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Hypophosphatasia: A Systemic Skeletal Disorder Caused by Alkaline Phosphatase Deficiency

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<http://dx.doi.org/10.5772/intechopen.70597>

Abstract

Hypophosphatasia (HPP) is an inherited systemic bone disease caused by the deficiency of tissue-nonspecific alkaline phosphatase (TNAP). HPP is classified into six forms and the symptoms of HPP vary depending on the form. The pathophysiology of HPP is basically due to a defect of bone mineralization. TNAP is encoded by the *ALPL* gene, and the TNAP protein expressed in bone, kidney, liver, and neuronal cells and is linked to the cell membrane via a glycosylphosphatidylinositol anchor. TNAP is an ectoenzyme hydrolyzing phosphate compound such as inorganic pyrophosphate. TNAP plays an important role in mineralization of hard tissues. Defect of mineralization process causes hypomineralization of hard tissues, which leads to rickets or osteomalacia and dental manifestations. In addition, hypomineralization of the ribs results in respiratory failure in the severe forms, which is the main cause of death. Inheritance of HPP is autosomal recessive, but autosomal dominant cases have been reported in the milder forms. To date, a total of 335 mutations in the *ALPL* gene have been reported, and mutation sites are scattered throughout the gene. Recent development of enzyme replacement therapy has opened up a new vista on the treatment of this previously untreatable disease.

Keywords: hypophosphatasia, alkaline phosphatase, mineralization, bone, enzyme replacement therapy

1. Introduction

Hypophosphatasia (HPP; Online Mendelian Inheritance in Man (OMIM) #241500,241,510, 146,300) is an inherited systemic bone disease that is due to a deficiency of tissue-nonspecific alkaline phosphatase (TNAP) [1–3]. The first case of HPP was reported by the Canadian pediatrician John Campbell Rathbun in 1948 as a new developmental anomaly [4]. That case was an infantile form, and the patient's mutations were identified 50 years later using DNA

of the surviving parents as a compound heterozygote of p.A114T and p.D294A [5]. Since then, a total of 335 mutations in the gene for TNAP (the *ALPL* gene) have been reported [6]. The symptoms of HPP vary and are classified into six HPP forms [1, 2]. The pathophysiology of HPP is basically due to a defect of bone mineralization. In severe forms, the patients show skeletal manifestations and respiratory failure derived from costal bone insufficiency, whereas in the mildest forms, they show only dental manifestations [1]. Recent development of enzyme replacement therapy (ERT) has opened up a new vista on the treatment of this previously untreatable disease [7].

2. TNAP: gene, structure of the protein, and its function as an enzyme

There are four human alkaline phosphatase (ALP) isoenzymes (**Table 1**): TNAP, placental alkaline phosphatase (PLAP), intestinal alkaline phosphatase (IAP), and germ cell ALP [8, 9]. The latter three ALPs are tissue-specific and are expressed in the placenta, intestine, and germ cells (embryonic and cancer cells), respectively [9]. TNAP, also known as the liver/bone/kidney (LBK) alkaline phosphatase, is expressed ubiquitously; liver, bone, kidney, neuronal cells, and white blood cells in particular are tissues that show marked expression [10].

Human TNAP is encoded by the *ALPL* gene that is located on the short arm of chromosome 1 (1p36.1–34). The coding region of the gene is approximately 1.5 kb in length, and it is extended over more than 50 kb of genomic DNA [11]. The *ALPL* gene consists of 12 exons of which exons 2–12 are coding exons and there exist two alternative noncoding exons 1 (bone type and liver type) [12, 13]. The promoter region of the gene includes a TATA box, an Sp1 binding site, and a retinoic acid responsive element (RARE) [14, 15].

Common name	Protein name	Gene	Chromosomal location	Sites of expression	Function
Tissue-nonspecific (liver/bone/kidney)	TNAP (TNSALP)	<i>ALPL</i>	1p36.1–34	Ubiquitous	Mineralization entrance of pyridoxal phosphate into the neuronal cells
Intestinal	IAP	<i>ALPI</i>	2q34–37	Intestine	Degradation of LPS* lipid absorption
Placental	PLAP (PAP)	<i>ALPP</i>	2q34–37	Placenta	Degradation of LPS* (?)
Germ cell (placental like)	—	<i>ALPP2</i>	2q34–37	Germ cells Cancer cells	

*Lipopolysaccharides.

Table 1. Isoenzymes of human ALP.

Retinoic acid regulates the expression of TNAP via RARE [15], whereas another fat-soluble vitamin, active vitamin D (1,25-dihydroxycholecalciferol), regulates the expression of TNAP by modification of the stability of TNAP mRNA [16]. Furthermore, phosphates derived from ALP enzymatic activity are considered to regulate TNAP expression [17]. Epigenetic regulation by methylation of some of the promoter regions of the gene has been reported [18]. However, the precise regulatory mechanism of the *ALPL* gene regulation, especially its tissue-specific regulation, is not known. On the other hand, the genes encoding tissue-specific ALPs are located on the long arm of chromosome 2 and have a more compact gene structure [19–22].

The TNAP protein, which has a molecular weight of approximately 80 kDa, is linked to the outer cell membrane through a glycosylphosphatidylinositol (GPI) anchor [9]. The TNAP protein is initially synthesized as a 66 kDa peptide, and then O- and N-glycosides are attached in the endoplasmic reticulum. Eventually, TNAP is localized on the outer membrane of the cells via a GPI anchor [23]. This GPI anchor is added after hydrophobic amino acid residues at the C-terminus are eliminated. The GPI anchor consists of an ethanolamine phosphate, three residues of mannose, a glucosamine, and a phosphatidylinositol [9]. The precise amino acid residue in TNAP to which the GPI anchor is added has not been elucidated, whereas it is known to be an aspartate residue (D484) in PLAP [24, 25]. An active enzyme consists of a dimer and acts as an ectoenzyme. Approximately 58% of the amino acid residues in human TNAP sequences are conserved among mammalian ALPs [26]. On the other hand, approximately 90% of the amino acid residues are conserved among mammalian TNAPs, which allow prediction of missense mutations responsible for HPP [26]. Since the three dimensional structure of TNAP has not been solved, a simulation model based on human PLAP or mouse IAP is used to discuss TNAP structure [27–29]. The active site of the enzyme comprises a catalytic serine residue (S92 in the human PLAP), two Zn^{2+} -binding sites, and an Mg^{2+} -binding site. Ca^{2+} is also necessary as a cofactor. The crown domain is characteristic of mammalian ALPs and is considered to interact with extracellular proteins including collagen [30]. There are also isoforms of TNAP itself that depend on the tissue origin. Since these isoforms have different O-linked sugar chains, they show different patterns on the electrophoresis. [9, 31].

