapen H, Bulitta M, Peters WH et al (2012) Alkaline phosphatase for treatment of sepsis-induced acute kidney injury: a prospective randomized double-blind placebo-controlled trial. Crit Care 16(1):R14

Poryo M, Meyer S, Eymann R, Yilmaz U, Nemat S, Rohrer T (2016) Clinical images: a cloudy skull-hypophosphatasia as reason for copper-beaten skull. Neuropediatrics 47(6):410

Poyrazoglu HG, Bas VN, Arslan A, Bastug F, Canpolat M, Per H, Gumus H, Kumandas S (2017) Bone mineral density and bone metabolic markers' status in children with neurofibromatosis type 1. J Pediatr Endocrinol Metab: JPEM 30(2):175–180

Reibel A, Maniere MC, Clauss F, Droz D, Alembik Y, Mornet E, Bloch-Zupan A (2009) Orodental phenotype and genotype findings in all subtypes of hypophosphatasia. Orphanet J Rare Dis 4:6

Remde H, Cooper MS, Quinkler M (2017) Successful asfotase alfa treatment in an adult dialysis patient with childhood-onset hypophosphatasia. J Endocr Soc 1(9):1188–1193

Righetti M, Wach J, Desmarchelier R, Coury F (2018) Teriparatide treatment in an adult patient with hypophosphatasia exposed to bisphosphonate and revealed by bilateral atypical fractures. Joint Bone Spine 85(3):365–367

Robison R (1923) The possible significance of hexosephosphoric esters in ossification. Biochem J 17(2):286–293

Robison R, Soames KM (1924) The possible significance of hexosephosphoric esters in ossification: part ii. The phosphoric esterase of ossifying cartilage. Biochem J 18(3–4):740–754

Robison R, Soames KM (1925) A chemical study of defective ossification in rachitic animals. Biochem J 19(1):153–161

Robison R, Macleod M, Rosenheim AH (1930) The possible significance of hexosephosphoric esters in ossification: calcification in vitro. Biochem J 24(6):1927–1941

Rodari G, Scuvera G, Ulivieri FM, Profka E, Menni F, Saletti V, Esposito S, Bergamaschi S, Ferrante E, Eller-Vainicher C et al (2018) Progressive bone impairment with age and pubertal development in neurofibromatosis type i. Arch Osteoporos 13(1):93

Rodrigues TL, Nagatomo KJ, Foster BL, Nociti FH, Somerman MJ (2011) Modulation of phosphate/pyrophosphate metabolism to regenerate the periodontium: a novel in vivo approach. J Periodontol 82(12):1757–1766

Rodrigues TL, Georgetti AP, Martins L, Pereira Neto JS, Foster BL, Nociti FH Jr (2012, June) Clinical correlate: Cementum and periodontal defects resulting from odontohypophosphatasia predispose for premature tooth loss. In: Somerman MJ, LK MC (eds) Mineralized tissues in Oral and craniofacial science: biological principles and clinical correlates

Rougier H, Desrumaux A, Bouchon N, Wroblewski I, Pin I, Nugues F, Mornet E, Baujat G (2018) Enzyme-replacement therapy in perinatal hypophosphatasia: case report and review of the literature. Arch Pediatr 25(7):442–447

Royce PM, Blumberg A, Zurbrugg RP, Zimmermann A, Colombo JP, Steinmann B (1988) Lethal osteogenesis imperfecta: abnormal collagen metabolism and biochemical characteristics of hypophosphatasia. Eur J Pediatr 147(6):626–631

Rush ET (2018) Childhood hypophosphatasia: to treat or not to treat. Orphanet J Rare Dis 13(1):116

Rutsch F, Vaingankar S, Johnson K, Goldfine I, Maddux B, Schauerte P, Kalhoff H, Sano K, Boisvert W, Superti-Furga A et al (2001) Pc-1 nucleoside triphosphate pyrophosphohydrolase deficiency in idiopathic infantile arterial calcification. Am J Pathol 158(2):543–554

Rutsch F, Ruf N, Vaingankar S, Toliat MR, Suk A, Hohne W, Schauer G, Lehmann M, Roscioli T, Schnabel D et al (2003) Mutations in enpp1 are associated with 'idiopathic' infantile arterial calcification. Nat Genet 34(4):379–381

