Hypophosphatasia — aetiology, nosology, pathogenesis, diagnosis and treatment

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Abstract | Hypophosphatasia is the inborn error of metabolism characterized by low serum alkaline phosphatase activity (hypophosphatasaemia). This biochemical hallmark reflects loss-of-function mutations within the gene that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP). TNSALP is a cell-surface homodimeric phosphohydrolase that is richly expressed in the skeleton, liver, kidney and developing teeth. In hypophosphatasia, extracellular accumulation of TNSALP natural substrates includes inorganic pyrophosphate, an inhibitor of mineralization, which explains the dento-osseous and arthritic complications featuring tooth loss, rickets or osteomalacia, and calcific arthopathies. Severely affected infants sometimes also have hypercalcaemia and hyperphosphataemia due to the blocked entry of minerals into the skeleton, and pyridoxine-dependent seizures from insufficient extracellular hydrolysis of pyridoxal 5′-phosphate, the major circulating form of vitamin B_6, required for neurotransmitter synthesis. Autosomal recessive or dominant inheritance from ~300 predominantly missense *ALPL* (also known as *TNSALP*) mutations largely accounts for the remarkably broad-ranging expressivity of hypophosphatasia. High serum concentrations of pyridoxal 5′-phosphate represent a sensitive and specific biochemical marker for hypophosphatasia. Also, phosphoethanolamine levels are usually elevated in serum and urine, though less reliably for diagnosis. *TNSALP* mutation detection is important for recurrence risk assessment and prenatal diagnosis. Diagnosing paediatric hypophosphatasia is aided by pathognomic radiographic changes when the skeletal disease is severe. Hypophosphatasia was the last type of rickets or osteomalacia to await a medical treatment. Now, significant successes for severely affected paediatric patients are recognized using asfotase alfa, a bone-targeted recombinant TNSALP.

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In 1948, the Canadian physician John C. Rathbun coined the term 'hypophosphatasia' to describe the developmental anomaly that killed his infant patient who presented at 2 months of age with rickets and seizures and had paradoxically low alkaline phosphatase activity in serum as well as in bone and other tissues at autopsy[1]. Soon after, it became apparent that hypophosphatasia was a heritable condition[2]. In 1953, premature loss of deciduous teeth emerged as a cardinal feature in affected children[3]. Subsequent delineation of the nature of the alkaline phosphatase deficiency in tissues established hypophosphatasia as an inborn error of metabolism[4] and thereby linked the enzyme to skeletal mineralization, as postulated in 1923 by its discoverer, Robert Robison[5]. This Review discusses the aetiology, nosology, pathogenesis, diagnosis and treatment of hypophosphatasia while emphasizing its remarkably broad-ranging severity.

Aetiology

All animals have alkaline phosphatase (systematic name, orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1)[6]. In humans, four genes account for alkaline phosphatases[7]. Three genes (*ALPI*, *ALPP* and *ALPPL2*) encode the tissue-specific intestinal, placental and germ-cell alkaline phosphatases, respectively, whereas the fourth gene (*ALPL*; also known as *TNSALP*) encodes tissue-nonspecific alkaline phosphatase (TNSALP), which is abundant in the skeleton, liver, kidney and developing teeth[7,8]. TNSALP is actually a family of isoforms that differ by post-translational modifications[7]. Although the official human gene mapping symbol is *ALPL*, which denotes alkaline phosphatase-liver, whether TNSALP has a function in the liver is not known[7].

TNSALP has 12 exons, 11 of which encode the 524 amino acid residue monomer[7,9], which contains one active

Key points

- Hypophosphatasia is the autosomal dominant or autosomal recessive inborn error of metabolism with an extraordinary range of severity caused by loss-of-function mutations within the gene that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP)
- Extracellular accumulation of the TNSALP substrate inorganic pyrophosphate results in defective mineralization of the dentition causing tooth loss and often of the skeleton causing rickets or osteomalacia
- Hypophosphatasaemia (low serum alkaline phosphatase activity) for age and sex is the biochemical hallmark
- An elevated serum level of the TNSALP substrate pyridoxal 5′-phosphate (the major circulating form of vitamin B_6) is expected
- TNSALP gene (*ALPL*; also known as *TNSALP*) mutation analysis is necessary to understand recurrence risks and for prenatal diagnosis
- Recombinant, bone-targeted TNSALP replacement has been shown to be effective for paediatric-onset hypophosphatasia

site[10] generated by evolutionarily conserved nucleotide sequences[11]. The bone and liver isoforms feature transcripts with different 5′-untranslated regions[12]. Basal expression of *TNSALP* reflects 'housekeeping' promoter activity, whereas enhanced expression involves post-transcriptional regulation[13]. Homodimerization of TNSALP monomers is required for the enzyme to have catalytic activity[7].