The systematic name of ALP is orthophosphoric-monoester phosphohydrolase [alkaline optimum] (EC 3.1.3.1) that hydrolyzes monophosphate esters, and the optimal pH is between 8 and 10 [9]. Inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP) are considered to be natural substrates of the enzyme [32]. PPi is an inhibitor of hydroxyapatite formation, which is essential for bone mineralization. PLP is an active vitamin B₆ and is necessary in neuronal cells for the biosynthesis of γ -aminobutyric acid (GABA), which acts as an inhibitory neurotransmitter. PLP on the outside of neuronal cells must be dephosphorylated by TNAP at the cell membrane before it can enter the neuronal cells, and it is then be rephosphorylated within the neuronal cells [32, 33]. In laboratory testing, ALP enzymatic activity is usually estimated using *p*-nitrophenylphosphate as an artificial substrate [9].

3. Molecular process of mineralization and the role of TNAP in mineralization

Biom mineralization in hard tissues including bone occurs in a two-step process [34]. Hypertrophic chondrocytes, osteoblasts, and odontocytes in the bone and dental tissues bud matrix vesicles (MVs) from the cell membrane [2, 35]. MVs are 50–200 nm in diameter and are enclosed by a membrane. MVs are a type of extracellular vesicles; however, the difference between MVs and exosomes, which are secreted by cells in the nonmineralized condition, is unclear [36]. TNAP is one of the most abundant proteins on the membrane of an MV [34]. The other proteins that are abundant in MVs are annexins A2, A5, and A6, Ca^{2+} -ATPase, nucleotide pyrophosphatase phosphodiesterase 1 (NPP1), Pit-1 (a sodium-phosphate cotransporter), and PHOSPHO1, all of which have important roles in mineralization [9, 34]. Biologically, mineralization is defined as the deposition of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) crystals among the collagen fibers. If this process is insufficient, extracellular spaces are not mineralized, which leads to the formation of an abnormal soft tissue called osteoid tissue. In the first step of the mineralization, hydroxyapatite is formed in an MV. The membrane lipids of the MV provide a source of phosphate; of these lipids, phosphatidylcholine and phosphatidylethanolamine are hydrolyzed by phospholipase C (PLC), yielding phosphocholine (PCho) and phosphoethanolamine (PEA), respectively [37]. Subsequently, PCho and PEA are hydrolyzed by PHOSPHO1, a cytosolic phosphatase abundant in MVs [38]. The phosphate transporter, Pit-1, provides another source of phosphate. On the other hand, calcium is incorporated into MVs via an annexin calcium channel, which consists of annexins A2, A5, and A6 [34, 35]. When the concentration of calcium phosphate rises beyond the solubility of calcium phosphate, hydroxyapatite crystal formation begins. Subsequently, hydroxyapatite crystals penetrate the MV membrane and elongate in the extracellular space [34, 35]. For the elongation of hydroxyapatite, calcium and phosphate should be provided by the extracellular space. Although calcium ions may be abundant in this milieu, phosphate is provided mainly by the TNAP on the MV membrane, which hydrolyzes PPi to yield inorganic phosphate (Pi) [2, 8, 34]. This hydrolysis by TNAP has dual roles; it supplies a source of phosphate for hydroxyapatite formation and degrades an inhibitor of hydroxyapatite formation (PPi). Ultimately, formed hydroxyapatite crystals deposit among collagen fibers, and mineralization is complete (**Figure 1**). Although the crown domain of TNAP can bind collagen and is suggested to have a role in hydroxyapatite deposition, it has not been elucidated whether TNAP plays a direct role in hydroxyapatite deposition.

Extracellular PPi is formed by NPP1 on the MV membrane by hydrolysis of ATP and also provided by a membrane transporter of PPi , ANKH (the human homolog of ANK, the mouse progressive ankylosis gene product). Therefore, mineralization is regulated by the balance of the activities of these three molecules: TNAP, NPP1, and ANKH [9, 39, 40]. Experiments using mice with knockout of these three genes showed that loss of activity of NPP1 or ANKH leads to hypercalcification (ectopic calcification of aorta and/or vertebrae and joints), whereas that of TNAP causes hypomineralization [41].

Figure 1. Mineralization process focusing on the matrix vesicle.

4. Clinical features of HPP including laboratory tests

HPP is classified into six forms depending on the onset age and the clinical severity (**Table 2**): perinatal (lethal) form, perinatal benign form, infantile form, childhood form, adult form, and odontohypophosphatasia [3]. The perinatal form occurs in utero and exhibits the most severe manifestations. Patients are stillborn or die during the early postnatal period. They show hypomineralization of the cranial bone and shortened and deformed limbs during gestation, which are easily revealed by ultrasonic examination. The hypomineralization of bones causes a membranous cranium and early craniosynostosis as well as musculoskeletal disorder after birth. The ribs are also hypomineralized, leading to respiratory failure after birth, which often requires respiratory aid. Failure of respiratory management often causes respiratory infections, which are the main cause of death. Epileptic seizures sometimes occur due to a deficit of PLP in neuronal cells, since PLP needs TNAP to enter neuronal cells. A deficit of PLP in neuronal cells causes a decrease in the inhibitory neurotransmitter GABA, leading to epileptic seizures. The perinatal benign form is a recently reported form [42]. Although the symptoms are recognized in gestation, prognosis is good and nonlethal. The infantile form occurs before 6 months of age and also shows severe manifestations. Patients display rickets and deformity of ribs and limbs, and fail to thrive. They also exhibit respiratory failure due to hypomineralization of the ribs, which requires respiratory aid. Recent progress in respiratory management elongates their lifespan. In addition, they often show hypercalcemia and hypercalciuria, leading to nephrocalcinosis. The childhood form shows manifestations after 6 months of age, whose symptoms are milder and not life-threatening. Patients show deformity of limbs, delayed walking, waddling gait, and muscle weakness. Craniosynostosis and