Saraff V, Narayanan VK, Lawson AJ, Shaw NJ, Preece MA, Hogler W (2016) A diagnostic algorithm for children with low alkaline phosphatase activities: lessons learned from laboratory screening for hypophosphatasia. J Pediatr 172:181–186.e181

Schmidt T, Mussawy H, Rolvien T, Hawellek T, Hubert J, Rüther W, Amling M, Barvencik F (2017) Clinical, radiographic and biochemical characteristics of adult hypophosphatasia. Osteoporos Int 28(9):2653–2662

Schnabel C, Jett K, Friedman JM, Frieling I, Kruse HP, Mautner V (2013) Effect of vitamin d3 treatment on bone density in neurofibromatosis 1 patients: a retrospective clinical study. Joint Bone Spine 80(3):315–319

Sebastian-Serrano A, Engel T, de Diego-Garcia L, Olivos-Ore LA, Arribas-Blazquez M, Martinez-Frailes C, Perez-Diaz C, Millan JL, Artalejo AR, Miras-Portugal MT et al (2016) Neurodevelopmental alterations and seizures developed by mouse model of infantile hypophosphatasia are associated with purinergic signalling deregulation. Hum Mol Genet 25(19):4143–4156

Seshia S, Derbyshire G, Haworth J, Hoogstraten J (1990) Myopathy with hypophosphatasia. Arch Dis Child 65(1):130–131

Shao J, Engle M, Xie Q, Schmidt R, Narisawa S, Millan J, Alpers D (2000) Effect of tissue non-specific alkaline phosphatase in maintenance of structure of murine colon and stomach. Microsc Res Tech 51(2):121–128

Shapiro JR, Lewiecki EM (2017) Hypophosphatasia in adults: clinical assessment and treatment considerations. J Bone Miner Res 32:1977–1980

Shimada Y, Yoshiya I, Tanaka K, Yamazaki T, Kumon K (1979) Crying vital capacity and maximal inspiratory pressure as clinical indicators of readiness for weaning of infants less than a year of age. Anesthesiology 51(5):456–459

Siller AF, Whyte MP (2018) Alkaline phosphatase: discovery and naming of our favorite enzyme. J Bone Miner Res 33(2):362–364

Skoultchi AI, Nandi A, Roginski RS, Gregg RG, Smithies O (1987) Expression of genes inserted at the human beta-globin locus by homologous recombination. Prog Clin Biol Res 251:581–594

Sofronescu AG, Ross M, Rush E, Goldner W (2018) Spurious testosterone laboratory results in a patient taking synthetic alkaline phosphatase (asfotase alfa). Clin Biochem 58:118–121

Staines KA, MacRae VE, Farquharson C (2012) The importance of the sibling family of proteins on skeletal mineralisation and bone remodelling. J Endocrinol 214(3):241–255

Street SE, Kramer NJ, Walsh PL, Taylor-Blake B, Yadav MC, King IF, Vihko P, Wightman RM, Millan JL, Zylka MJ (2013) Tissue-nonspecific alkaline phosphatase acts redundantly with pap and nt5e to generate adenosine in the dorsal spinal cord. J Neurosci 33(27):11314–11322

Sugano H, Matsumoto T, Miyake K, Watanabe A, Iijima O, Migita M, Narisawa S, Millan JL, Fukunaga Y, Shimada T (2012) Successful gene therapy in utero for lethal murine hypophosphatasia. Hum Gene Ther 23(4):399–406

Sutton RA, Mumm S, Coburn SP, Ericson KL, Whyte MP (2012) "Atypical femoral fractures" during bisphosphonate exposure in adult hypophosphatasia. J Bone Miner Res 27(5):987–994

Terkeltaub R (2001) Inorganic pyrophosphate generation and disposition in pathophysiology. Am J Phys Cell Physiol 281(1):C1–C11

Thakker RV, Whyte MP, Eisman J, Igarashi T (2017) Genetics of bone biology and skeletal disease. Academic, San Diego

Thomas KR, Capecchi MR (1987) Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell 51(3):503–512

Tomazos IC, Moseley S, L'Italien G, Da Silva HG, Phillips D (2017) Improvements in the 6-minute walk test and correlation with quality-of-life measures in children and adults with hypophosphatasia treated with asfotase alfa. Paper presented at: ENDO 2017. Orlando

Ucakturk SA, Elmaogullari S, Unal S, Gonulal D, Mengen E (2018) Enzyme replacement therapy in hypophosphatasia. J Coll Physicians Surg Pak 28(9):S198–S200