In vitro, alkaline phosphatases demonstrate broad specificity, pH optima and concentrations for the phosphocompound substrates they hydrolyse[6,14]. The rate-limiting step involves dissociation of covalently linked phosphate from a serine residue. Inorganic phosphate inhibits[6,10], yet stabilizes[15], the enzyme. In tissues, alkaline phosphatases tether at the cell surface to the polar head group of phosphatidylinositol-glycan, which is anchored within the plasma membrane[16]. Release of alkaline phosphatases from tissues occurs with exposure to phosphatidylinositol-specific phospholipase[7,16]. Soluble, lipid-free, homodimeric alkaline phosphatases in the circulation are presumably cleared by the liver[17]. In healthy adults, serum alkaline phosphatase predominantly comprises equal amounts of bone and liver TNSALP[18]. In healthy infants and children, and especially adolescents, the circulation is enriched with bone TNSALP[6].

Autopsy studies of hypophosphatasia years ago delineated the enzymopathy and thereby predicted its genetic basis[8]. Deficiency of alkaline phosphatase activity was found in bone, liver and kidney, whereas alkaline phosphatase activity in the intestine and placenta (in fetal trophoblasts) was normal[19–21]. This observation corroborated partial proteolytic peptide digest data from alkaline phosphatases purified from healthy organs[8] and indicated selective reduction of the activity of all TNSALP isoforms in hypophosphatasia[22].

In 1988, characterization of *TNSALP*[9] enabled discovery that same year of the first *TNSALP* mutation, found in a consanguineous infant with hypophosphatasia[23]. Now, at least 300 *TNSALP* mutations have been catalogued[24–26]. Autosomal dominant and autosomal recessive transmission of these defects generally explain mild versus severe hypophosphatasia, respectively[26]. Gene locus heterogeneity has not been reported for this disorder, and sporadic cases are rare[26]. Transfection studies have shown that certain *TNSALP* mutations diminish expression of the gene, compromise mRNA stability, lead to inactivation of the enzyme by altering its various domains, or result in intracellular sequestering of the nascent protein[27,28]. Some *TNSALP* mutations exert a dominant-negative effect, helping to explain instances of autosomal dominant inheritance of hypophosphatasia[27].

Nosology

Hypophosphatasia prevalence is highest among Mennonites in Manitoba, Canada, where approximately 1 in 25 individuals carries a *TNSALP* founder mutation and approximately 1 in 2,500 neonates manifests lethal hypophosphatasia[29]. In 1957, a report from Toronto, Canada, suggested that, more generally, severe hypophosphatasia affects 1 in 100,000 live births[2]. In the USA, hypophosphatasia seems to be significantly more prevalent in white than in black individuals[26,30], and two *TNSALP* missense defects account for many instances of autosomal dominant hypophosphatasia[31]. Perhaps several thousand children in the USA are affected, but predominantly with mild manifestations[26,31]. In 2011, *TNSALP* mutation analysis indicated severe and moderately severe hypophosphatasia in 1 in 300,000 and 1 in 6,370 Europeans, respectively[32]. Hypophosphatasia has been reported in Hispanic and Chinese people, and two *TNSALP* founder mutations have been identified in Japanese individuals[33].

Studies of at least several hundred patients with hypophosphatasia have delineated the disorder's major clinical, radiographic and biochemical features, as well as its extraordinary range of severity that spans from death *in utero* due to an unmineralized skeleton to dental complications or arthropathy without bone disease in adult life[8,26]. I regard hypophosphatasia as having the broadest expressivity of all skeletal diseases. Its prognosis is conditioned principally by the skeletal complications, which generally reflect patient age at presentation[2,8,22]. The clinical nosology of hypophosphatasia initiated by Donald Fraser in 1957 (REF. 2) has been expanded and refined[8,26]. Now, seven major forms guide recurrence risk estimation and prognostication[26]. Dental complications alone represent odontohypophosphatasia[3]. Then, ranked in order of increasing severity, 'adult', 'childhood', 'infantile' and 'perinatal' hypophosphatasia denote the patient's age when skeletal disease and other complications present[2,8]. 'Benign prenatal' hypophosphatasia importantly refers to skeletal deformity *in utero* or at birth that subsequently improves spontaneously *ex utero*[34]. In 2015, 'mild' versus 'severe' forms of childhood hypophosphatasia were differentiated after investigation of 173 affected children[26]. Extraordinarily rare 'pseudohypophosphatasia' resembles infantile hypophosphatasia, with the exception that serum alkaline phosphatase activity is normal or elevated when measured in the clinical laboratory[35,36]. Individuals with a defective *TNSALP* allele (some with biochemical features of hypophosphatasia), yet without

dento-osseous or arthritic complications, are 'carriers'[8]. Who among such carriers will eventually manifest complications and be diagnosed with hypophosphatasia might come from investigation of older family members[37,38]. Although this extant nosology for hypophosphatasia is useful, the principal forms described below all range in severity[26].