Form	Inheritance pattern	Onset	Symptoms	Prognosis
Perinatal	AR	In utero	Deformity of extremities Membranous cranium Respiratory failure Epileptic seizures	Lethal
Perinatal benign	AR or AD	In utero	Rickets	Benign
Infantile	AR	After birth Before 6 months of age	Rickets, Craniosynostosis Respiratory failure Failure to thrive Epileptic seizures Premature loss of deciduous teeth	Mostly lethal
Childhood	AR or AD	After 6 months of age	Rickets Musculoskeletal weakness Premature loss of deciduous teeth	Benign
Adult	AR or AD	Middle age	Osteomalacia Stress fractures	Benign
Odontohypophosphatasia	AR or AD		Premature loss of deciduous teeth Dental caries	Benign

AR: autosomal recessive, AD: autosomal dominant.

Table 2. Clinical features of hypophosphatasia.

high intracranial pressure sometimes occur. These patients also show premature loss of deciduous teeth due to failure of cementum formation [43]. Radiologically, childhood form patients exhibit a characteristic tongue-like radiolucent projection from the rachitic growth plate into the metaphysis due to a focal bone defect at the ends of long bones [1, 3]. The adult form occurs during middle age. Although the natural history of the adult form has not been well characterized, patients sometimes have a history of rickets and/or premature loss of deciduous teeth [44]. In the adult form, osteomalacia develops with pain associated with often recurring metatarsal stress fractures. In some patients, calcium pyrophosphate dehydrate crystals are deposited on articular cartilage due to an increase in concentrations of endogenous PPI [1]. Odontohypophosphatasia manifests only dental symptoms such as premature loss of deciduous teeth without skeletal symptoms due to rickets or osteomalacia.

General

Failure to thrive
Poor feeding
Weakness

Skeletal

Hypomineralization
Rickets/osteomalacia
Short, deformed limbs
Membranous cranium
Craniosynostosis
Deformed ribs
Skeletal pain
Short stature

Muscular

Muscle weakness
Gait disturbances; delayed walking, waddling gait

Neuronal

Epileptic seizures (pyridoxine dependent)
Irritability

Respiratory

Respiratory failure

Renal

Nephrocalcinosis

Dental

Premature loss of deciduous teeth
Dental caries

Blood examination

Reduced serum ALP
Elevated plasma PPi, PLP and PEA
Elevated plasma Ca^{2+}

Urinalysis

Elevated urine PEA
Elevated urine Ca^{2+}

Different presentation of symptoms is exhibited depend on the forms.

Table 3. Signs and symptoms of HPP.

A common histopathological feature of HPP is hypomineralization of bone and teeth [1]. Extracellular hydroxyapatite crystals are reduced, although mineralization occurs within the MV, because PHOSPHO1 acts in the MV. Elongation of hydroxyapatite is impaired. Osteoid tissues are increased in bone, which contains nonmineralized extracellular matrix, and they cause rickets or osteomalacia [45].

For all forms, a characteristic laboratory finding is low serum alkaline phosphatase activity, in which the bone isozyme is reduced [1]. In addition, the natural enzyme substrates, plasma PPi and PLP are elevated. Urine PEA is also elevated, although it is doubtful whether this compound is a natural substrate of TNAP. However, because urine PEA is easy to evaluate by using high-performance liquid chromatography (HPLC), the measurement of PEA is widely used for the diagnosis [2]. The combination of low ALP activity with elevated PPi or PEA is strong evidence for HPP. In some milder cases, however, an increase in PEA is not shown, and, in some cases, PEA is slightly elevated in carriers [46]. Signs and symptoms of HPP are summarized in **Table 3**.

5. Genetic aspect of HPP

5.1. Inheritance of HPP

HPP is an autosomal recessive inherited disease [1]. Carriers usually do not exhibit any manifestations. Sometimes, however, carriers show subnormal serum ALP activity and slightly higher urine PEA values [2, 46]. The penetrance differs among forms. In some milder cases, an autosomal dominant cases have been reported [47, 48], and the dominant negative effect accounts for some autosomal dominant cases [48]. In addition, different severity of the symptoms within the same family has been reported [49, 50], suggesting the involvement of epigenetic mechanisms.

5.2. Prevalence of HPP

The prevalence of HPP was estimated as 1 in 100,000 live births in the Toronto area in Canada, where the first case was found [51]. In Manitoba, Canada, the prevalence is higher in the Mennonite group, being 1 in 2500, according to a founder effect of a particular mutation [52]. In Europe, the prevalence of severe cases is estimated as 1 in 300,000 [53], whereas in Japan it is 1 in 450,000 for patients who have the c.1559delT allele [46]. This particular allele is a severe allele and is characteristic of Japanese families (46.8% of Japanese patients with HPP have this deletion allele) [46].

5.3. Genetic diagnosis

When HPP is suspected, collection of the family history and the making of a pedigree are important for genetic counseling [54]. Clinical diagnosis can be done by laboratory biochemical examinations and ultrasonic and radiographic findings. Definitive diagnosis is performed by genetic testing. Genomic DNA of the patient is amplified, and the nucleotide sequences

are determined. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP), PCR-denaturing gradient gel electrophoresis (PCR-DGGE), and high-resolution melting curve analysis (HRM) methods used to be employed for this purpose, but direct nucleotide sequencing may be the most effective current method of analysis. Once the mutation of the proband is determined, the inheritance can be pursued by testing the parents' DNA, which makes it possible to give a genetic counseling, because the inheritance pattern of HPP is basically Mendelian inheritance [54, 55]. However, as mentioned above, the same mutation can result in different phenotypes in some families. In addition, a rare case of paternal uniparental isodisomy has been reported [56]. Once a genetic diagnosis is established, enzymatic activity and mineralization activity can be evaluated [57]. An expression plasmid containing the mutant cDNA is transfected into U2OS cells, which are osteoblast-like cells that lack ALP activity. The cells are then cultured for an appropriate period, and enzymatic activity is estimated. For the mineralization assay, the transfected cells are cultured in a mineralization medium that contains β -glycerophosphate as an artificial substrate for TNAP, with or without ascorbic acid. After about 5 days of culture, mineralization is estimated by Alizarin Red S staining [57].