Unger S, Mornet E, Mundlos S, Blaser S, Cole DE (2002) Severe cleidocranial dysplasia can mimic hypophosphatasia. Eur J Pediatr 161(11):623–626

Vallet M, Ralston SH (2016) Biology and treatment of paget's disease of bone. J Cell Biochem 117(2):289–299

van den Bos T, Handoko G, Niehof A, Ryan LM, Coburn SP, Whyte MP, Beertsen W (2005) Cementum and dentin in hypophosphatasia. J Dent Res 84(11):1021–1025

Vidmar AP, Ng C, Ganster A, Pitukcheewanont P (2017) Asfotase alfa treatment of an african-american infant with perinatal hypophosphatasia and homozygous hemoglobin sc disease. IBMS BoneKey 14

Wang W, Nyman JS, Ono K, Stevenson DA, Yang X, Elefteriou F (2011) Mice lacking nf1 in osteochondroprogenitor cells display skeletal dysplasia similar to patients with neurofibromatosis type i. Hum Mol Genet 20(20):3910–3924

Waymire KG, Mahuren JD, Jaje JM, Guilarte TR, Coburn SP, MacGregor GR (1995) Mice lacking tissue nonspecific alkaline phosphatase die from seizures due to defective metabolism of vitamin b-6. Nat Genet 11(1):45–51

Weber TJ, Sawyer EK, Moseley S, Odrljin T, Kishnani PS (2016) Burden of disease in adult patients with hypophosphatasia: results from two patient-reported surveys. Metabolism 65(10):1522–1530

Weiss MJ, Cole DE, Ray K, Whyte MP, Lafferty MA, Mulivor RA, Harris H (1988) A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia. Proc Natl Acad Sci U S A 85(20):7666–7669

Weiss MJ, Cole DE, Ray K, Whyte MP, Lafferty MA, Mulivor R, Harris H (1989a) First identification of a gene defect for hypophosphatasia: evidence that alkaline phosphatase acts in skeletal mineralization. Connect Tissue Res 21(1–4):99–104. discussion 104–106

Weiss MJ, Ray K, Fallon MD, Whyte MP, Fedde KN, Lafferty MA, Mulivor RA, Harris H (1989b) Analysis of liver/bone/kidney alkaline phosphatase mrna, DNA, and enzymatic activity in cultured skin fibroblasts from 14 unrelated patients with severe hypophosphatasia. Am J Hum Genet 44(5):686–694

Wenkert D, McAlister WH, Coburn SP, Zerega JA, Ryan LM, Ericson KL, Hersh JH, Mumm S, Whyte MP (2011) Hypophosphatasia: nonlethal disease despite skeletal presentation in utero (17 new cases and literature review). J Bone Miner Res 26(10): 2389–2398

Wennberg C, Hessle L, Lundberg P, Mauro S, Narisawa S, Lerner UH, Millan JL (2000) Functional characterization of osteoblasts and osteoclasts from alkaline phosphatase knockout mice. J Bone Miner Res 15(10):1879–1888

Whyte MP (2012) Hypophosphatasia. In: Glorieux FH, Pettifor JM, Juppner H (eds) Pediatric bone: biology & diseases, 2nd edn. Academic, London, pp 771–794

Whyte MP (2016) Hypophosphatasia – aetiology, nosology, pathogenesis, diagnosis and treatment. Nat Rev Endocrinol 12(4):233

Whyte MP (2017) Hypophosphatasia: enzyme replacement therapy brings new opportunities and new challenges. J Bone Miner Res 32(4):667–675

Whyte MP, Valdes R Jr, Ryan LM, McAlister WH (1982) Infantile hypophosphatasia: enzyme replacement therapy by intravenous infusion of alkaline phosphatase-rich plasma from patients with paget bone disease. J Pediatr 101(3):379–386

Whyte MP, McAlister WH, Patton LS, Magill HL, Fallon MD, Lorentz WB Jr, Herrod HG (1984) Enzyme replacement therapy for infantile hypophosphatasia attempted by intravenous infusions of alkaline phosphatase-rich paget plasma: results in three additional patients. J Pediatr 105(6):926–933

Whyte MP, Magill HL, Fallon MD, Herrod HG (1986) Infantile hypophosphatasia: normalization of circulating bone alkaline phosphatase activity followed by skeletal remineralization. Evidence for an intact structural gene for tissue nonspecific alkaline phosphatase. J Pediatr 108(1):82–88