Odontohypophosphatasia

Odontohypophosphatasia, the mildest and probably most prevalent form of hypophosphatasia[26], features dental complications at any age without radiographic or histopathologic evidence of rickets or osteomalacia. Premature loss of one or more deciduous teeth (before the age of 5 years) occurs painlessly, bloodlessly and with tooth root intact, as the characteristic deficiency of mineralized cementum impairs tooth root linkage to the periodontal ligament[39]. Typically, lower and then upper incisors are shed first. Otherwise, individuals with odontohypophosphatasia have good health.

Adult hypophosphatasia

Adult hypophosphatasia typically presents during middle age[37,38,40]. However, some affected individuals know of early shedding of their deciduous teeth or a history of rickets. Loss of adult dentition is a common outcome[37,38]. Patients often describe how recurrent metatarsal stress fractures eventually failed to heal[37,38,40]. Subsequently, hip or thigh discomfort can indicate femoral pseudofractures (Looser's zones)[41], which are a hallmark of osteomalacia[42]. Femoral pseudofractures usually occur proximally and laterally in the subtrochanteric region in adults with hypophosphatasia[41–43] (FIG. 1), differing from their femoral neck predilection in other osteomalacias. In this way, these fractures in hypophosphatasia resemble the prodromal lesions of atypical femoral fractures sometimes encountered with antiresorptive therapy for osteoporosis[38,42]. Extracellular accumulation of inorganic pyrophosphate (discussed later in the article) can cause calcium pyrophosphate dihydrate (CPPD) deposition as well as pyrophosphate arthropathy, which includes pseudogout[44,45]. Calcific periarthritis represents seemingly paradoxical deposition of hydroxyapatite crystals in the vicinity of joints[46]. Ossification of ligaments (syndesmophytes) can resemble spinal hyperostosis (Forestier disease)[44,47]. Adult hypophosphatasia can become debilitating due to recurrent fracturing, skeletal and joint pain, and muscle weakness[48].

Childhood hypophosphatasia

Childhood hypophosphatasia presents after 6 months of age with especially wide-ranging expressivity that can be considered mild or severe[2,26,49]. Premature loss of some deciduous teeth occurs almost invariably[26,30]. In severe childhood hypophosphatasia, occasionally all deciduous teeth are shed early[26]. Rachitic deformities can include a misshapen skull[50], beading of costochondral junctions, bowed legs or knock-knees and enlarged joints from metaphyseal flaring. Skeletal pain can be significant. Short stature is sometimes present[26]. Muscle weakness can cause stiffness, delayed walking and a waddling gait[2,51]. Rarely, joint swelling and discomfort and the associated radiological findings mimic chronic recurrent multifocal osteomyelitis[52].

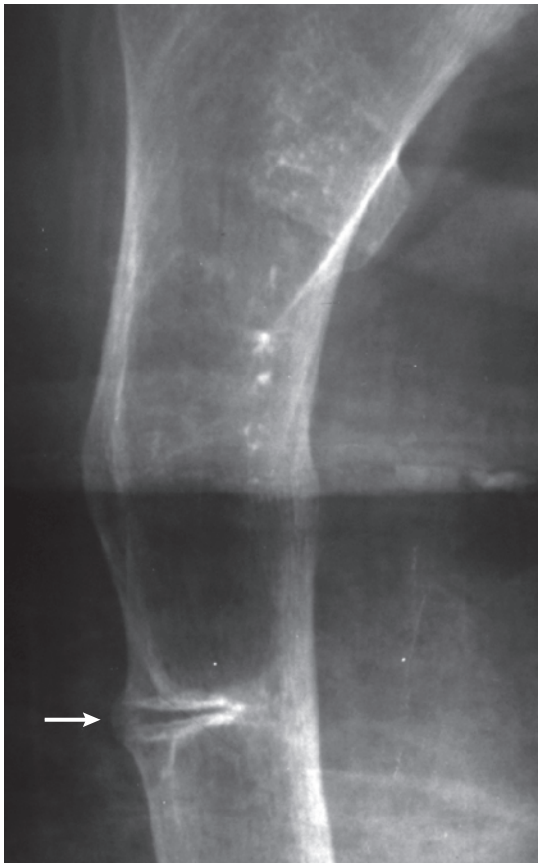

Figure 1 | **Adult hypophosphatasia.** Radiograph of a 57-year-old woman with an osteopenic proximal right femur and a characteristic pseudofracture traversing its lateral subtrochanteric cortex (arrow).