5.4. Prenatal diagnosis

Prenatal diagnosis by ALP enzymatic assay or by immunological detection using amniotic fluid and chorionic villus has been reported, but their diagnostic value is low [3] because of contamination of fetal intestinal ALP and maternal ALP. HPP can be diagnosed using ultrasonography and radiography during the second trimester, but the differential diagnosis is complicated. DNA-based diagnosis using chorionic villus is accurate if information about the nucleotide sequences within the family has been obtained [54, 58]. However, prediction of the prognosis of the disease is not easy, because of the fact that the same mutations can cause different phenotypes even in the same family. In addition, ethical considerations including genetic counseling are very important when prenatal genetic diagnosis is performed [54].

6. Mutations in the *ALPL* gene

To date, a total of 335 mutations in the *ALPL* gene have been reported [6]. The TNAP gene mutations' databases (http://www.sesep.uvsq.fr/03_hypo_mutations.php) of the University of Versailles-Saint Quentin en Yvelines provide up-to-date information regarding mutations [55]. Almost all of these mutations are located within the exons, although some mutations are in the promoter region, exon-intron boundaries and introns. In addition, over 70% of the mutations are missense mutations, 11% are small deletions, 6% are splicing mutations, 5% are nonsense mutations, 3% are small insertions, and 3% are large deletions [6]. Only one regulatory mutation has been reported [59]. Many of the patients are compound heterozygotes. Generally, the interaction between the mutant alleles determines the phenotypes of the patients. Residual activities of mutant TNAPs influence the enzymatic activity and the mineralizing activity of the compound heterozygotes. However, the relationships of genotype and phenotype are rather complicated, and the phenotypes are not always estimated

from the combination of the genotypes. Mutation sites are scattered throughout the gene, but there are some “hot spots.” In Caucasian patients, p.E191K (a moderate allele with a dominant negative effect) and p.D378V (a severe allele) are frequent mutations [60, 61], whereas c.1559delT (p.L520RfsX86; a severe allele) and p.F327L (a moderate allele) are frequent in Japanese patients [62, 63]. c.1559delT also has founder effects, and the frequency of c.1559delT is mentioned above [46, 62].

7. Structure and function of mutant TNAP

Mutation sites of TNAP proteins are classified by its domain structure [30]. Severe phenotypes are associated with the mutations that are located in the active site and its vicinity, the homodimer interface, the crown domain, and the calcium-binding domain. Mutations in the active site valley (the entry site of the substrate into the active site) resulted in less severe phenotypes [30]. Mutations in the other regions of the protein are inclined to show residual enzymatic activity and are, therefore, milder phenotypes.

Because most of the patients are compound heterozygotes, the residual activity and phenotype are determined by the interaction of two mutant proteins [55]. In some cases, especially in autosomal dominant cases, dominant negative mechanisms are suggested, in which cases the mutant proteins affect the function of the wild-type enzymes [48]. Those interactions have not been precisely elucidated and need to be explored in more detail in order to reveal the genotype–phenotype interrelationships and pathophysiology of HPP.

8. Treatment of HPP based on the pathophysiology of the disease

There have been several trials for the treatment of HPP. Respiratory aid somehow succeeds in saving life in the perinatal and the infantile forms, although it is a symptomatic treatment. Other symptomatic treatments are diet therapies, including calcium restriction and vitamin D supplementation, and surgical operations for bone fractures and craniosynostosis [1]. In terms of treatment based on the pathophysiology of HPP, ERT has been attempted. Whyte et al. used the serum of Paget’s disease patients who exhibited a high level of TNAP for enzyme replacement [64]. Infusion of PLAP has also been attempted based on the observation that, when the patients with mild forms become pregnant, which causes a high serum ALP level according to an increase in PLAP, they sometimes show improvement of symptoms. Those ERT attempts, however, failed to improve the symptoms [3]. Bone marrow transplantation (BMT) and mesenchymal cell transplantation have also been attempted. Those trials showed a slight improvement but an insufficient effect [65]. Successful ERT was reported in 2012, in which bioengineered TNAP was administered [7]. The C-terminal membrane-bound region of human TNAP was eliminated and replaced with the Fc region of human IgG and deca-aspartate sequences [66]. This bioengineered TNAP is, therefore, soluble, can be easily purified using the Fc region, and has high affinity for hydroxyapatite through acidic peptides such as deca-aspartate [66]. Before the trial, an animal experiment using the bioengineered