Whyte MP, Kurtzberg J, McAlister WH, Mumm S, Podgornik MN, Coburn SP, Ryan LM, Miller CR, Gottesman GS, Smith AK et al (2003) Marrow cell transplantation for infantile hypophosphatasia. J Bone Miner Res 18(4):624–636

Whyte MP, Mumm S, Deal C (2007) Adult hypophosphatasia treated with teriparatide. J Clin Endocrinol Metab 92(4):1203–1208

Whyte MP, Wenkert D, McAlister WH, Mughal MZ, Freemont AJ, Whitehouse R, Baildam EM, Coburn SP, Ryan LM, Mumm S (2009) Chronic recurrent multifocal osteomyelitis mimicked in childhood hypophosphatasia. J Bone Miner Res 24(8):1493–1505

Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D, Van Sickle BJ, Simmons JH, Edgar TS, Bauer ML (2012) Enzyme-replacement therapy in life-threatening hypophosphatasia. N Engl J Med 366(10):904–913

Whyte MP, Zhang F, Wenkert D, McAlister WH, Mack KE, Benigno MC, Coburn SP, Wagy S, Griffin DM, Ericson KL (2015a) Hypophosphatasia: validation and expansion of the clinical nosology for children from 25years experience with 173 pediatric patients. Bone 75:229–239

Whyte MP, Zhang F, Wenkert D, McAlister WH, Mack KE, Benigno MC, Coburn SP, Wagy S, Griffin DM, Ericson KL et al (2015b) Hypophosphatasia: validation and expansion of the clinical nosology for children from 25 years experience with 173 pediatric patients. Bone 75:229–239

Whyte MP, Madson KL, Phillips D, Reeves AL, McAlister WH, Yakimoski A, Mack KE, Hamilton K, Kagan K, Fujita KP (2016a) Asfotase alfa therapy for children with hypophosphatasia. JCI Insight 1(9):e85971

Whyte MP, Rockman-Greenberg C, Ozono K, Riese R, Moseley S, Melian A, Thompson DD, Bishop N, Hofmann C (2016b) Asfotase alfa treatment improves survival for perinatal and infantile hypophosphatasia. J Clin Endocrinol Metab 101(1):334–342

Whyte MP, Coburn SP, Ryan LM, Ericson KL, Zhang F (2018) Hypophosphatasia: biochemical hallmarks validate the expanded pediatric clinical nosology. Bone 110:96–106

Williams DK, Pinzon C, Huggins S, Pryor JH, Falck A, Herman F, Oldeschulte J, Chavez MB, Foster BL, White SH et al (2018) Genetic engineering a large animal model of human hypophosphatasia in sheep. Sci Rep 8(1):16945

Wyckoff MH, El-Turk C, Laptook A, Timmons C, Gannon FH, Zhang X, Mumm S, Whyte MP (2005) Neonatal lethal osteochondrodysplasia with low serum levels of alkaline phosphatase and osteocalcin. J Clin Endocrinol Metab 90(2):1233–1240

Yadav MC, Lemire I, Leonard P, Boileau G, Blond L, Beliveau M, Cory E, Sah RL, Whyte MP, Crine P et al (2011) Dose response of bone-targeted enzyme replacement for murine hypophosphatasia. Bone 49(2):250–256

Yadav MC, de Oliveira RC, Foster BL, Fong H, Cory E, Narisawa S, Sah RL, Somerman M, Whyte MP, Millan JL (2012) Enzyme replacement prevents enamel defects in hypophosphatasia mice. J Bone Miner Res 27(8):1722–1734

Yamamoto S, Orimo H, Matsumoto T, Iijima O, Narisawa S, Maeda T, Millan JL, Shimada T (2011) Prolonged survival and phenotypic correction of akp2(−/−) hypophosphatasia mice by lentiviral gene therapy. J Bone Miner Res 26(1):135–142

Zankl A, Mornet E, Wong S (2008) Specific ultrasonographic features of perinatal lethal hypophosphatasia. Am J Med Genet A 146A(9):1200–1204

Zweifler LE, Patel MK, Nociti FH Jr, Wimer HF, Millan JL, Somerman MJ, Foster BL (2015) Counter-regulatory phosphatases tnap and npp1 temporally regulate tooth root cementogenesis. Int J Oral Sci 7(1):27–41