Radiographs of major long bones typically reveal characteristic 'tongues' of lucency projecting from growth plates into metaphyses[22,53] (FIG. 2a). Physes can be wide with irregularity of the provisional zone of calcification, and metaphyses can be flared with patchy osteopenia and osteosclerosis. Bony craniosynostosis can cause proptosis, raised intracranial pressure and cerebral damage[50]. The calvarium often features a 'beaten-copper' appearance (FIG. 2b). Pulp chambers and root canals can be enlarged, causing 'shell teeth'.

The manifestations of childhood hypophosphatasia are typically persistent during growth[54], but sometimes symptoms improve during young adult life, perhaps owing to fusion of growth plates. Permanent teeth fare well early on[55], but poorly characterized dental problems and skeletal disease featuring osteomalacia can eventually re-emerge[54].

Infantile hypophosphatasia

Infantile hypophosphatasia presents before 6 months of age[2,8]. Postnatal development can seem normal until poor feeding, failure to thrive, or weakness with delayed motor milestones accompany signs of rickets[56]. Fontanels can appear wide. Proptosis, mild hypertelorism, and

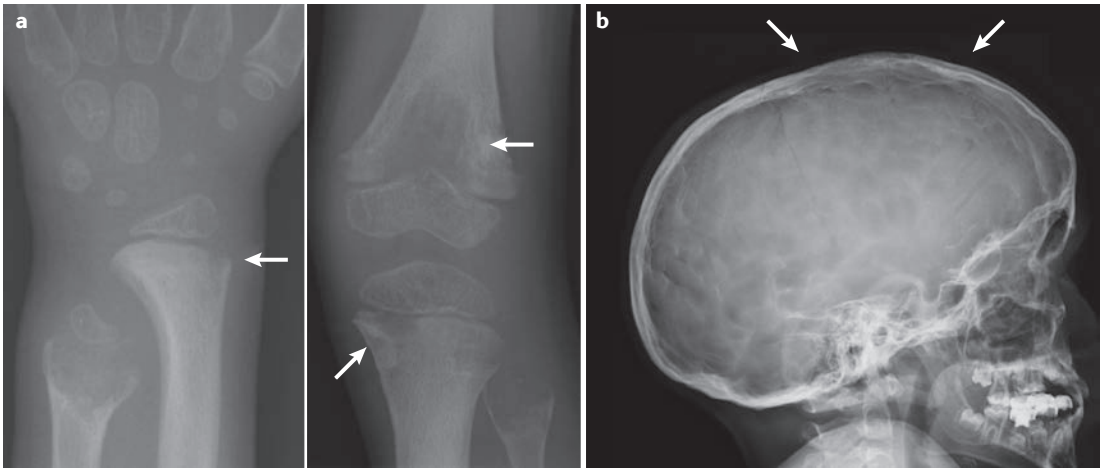

Figure 2 | **Childhood hypophosphatasia. a** | Radiographs of a 3-year-old girl showing characteristic changes of childhood hypophosphatasia, which include 'tongues' of radiolucency (arrows) projecting from physes into metaphyses at the left wrist (left panel) and left knee (right panel). Marked hypomineralization of the metaphyses of the distal ulna and the proximal fibula are also evident. **b** | Lateral radiograph of a 9-year-old boy showing an elongated (dolichocephalic) skull with the 'beaten-copper' appearance characteristic of pan-suture closure. A craniectomy site (arrows) is filling-in with bone. Wide pulp chambers in his teeth are consistent with the dental complications of hypophosphatasia.

brachycephaly can develop[50] (FIG. 3). Intracranial pressure can rise and lead to papilloedema. Hypercalcaemia and hypercalciuria resulting from blocked mineral entry into the skeleton can cause vomiting and sometimes nephrocalcinosis and renal compromise[2,56,57]. Progressive deformity of the thorax, rib fractures and tracheomalacia will predispose to pneumonia. Historically, it has been estimated that ~50% of babies with infantile hypophosphatasia will die in infancy[8,22].

Together, the radiographic changes are pathognomonic[53,56,57]. Abrupt transition from unremarkable diaphyses to poorly calcified metaphyses suggests a sudden metabolic deterioration (FIG. 4a). Fractures and bone deformities can accompany progressive skeletal demineralization[56,57]. Skeletal scintigraphy indicates functional closure of cranial sutures if decreased tracer uptake is associated with their 'widened' appearance on radiographs[58] (FIG. 4b). Premature bony fusion of sutures can follow after infancy[50,53].

Now, a lethal outcome can be predicted if there are pyridoxine-dependent seizures, which sometimes manifest before skeletal changes[59], or worsening skeletal disease that will probably cause respiratory complications[56,60].