TNAP in a knockout mouse (*Akp2*^{-/-}; *AKP2* is the mouse homolog of the *ALPL*) that is a good mimic of the perinatal form of HPP, showed elongation of life and improvements in bone and dental defects without respiratory failure [66, 67]. The clinical trial with the bioengineered TNAP (ENB-0040; asfotase alfa) was conducted with five perinatal and six infantile patients [7]. It was administered first as a single intravenous infusion of 2 mg/kg, which was then followed by subcutaneous injections three times per week at a dose of 1 mg/kg for 48 weeks. With the exception of one case who died of respiratory failure that was unrelated with asfotase alfa, the recruited patients showed improvements in rickets and respiratory failure [7]. Asfotase alfa (StrensiqST; Alexion Pharmaceuticals, Inc.) was approved in Japan, the EU, Canada, and the USA in that order in 2015 [2]. Asfotase alfa has dramatically changed the treatment of HPP [68]. Asfotase alfa is indicated for the treatment of patients with perinatal-, infantile- and juvenile-onset HPP [69], in which juvenile-onset HPP means almost the same as the childhood form. The current protocol of the recommended administration is subcutaneous injection six times a week at a dose of 1 mg/kg or three times a week at 2 mg/kg, and the maximal volume of injection is 1 ml [69]. The half-life of asfotase alfa is 5 days in the case of subcutaneous administration. The most common adverse reactions ($\geq 10\%$) are injection site reactions, lipodystrophy, ectopic calcifications, and hypersensitivity reactions. Patients with HPP are at increased risk for developing ectopic calcifications, especially of the eye including the cornea and conjunctiva, and the kidneys (nephrocalcinosis). Although ectopic calcification of the blood vessels has not been reported, it is conceivable that long-term administration may cause medial artery calcification. Medial artery calcification or Mönckeberg-type calcification is often shown as a lethal complication in chronic kidney disease (CKD) patients [70]. In CKD patients, hyperphosphatemia triggers transformation of smooth muscle cells in the media into osteoblastic cells that express elevated TNAP, which then stimulates calcification in the medial artery by a mechanism similar to that of bone mineralization [71, 72]. Asfotase alfa is still not indicated for milder form HPP patients. In this regards, the natural history of the adult form has not been well elucidated [44], and more study is needed. Similarly, odontohypophosphatasia may be still underdiagnosed, because dentists usually do not evaluate the serum ALP value. There should be more investigation into the feasibility of using asfotase alfa for those milder forms.

9. Future perspective

Although current ERT has drastically changed the treatment of HPP, many problems are indicated regarding asfotase alfa administration. First of all, two or three injections per week are needed for this ERT treatment, which burdens patients with injections and parents with administration fees. The interval between injections can be elongated by introducing some modifications into the enzyme preparation. Other possible therapies are bone marrow stem-cell transplantation and/or combination therapy of such transplantation with ERT. Another possible trial is a trial of gene therapy. Using viral vectors, gene therapy was successfully used to treat knockout mice (*AKP2*^{-/-}) [73, 74]. Since a viral vector containing *ALPL* cDNA that is injected into blood cannot maintain an effective concentration, gene therapy in combination with stem-cell transplantation (*ex vivo* gene therapy) may be more effective [75].

Once gene-transferred stem cells are transplanted, no other injection may be necessary [2]. Although gene therapy seems to be a promising procedure, results have so far only been obtained for mouse models, and its feasibility and safety in humans must be investigated.

10. Conclusions

HPP is a systemic skeletal disorder that is caused by TNAP deficiency. Human TNAP is one of the four isoenzymes of alkaline phosphatase and is expressed ubiquitously. The TNAP protein is linked to the outer membrane of cells via a GPI anchor and works as an enzyme in a homodimer state. TNAP is essential for biomineralization; it is located on the MV membrane and plays a role in the elongation of hydroxyapatite crystals into the extracellular space.

HPP is classified into six forms and clinical severity varies among the forms. Hypomineralization of hard tissues is a common feature of HPP. In the severe forms, patients show rickets and respiratory failure that cause death. Milder forms exhibit musculoskeletal disorder or teeth problems. Although low serum ALP activity and an elevated urine PEA value are characteristic of HPP, genetic diagnosis is the definitive diagnosis. ERT using a genetically modified enzyme (asfotase alfa) opens up a new vista in the therapy of HPP, especially for severe forms of HPP. Although asfotase alfa has drastically changed the treatment of HPP, there remain still several problems with its use that need to be resolved.

Conflict of interest

The author has received honoraria from Alexion Pharmaceuticals, Inc. The author reports no other conflict of interest in this work.

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References

- [1] Whyte MP. Hypophosphatasia: Nature's Window on alkaline phosphatase function in man. In: Bilezikian JP, Raisz LG, Rodan GA, editors. *Principles of Bone Biology*. 2nd ed. San Diego, CA: Academic Press; 2002. p. 1229-1248

- [2] Orimo H. Pathophysiology of hypophosphatasia and the potential role of asfotase alpha. *Therapeutics and Clinical Risk Management*. 2016;**12**:777-786
- [3] Whyte MP. Hypophosphatasia: An overview for 2017. *Bone*. 2017;**102**:15-25. DOI: 10.1016/j.bone.2017.02.011
- [4] Rathbun JC. "Hypophosphatasia": A new developmental anomaly. *American Journal of Diseases of Children*. 1948;**75**:822-831
- [5] Mumm S, Jones J, Finnegan P, Whyte MP. Hypophosphatasia: Molecular diagnosis of Rathbun's original case. *Journal of Bone and Mineral Research*. 2001;**16**:1724-1727
- [6] The Tissue-Nonspecific Alkaline Phosphatase Gene Mutations Database [Internet]. Available from: http://www.sesep.uvsq.fr/03_hypo_mutations.php [Accessed: 31 May 2017]
- [7] Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D, Van Sickle BJ, Simmons JH, Edgar TS, Bauer ML, Hamdan MA, Bishop N, Lutz RE, McGinn M, Craig S, Moore JN, Taylor JW, Cleveland RH, Cranley WR, Lim R, Thacher TD, Mayhew JE, Downs M, Millán JL, Skinner AM, Crine P, Landy H. Enzyme-replacement therapy in life-threatening hypophosphatasia. *New England Journal of Medicine*. 2012;**366**:904-913
- [8] Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *Journal of Nippon Medical School*. 2010;**77**:4-12
- [9] Millán JL. *Mammalian Alkaline Phosphatases: From Biology to Applications in Medicine and Biotechnology*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co.; 2006
- [10] Harris H. The human alkaline phosphatases: What we know and what we don't know. *Clinica Chimica Acta*. 1989;**186**:133-150
- [11] Smith M, Weiss MJ, Griffin CA, Murray JC, Buetow KH, Emanuel BS, Henthorn PS, Harris H. Regional assignment of the gene for human liver/bone/kidney alkaline phosphatase to chromosome 1p36.1-34. *Genomics*. 1988;**2**:139-143
- [12] Weiss MJ, Ray K, Henthorn PS, Lamb B, Kadesch T, Harris H. Structure of the human liver/bone/kidney alkaline phosphatase gene. *Journal of Biological Chemistry*. 1988; **263**:12002-12010
- [13] Matsuura S, Kishi F, Kajii T. Characterization of a 5'-flanking region of the human liver/bone/kidney alkaline phosphatase gene: Two kinds of mRNA from a single gene. *Biochemical and Biophysical Research Communications*. 1990;**168**:993-1000
- [14] Kiledjian M, Kadesch T. Analysis of the human liver/bone/kidney alkaline phosphatase promoter *in vivo* and *in vitro*. *Nucleic Acids Research*. 1990;**18**:957-961
- [15] Orimo H, Shimada T. Regulation of the human tissue-nonspecific alkaline phosphatase gene expression by all-*trans*-retinoic acid in SaOS-2 osteosarcoma cell line. *Bone*. 2005;**36**:866-876

- [16] Orimo H, Shimada T. Posttranscriptional modulation of the human tissue-nonspecific alkaline phosphatase gene expression by 1,25-dihydroxyvitamin D3 in MG-63 osteoblastic osteosarcoma cells. *Nutrition Research*. 2006;**26**:227-234
- [17] Orimo H, Shimada T. The role of tissue-nonspecific alkaline phosphatase in phosphate-induced activation of alkaline phosphatase and mineralization in SaOS-2 human osteoblast-like cells. *Molecular and Cellular Biochemistry*. 2008;**315**:51-60
- [18] Delgado-Calle J, Sañudo C, Sánchez-Verde L, García-Renedo RJ, Arozamena J, Riancho JA. Epigenetic regulation of alkaline phosphatase in human cells of the osteoblastic lineage. *Bone*. 2011;**49**:830-838
- [19] Henthorn PS, Raducha M, Kadesch T, Weiss MJ, Harris H. Sequence and characterization of the human intestinal alkaline phosphatase gene. *Journal of Biological Chemistry*. 1988;**263**:12011-12019
- [20] Knoll BJ, Rothblum KN, Longley M. Nucleotide sequence of the human placental alkaline phosphatase gene: Evolution of the 5' flanking region by deletion/substitution. *Journal of Biological Chemistry*. 1988;**263**:12020-12027
- [21] Millán JL, Manes T. Seminoma-derived Nagao isozyme is encoded by a germ-cell alkaline phosphatase gene. *Proceedings of the National Academy of Sciences of the United States of America*. 1988;**85**:3124-3028
- [22] Griffin CA, Smith M, Henthorn PS, Harris H, Weiss MJ, Raducha M, Emanuel BS. Human placental and intestinal alkaline phosphatase genes map to 2q34-q37. *American Journal of Human Genetics*. 1987;**41**:1025-1034
- [23] Shibata H, Fukushi M, Igarashi A, Misumi Y, Ikehara Y, Ohashi Y, Oda K. Defective intracellular transport of tissue-nonspecific alkaline phosphatase with an Ala162→Thr mutation associated with lethal hypophosphatasia. *Journal of Biochemistry*. 1998;**123**:968-977
- [24] Micanovic R, Bailey CA, Brink L, Gerber L, Pan Y-CE, Hulmes JD, Udenfriend S. Aspartic acid-484 of nascent placental alkaline phosphatase condenses with a phosphatidylinositol glycan to become the carboxyl terminus of the mature enzyme. *Proceedings of the National Academy of Sciences of the United States of America*. 1988;**85**:1398-1402
- [25] Micanovic R, Gerber LD, Berger J, Kodukula K, Udenfriend S. Selectivity of the cleavage/attachment site of phosphatidylinositol-glycan-anchored membrane proteins determined by site-specific mutagenesis at Asp-484 of placental alkaline phosphatase. *Proceedings of the National Academy of Sciences of the United States of America*. 1990;**87**:157-161
- [26] Silvent J, Gasse B, Mornet E, Sire JY. Molecular evolution of the tissue-nonspecific alkaline phosphatase allows prediction and validation of missense mutations responsible for hypophosphatasia. *Journal of Biological Chemistry*. 2014;**289**:24168-24179
- [27] Le Du MH, Stigbrand T, Taussig MJ, Ménez A, Stura EA. Crystal structure of alkaline phosphatase from human placenta at 1.8Å Resolution. *Journal of Biological Chemistry*. 2001;**276**:9158-9165

- [28] Ghosh K, Tagore DM, Anumula R, Lakshmaiah B, Kumar PPBS, Singaram S, Matan T, Kallipatti S, Selvam S, Krishnamurthy P, Ramarao M. Crystal structure of rat intestinal alkaline phosphatase – Role of crown domain in mammalian alkaline phosphatases. *Journal of Structural Biology*. 2013;**184**:182-192
- [29] Le Du MH, Millán JL. Structural evidence of functional divergence in human alkaline phosphatases. *Journal of Biological Chemistry*. 2002;**277**:49808-49814
- [30] Mornet E, Stura E, Lia-Baldini A-S, Stigbrand T, Ménez A, Le Du M-H. Structural evidence for a functional role of human tissue nonspecific alkaline phosphatase in bone mineralization. *Journal of Biological Chemistry*. 2001;**276**:31171-31178
- [31] Nosjean O, Koyama I, Goseki M, Roux B, Komoda T. Human tissue-nonspecific alkaline phosphatases: Sugar-moiety-induced enzymic and antigenic modulation and genetic aspects. *Biochemical Journal*. 1997;**321**:297-303
- [32] Fedde KN, Whyte MP. Alkaline phosphatase (tissue-nonspecific isoenzyme) is a phosphoethanolamine and pyridoxal-5'-phosphate ectophosphatase: Normal and hypophosphatasia fibroblast study. *American Journal of Human Genetics*. 1990;**47**:767-775
- [33] Guilarte TR. Regional changes in the concentrations of glutamate, glycine, taurine, and GABA in the vitamin B-6 deficient developing rat brain: Association with neonatal seizures. *Neurochemical Research*. 1989;**14**:889-897
- [34] Anderson HC. The role of matrix vesicles in physiological and pathological calcification. *Current Opinion in Orthopaedics*. 2007;**18**:428-433
- [35] Cui L, Houston DA, Farquharson C, MacRae VE. Characterisation of matrix vesicles in skeletal and soft tissue mineralisation. *Bone*. 2016;**87**:147-158
- [36] Shapiro IM, Landis WJ, Risbud MV. Matrix vesicles: Are they anchored exosomes? *Bone*. 2015;**79**:29-36
- [37] Mebarek S, Abousalham A, Magne D, De LD, Bandorowicz-Pikula J, Pikula S, Buchet R. Phospholipases of mineralization competent cells and matrix vesicles: Roles in physiological and pathological mineralizations. *International Journal of Molecular Sciences*. 2013;**14**:5036-5129
- [38] Roberts S, Narisawa S, Harmey D, Millán JL, Farquharson C. Functional involvement of PHOSPHO1 in matrix vesicle-mediated skeletal mineralization. *Journal of Bone and Mineral Research*. 2007;**22**:617-627
- [39] Hessle L, Johnson KA, Anderson HC, Narisawa S, Goding JW, Terkeltaub R, Millán JL. Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;**99**:9445-9449
- [40] Yadav MC, Sper Simão AM, Narisawa S, Huesa C, McKee MD, Farquharson C, Millán JL. Loss of skeletal mineralization by the simultaneous ablation of PHOSPHO1 and alkaline phosphatase function: A unified model of the mechanisms of initiation of skeletal calcification. *Journal of Bone and Mineral Research*. 2011;**26**:286-297