Perinatal hypophosphatasia

Perinatal hypophosphatasia, the most extreme form of hypophosphatasia, manifests *in utero*, is obvious at birth, and is nearly always fatal soon after[56,60]. Caput membraneceum and limbs that are short and deformed reflect profound skeletal hypomineralization (FIG. 5). A high-pitched cry, pyridoxine-dependent seizures, periodic apnea with cyanosis and bradycardia, unexplained fever, irritability, myelophthisic anaemia and intracranial haemorrhage are additional features[61–63]. Sometimes the lungs are hypoplastic[63].

The radiographic findings are pathognomonic[53,56], excluding severe osteogenesis imperfecta and skeletal dysplasias, yet vary from patient to patient[62]. Occasionally, nearly all bones appear completely unmineralized (FIG. 6a), or extreme rickets occurs where poorly calcified epiphyses accompany large tongues of radiolucency projecting into metaphyses (FIG. 6b). Calvarial bones can appear mineralized only centrally, suggesting wide cranial sutures that instead are functionally closed[53].

Pseudohypophosphatasia

Pseudohypophosphatasia resembles infantile hypophosphatasia, with the exception that serum alkaline phosphatase activity is within the normal range or increased by the nonphysiological conditions used in clinical laboratories to assay this enzyme[35,36,62,64]. Most reports of pseudohypophosphatasia, however, seem to represent failure to recognize the need for age-dependent reference ranges for assessing serum alkaline phosphatase activity, perhaps transient correction of hypophosphatasaemia as a result of fracture or intercurrent illness, or overemphasis concerning slight elevations in levels of TNSALP substrates[65,66].

Benign prenatal hypophosphatasia

In 2011, an assessment of 17 patients with benign prenatal ('bent but not broken') hypophosphatasia plus a review of the literature showed that sometimes bone deformity *in utero*, although worrisome for perinatal hypophosphatasia, improves postnatally with a clinical course instead ranging from infantile hypophosphatasia to odontohypophosphatasia[34]. Ultrasonography early in pregnancy had considerable uncertainty for hypophosphatasia prognostication[34].

Pathogenesis

Delineation of the pathogenesis of hypophosphatasia has provided the greatest understanding of alkaline phosphatase physiology[22]. However, the discoverer of this enzyme, Robert Robison[5], knew in 1923 that his assay for alkaline phosphatase using colorimetric substrates at high

pH was not physiological, and called the enzyme 'bone phosphatase' (REF. 67). The term alkaline phosphatase was coined afterwards, probably when the method was applied to serum to detect and monitor patients with high levels of the enzyme as a result of skeletal and hepatobiliary disease[6]. Now, nearly a century later, clinical and research laboratory assays for alkaline phosphatase persist in using high concentrations (in the mM range) of substrates like *p*-nitrophenylphosphate in inorganic phosphate-free buffers (pH 9.2–10.5)[6,22]. Actually, the pH optima of alkaline phosphatases are less basic for natural substrates at physiologic concentrations[6,22]. The term alkaline phosphatase is misleading, yet entrenched.

Alkaline phosphatase and mineralization

By 1932, Robert Robison hypothesized that skeletal mineralization is regulated not only by local increases in inorganic phosphate concentrations due to the phosphohydrolase action of bone phosphatase[5], but also by a second important, yet unknown, factor[67]. This factor, as explained below, would prove to be inorganic pyrophosphate, which alkaline phosphatases hydrolyse[8,22]. In 2005, the necessity for alkaline phosphatase to be near fibrillar collagens for biomineralization explained why alkaline phosphatase-rich tissues such as liver, intestine and placenta do not calcify[68].

Healthy osteoblasts and hypertrophic chondrocytes richly express the bone isoform of TNSALP[7]. In the 1960s, H. Clarke Anderson used electron microscopy to reveal that hydroxyapatite crystal formation in the skeleton begins within alkaline phosphatase-rich structures called matrix vesicles representing buds from their plasma membrane[69]. Hydroxyapatite crystal growth then ruptures the matrix vesicles, continues extravesicularly and mineralizes osteoid[69]. Thus, skeletal mineralization normally comprises phase 1 (within matrix vesicles) and phase 2 (nearby)[70].

Impaired mineralization of skeletal matrix in neonates, infants, children and adolescents, irrespective of cause, disrupts endochondral and intramembranous bone formation and causes rickets. Onset in adults (that is, after physeal fusion) causes osteomalacia. Nearly all types of rickets and osteomalacia feature low extracellular levels of calcium and/or inorganic phosphate and an increase, seemingly compensatory, in bone and circulating alkaline phosphatase activity (hyperphosphatasaemia). Hypophosphatasia is a striking and contrary exception.