- [41] Harmey D, Hessle L, Narisawa S, Johnson KA, Terkeltaub R, Millán JL. Concerted regulation of inorganic pyrophosphate and osteopontin by *Akp2*, *Enpp1*, and *Ank*. *American Journal of Pathology*. 2004;**164**:1199-1209
- [42] Wenkert D, McAlister WH, Coburn SP, Zerega JA, Ryan LM, Ericson KL, Hersh JH, Mumm S, Whyte MP. Hypophosphatasia: Nonlethal disease despite skeletal presentation in utero (17 new cases and literature review). *Journal of Bone and Mineral Research*. 2011;**26**:2389-2398
- [43] van den Bos T, Handoko G, Niehof A, Ryan LM, Coburn SP, Whyte MP, Beertsen W. Cementum and dentin in hypophosphatasia. *Journal of Dental Research*. 2005;**84**:1021-1025
- [44] Berkseth KE, Tebben PJ, Drake MT, Hefferran TE, Jewison DE, Wermers RA. Clinical spectrum of hypophosphatasia diagnosed in adults. *Bone*. 2013;**54**:21-27
- [45] Anderson HC, Hsu HH, Morris DC, Fedde KN, Whyte MP. Matrix vesicles in osteomalacic hypophosphatasia bone contain apatite-like mineral crystals. *American Journal of Pathology*. 1997;**151**:1555-1561
- [46] Watanabe A, Karasugi T, Sawai H, Naing BT, Ikegawa S, Orimo H, Shimada T. Prevalence of c.1559delT in ALPL, a common mutation resulting in the perinatal (lethal) form of hypophosphatasia in Japanese and effects of the mutation on heterozygous carriers. *Journal of Human Genetics*. 2011;**56**:166-168
- [47] Silverman JL. Apparent dominant inheritance of hypophosphatasia. *Archives of Internal Medicine*. 1962;**110**:191-198
- [48] Fauvert D, Brun-Heath I, Lia-Baldini AS, Bellazi L, Taillandier A, Serre J-L, de Mazancourt P, Mornet E. Mild forms of hypophosphatasia mostly result from dominant negative effect of severe alleles or from compound heterozygosity for severe and moderate alleles. *BMC Medical Genetics*. 2009;**10**:51
- [49] Macfarlane JD, Kroon HM, van den Harten JJ. Phenotypically dissimilar hypophosphatasia in two sibships. *American Journal of Medical Genetics Part A*. 1992;**42**:117-121
- [50] Peach CA, Zhang Y, Wordsworth BP. Mutations of the tissue-nonspecific alkaline phosphatase gene (TNAP) causing a non-lethal case of perinatal hypophosphatasia. *Rheumatology*. 2007;**46**:1037-1040
- [51] Fraser D. Hypophosphatasia. *American Journal of Medicine*. 1957;**22**:730-746
- [52] Greenberg CR, Taylor CL, Haworth JC, Seargeant LE, Philipps S, Triggs-Raine B, Chodirker BN. A homoallelic Gly³¹⁷→Asp mutation in ALPL causes the perinatal (lethal) form of hypophosphatasia in Canadian mennonites. *Genomics*. 1993;**17**:215-217
- [53] Mornet E, Yvard A, Taillandier A, Fauvert D, Simon-Bouy B. A molecular-based estimation of the prevalence of hypophosphatasia in the European population. *Annals of Human Genetics*. 2011;**75**:439-445

- [54] Watanabe A, Orimo H, Takeshita T, Shimada T. Prenatal diagnosis of severe perinatal (lethal) hypophosphatasia. In: Choy RKW, Leung TY, editors. *Prenatal Diagnosis – Morphology Scan and Invasive Methods*. Rijeka, Croatia: InTech; 2012. p. 27-32
- [55] Mornet E. Hypophosphatasia. *Orphanet Journal of Rare Diseases*. 2007;**2**:40
- [56] Watanabe A, Satoh S, Fujita A, Naing BT, Orimo H, Shimada T. Perinatal hypophosphatasia caused by uniparental isodisomy. *Bone*. 2014;**60**:93-99
- [57] Orimo H, Goseki-Sone M, Hosoi T, Shimada T. Functional assay of the mutant tissue-nonspecific alkaline phosphatase gene using U₂OS osteoblast-like cells. *Molecular Genetics and Metabolism*. 2008;**94**:375-381
- [58] Orimo H, Nakajima E, Hayashi Z, Kijima K, Watanabe A, Tenjin H, Araki T, Shimada T. First-trimester prenatal molecular diagnosis of infantile hypophosphatasia in a Japanese family. *Prenatal Diagnosis*. 1996;**16**:559-563
- [59] Taillandier A, Cozien E, Muller F, Merrien Y, Bonnin E, Fribourg C, Simon-Bouy B, Serre JL, Bieth E, Brenner R, Cordier MP, De Bie S, Fellmann F, Freisinger P, Hesse V, Hennekam RC, Josifova D, Kerein-Storarr L, Loporrier N, Zobot MT, Mornet E. Fifteen new mutations (–195C>T, L-12X, 298-2A>G, T117N, A159T, R229S, 997+2T>a, E274X, A331T, H364R, D389G, 1256delC, R433H, N461I, C472S) in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in patients with hypophosphatasia. *Human Mutation*. 2000;**15**:293
- [60] Hérasse M, Spentchian M, Taillandier A, Mornet E. Evidence of a founder effect for the tissue-nonspecific alkaline phosphatase (TNSALP) gene E174K mutation in hypophosphatasia patients. *European Journal of Human Genetics*. 2002;**10**:666-668
- [61] Whyte MP, Mumm S, Deal C. Adult hypophosphatasia treated with teriparatide. *Journal of Clinical Endocrinology & Metabolism*. 2007;**92**:1203-1208
- [62] Orimo H, Goseki-Sone M, Inoue M, Tsubakio Y, Sakiyama T, Shimada T. Importance of deletion of T at nucleotide 1559 in the tissue-nonspecific alkaline phosphatase gene in Japanese patients with hypophosphatasia. *Journal of Bone and Mineral Metabolism*. 2002;**20**:28-33
- [63] Michigami T, Uchihashi T, Suzuki A, Tachikwa K, Nakajima S, Ozono K. Common mutation F310L and T1559del in the tissue-nonspecific alkaline phosphatase gene are related to distinct phenotypes in Japanese patients with hypophosphatasia. *European Journal of Pediatrics*. 2005;**164**:277-282
- [64] Whyte MP, McAlister WH, Patton LS, Magill HL, Fallon MD, Lorentz WB, Herrod MD. Enzyme replacement therapy for infantile hypophosphatasia attempted by intravenous infusions of alkaline phosphatase-rich Paget plasma: Results in three additional patients. *The Journal of Pediatrics*. 1984;**105**:926-933
- [65] Taketani T, Oyama C, Mihara A, Tanabe Y, Abe M, Hirade T, Yamamoto S, Bo R, Kanai T, Tadenuma T, Michibata Y, Yamamoto S, Hattori M, Katsube Y, Ohnishi H, Sasao M, Oda Y,

- Hattori K, Yuba S, Ohgushi H, Yamaguchi S. Ex vivo expanded allogeneic mesenchymal stem cells with bone marrow transplantation improved osteogenesis in infants with severe hypophosphatasia. *Cell Transplantation*. 2015;**24**:1931-1943
- [66] Millán JL, Narisawa S, Lemire I, Loisel TP, Boileau G, Leonard P, Gramatikova S, Terkeltaub R, Camacho NP, McKee MD, Crine P, Whyte MP. Enzyme replacement therapy for murine hypophosphatasia. *Journal of Bone and Mineral Research*. 2008;**23**:777-787
- [67] McKee MD, Nakano Y, Masica DL, Gray JJ, Lemire I, Heft R, Whyte MP, Crine P, Millán JL. Enzyme replacement therapy prevents dental defects in a model of hypophosphatasia. *Journal of Dental Research*. 2011;**90**:470-476
- [68] Whyte MP, Rockman-Greenberg C, Ozono K, Riese R, Moseley S, Melian A, Thompson DD, Bishop N, Hofmann C. Asfotase alpha treatment improves survival for perinatal and infantile hypophosphatasia. *Journal of Clinical Endocrinology & Metabolism*. 2016;**101**:334-342
- [69] STRENSIQ™ (Asfotase Alfa) for Strensiq [Prescribing Information]. Cheshire, CT: Alexion Pharmaceuticals, Inc. [Internet]; 2015. Available from: <http://strensiq.com/images/pi.pdf> [Accessed: 15 May 2017]
- [70] Massy ZA, Drüeke TB. Vascular calcification. *Current Opinion in Nephrology and Hypertension*. 2013;**22**:405-412
- [71] Shioi A, Katagi M, Okuno Y, Mori K, Jono S, Koyama H, Nishizawa Y. Induction of bone-type alkaline phosphatase in human vascular smooth muscle cells: Roles of tumor necrosis factor- α and oncostatin M derived from macrophages. *Circulation Research*. 2002;**91**:9-16
- [72] Tani T, Orimo H, Shimizu A, Tsuruoka S. Development of a novel chronic kidney disease mouse model to evaluate the progression of hyperphosphatemia and associated mineral bone disease. *Scientific Reports*. 2017;**7**:2233. DOI: 10.1038/s41598-017-02351-6
- [73] Yamamoto S, Orimo H, Matsumoto T, Iijima O, Narisawa S, Maeda T, Millán JL, Shimada T. Prolonged survival and phenotypic correction of *Akp2*^{-/-} hypophosphatasia mice by lentiviral gene therapy. *Journal of Bone and Mineral Research*. 2011;**26**:135-142
- [74] Matsumoto T, Miyake K, Yamamoto S, Orimo H, Miyake N, Odagaki Y, Adachi K, Iijima O, Narisawa S, Millán JL, Fukunaga Y, Shimada T. Rescue of severe infantile hypophosphatasia mice by AAV-mediated sustained expression of soluble alkaline phosphatase. *Human Gene Therapy*. 2011;**22**:1355-1364
- [75] Iijima O, Miyake K, Watanabe A, Miyake N, Igarashi T, Kanokoda C, Nakamura-Takahashi A, Kinoshita H, Noguchi T, Abe S, Narisawa S, Millán JL, Okada T, Shimada T. Prevention of lethal murine hypophosphatasia by neonatal ex vivo gene therapy using lentivirally transduced bone marrow cells. *Human Gene Therapy*. 2015;**26**:801-881