In 1955, rachitic cartilage isolated from rats with vitamin D deficiency was reported to calcify in serum from patients with hypophosphatasia, but costochondral junctions isolated from patients with hypophosphatasia failed to mineralize in healthy serum or calcifying medium[71]. The explanation for the mineralization defect in hypophosphatasia thus seemed intrinsic to bone[71]. In 1966, Herbert Fleisch and Graham Russell discovered that inorganic pyrophosphate impaired hydroxyapatite crystal growth and inhibited mineralization[72,73]. Soon after, they found high blood and urinary levels of inorganic pyrophosphate in patients with hypophosphatasia[73]. Concurrently, TNSALP was recognized to be an inorganic pyrophosphatase[74]. Starting in 1988 (REF. 23), documentation of loss-of-function mutations within *TNSALP* in patients with hypophosphatasia proved, 65 years after its discovery, that alkaline phosphatase is required for bone and tooth mineralization[22]. Premature loss of the primary dentition occurs in other metabolic errors, toxicities and malignancies[75], but in hypophosphatasia is caused by deficiency of mineralized acellular cementum[39]. The numbers of deciduous teeth lost prematurely by children with hypophosphatasia parallels the severity of their disease overall[26].

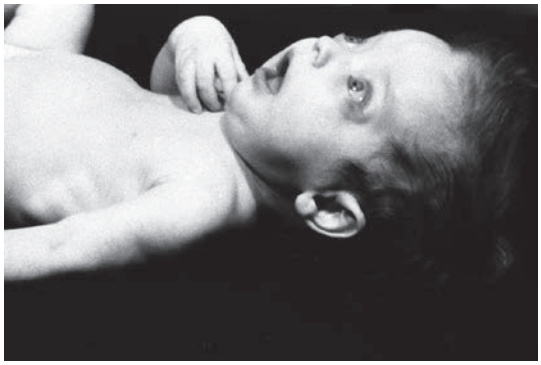

Figure 3 | **Infantile hypophosphatasia.** This 4-month-old girl has developed characteristics of infantile hypophosphatasia, which include a prominent anterior fontanel, proptotic eyes, a scaphoid chest and rachitic changes at the costochondral junctions.

Although the liver and adrenal glands contain considerable TNSALP activity[6], their functioning seems uncompromised in hypophosphatasia. Routine laboratory testing of liver and muscle health is usually normal. The purpose of the three tissue-specific alkaline phosphatases is not well understood[7].

Mineral homeostasis

In infantile hypophosphatasia[76], the blocked entry of minerals into the skeleton resulting from excessive extracellular levels of inorganic pyrophosphate often unmasks a type of absorptive hypercalcaemia and/or hypercalciuria in which serum parathyroid hormone (PTH) levels are appropriately reduced[56]. In childhood hypophosphatasia, this perturbation is less common and milder. Thus, circulating levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and PTH are usually unremarkable[77,78]. Although individuals with childhood or adult hypophosphatasia are typically eucalcaemic with normal serum PTH levels, most have above-average, and sometimes distinctly elevated, serum levels of inorganic phosphate. The hyperphosphataemia results from enhanced renal reclamation of inorganic phosphate (calculated as increased tubular maximum phosphorus (P)/glomerular filtration rate; that is, TmP/GFR)[43,79]. Perhaps, TNSALP directly facilitates renal excretion of inorganic phosphate[80]. Alternatively, excessive systemic or urinary inorganic pyrophosphate might somehow increase renal reclamation of inorganic phosphate in patients with hypophosphatasia[80]. Notably, in generalized arterial calcification of infancy[4], low extracellular (and perhaps urinary) inorganic pyrophosphate levels occur with acquired hypophosphataemic rickets[81].

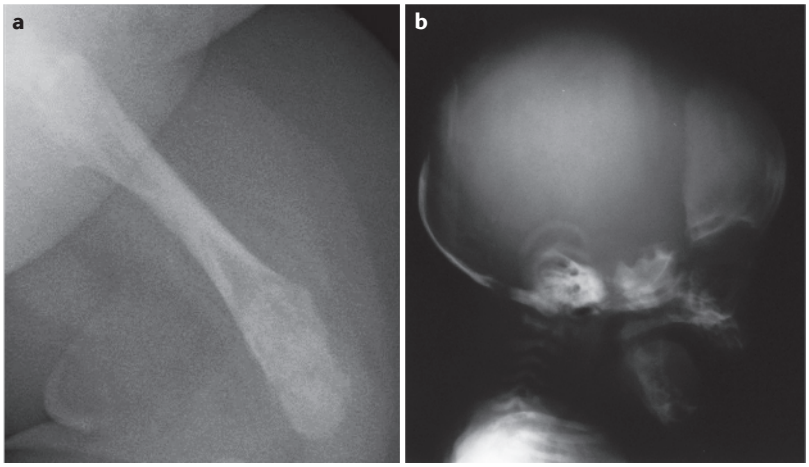

Figure 4 | **Radiographic features of infantile hypophosphatasia. a** | Radiograph of the left femur of a 3-month-old girl shows transition from a properly shaped segment of the diaphyseal shaft to hypomineralized and distorted proximal and distal metadiaphyses, which suggests a sudden metabolic change disrupting the more recently formed endochondral bone. **b** | Lateral radiograph of the skull of the same infant shows hypomineralization, especially posteriorly. Consequently, the fontanels appear wide.

Natural substrates of TNSALP

The discoveries of the three phosphocompounds that accumulate in hypophosphatasia clarified the disorder's metabolic basis and the physiological role of TNSALP[8,22]. First, in 1955, increased levels of phosphoethanolamine in urine became the second biochemical marker for hypophosphatasia[71,82]. Then, high levels of inorganic pyrophosphate were documented in 1965 and 1971 in the urine[83] and blood[73], respectively, of patients with hypophosphatasia. Lastly, in 1985, elevated plasma levels of pyridoxal 5′-phosphate[84] revealed that TNSALP has an important role in vitamin B_6 metabolism, and as a cell-surface enzyme[84,85]. Phosphoethanolamine and pyridoxal 5′-phosphate were subsequently also shown by *in vitro* studies to be substrates of TNSALP[86,87].

Phosphoethanolamine. In health, phosphoethanolamine is excreted with essentially no renal threshold[88]. Yet, in hypophosphatasia, blood and especially urinary levels of phosphoethanolamine are increased. Phosphoethanolamine might originate from degradation of the phosphatidylinositol moiety that couples proteins to cell surfaces[22]. Alternatively, the source could be the liver, which requires pyridoxal 5′-phosphate to catabolize phosphoethanolamine[89]. In a family with adult hypophosphatasia, urinary phosphoethanolamine excretion correlated inversely with serum levels of the liver but not the bone isoform of TNSALP[18].

Pyridoxal 5′-phosphate. In healthy individuals, the various dietary forms of vitamin B_6 undergo hepatic conversion to pyridoxal 5′-phosphate, which then circulates largely bound to albumin, but cannot traverse plasma membranes unless it is first dephosphorylated to pyridoxal[85] (FIG. 7). In the cytoplasm, pyridoxal is rephosphorylated in order to function as a cofactor for many enzymatic reactions[85]. Despite their unprecedented high levels of circulating pyridoxal 5′-phosphate, children and adults with hypophosphatasia lack symptoms of vitamin B_6 toxicity or deficiency[84], and respond normally to L-tryptophan loading used to detect vitamin B_6 deficiency (M. P. Whyte and S. P. Coburn, unpublished work). Furthermore, the vitameric forms of vitamin B_6 are present at normal levels in tissues[90] and fibroblasts[86] isolated from patients with hypophosphatasia, and urinary excretion of the vitamin B_6 degradation product 4-pyridoxic acid is normal[84]. These observations seem to reflect that all but the most severely affected patients with hypophosphatasia have, by some mechanism, normal circulating levels of pyridoxal[84]. The exceptions, some patients with perinatal or infantile hypophosphatasia, can have circulating pyridoxal levels too low for γ-carboxyglutamic acid neurotransmitter synthesis within their central nervous system, leading to pyridoxine-dependent seizures[59].

Inorganic pyrophosphate. The discovery of high circulating[73] as well as urinary[83] (and presumably skeletal) inorganic pyrophosphate levels in hypophosphatasia explained the impaired mineralization that causes rickets and osteomalacia[72]. Inorganic pyrophosphate adsorbs to amorphous calcium phosphate and prevents its transformation into hydroxyapatite crystals[72]. Additionally, hydroxyapatite crystal growth and dissolution are impaired. The high levels of inorganic pyrophosphate in hypophosphatasia also explain the associated CPPD deposition, chondrocalcinosis, pseudogout and pyrophosphate arthropathy[14,44,45]. Alkaline phosphatases can dissolve CPPD crystals *in vitro*[14], reflecting an additional pathogenetic factor in hypophosphatasia. Furthermore, increased levels of inorganic pyrophosphate in certain tissues seem paradoxically to enhance the formation of amorphous calcium phosphate[91] and explain the calcific periarthritis and ligamentous calcification of some adults with hypophosphatasia[44–46].

Although the pathogenesis of muscle weakness in severe hypophosphatasia is uncertain[22,51], toxicity from especially the first-generation inorganic pyrophosphate analogue, etidronate, recapitulates key features of hypophosphatasia (that is, defective skeletal mineralization causing rickets or osteomalacia, muscle weakness with a waddling gait and increased TmP/GFR with hyperphosphataemia)[80].

Alkaline phosphatase in the circulation

Alkaline phosphatase within the circulation seems physiologically unimportant[22]. In the 1980s, critically ill babies with infantile hypophosphatasia infused intravenously with soluble bone or placental alkaline phosphatase achieved normal or high serum alkaline phosphatase activity, but this attempted treatment failed to correct elevated urinary phosphoethanolamine or inorganic pyrophosphate levels or high plasma pyridoxal 5′-phosphate concentrations and there was no significant clinical or radiographic improvement[22,57].

Epigenetic and nongenetic effects

Although hypophosphatasia generally 'breeds true', significant discordance for hypophosphatasia sometimes accompanies an identical *TNSALP* genotype within sibships[92,93].

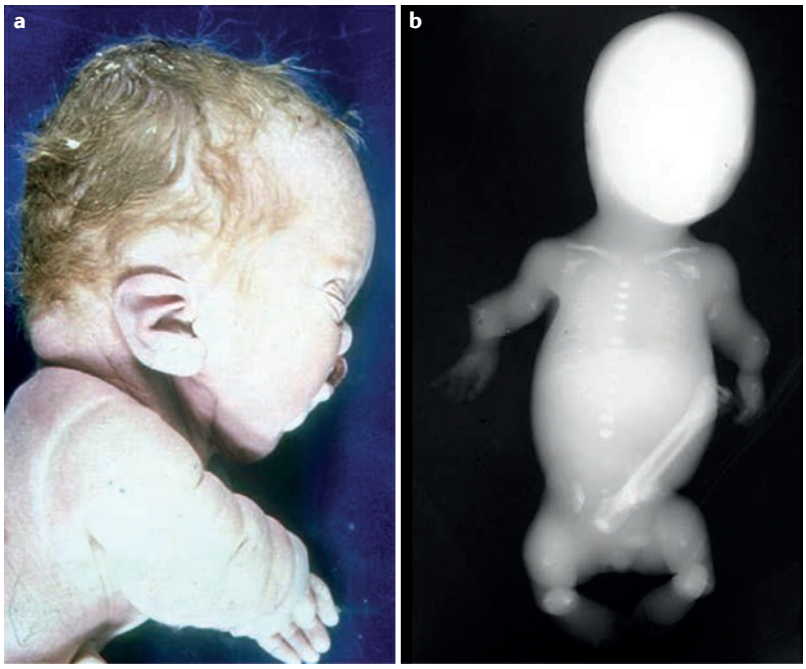

Figure 5 | **Perinatal hypophosphatasia. a** | Stillborn neonate showing profound deformities that cause accordion-like skinfolds of the arm, which are explained by lack of underlying bony structure. **b** | Almost no mineralization of the skeleton is revealed by radiographic examination.

Thus, additional factors can considerably affect hypophosphatasia expressivity. Generation of extracellular inorganic pyrophosphate is complex and involves a variety of genes in addition to *TNSALP* that need to be studied in this regard[94]. Hypothetically, dietary calcium and inorganic phosphate levels could affect circulating PTH levels and thereby condition TNSALP biosynthesis by chondrocytes and osteoblasts. Similarly, inorganic phosphate is a competitive inhibitor of TNSALP[22], and dietary levels could influence its extracellular concentration. Investigation of benign prenatal hypophosphatasia suggested that mechanical forces (for example, 'fetal packing'), as well as the biochemical environment of a carrier or affected mother, can harm an affected fetus[34]. Possibly, the physiological decrease in serum alkaline phosphatase activity after puberty or senescence of bone cells with ageing accounts for emergence of osteomalacia in former paediatric patients or 'carriers'.

Mouse models of hypophosphatasia

In the 1990s, use of homologous recombination to knock out *Alpl* (encoding murine TNSALP) generated two mouse models of lethal infantile hypophosphatasia[95]. These mice accumulate inorganic pyrophosphate, phosphoethanolamine and pyridoxal 5′-phosphate endogenously, and develop severe rickets and pyridoxine-dependent seizures soon after birth[95]. Cross-breeding these mice with mice possessing other genetic disturbances has clarified how inorganic pyrophosphate metabolism is regulated[7,94]. In 2007, an *N*-ethyl-*N*-nitrosourea murine model exhibited late-onset defective endochondral bone mineralization and knee and shoulder arthropathy[96,97]. Newer murine models for the various milder clinical forms of hypophosphatasia are now being generated, and will be helpful for assessing medical treatment for this disorder[96,98].

Diagnosis

Hypophosphatasia has been diagnosed reliably for nearly 70 years when persistent hypophosphatasaemia matches a medical history, physical examination, routine laboratory studies and radiographic findings consistent with the diagnosis[8]. Even odontohypophosphatasia is identified in this way. The hypophosphatasaemia stands out as paradoxical for a form of rickets or osteomalacia universally known to be associated with hyperphosphatasaemia. For perinatal and infantile hypophosphatasia, umbilical cord blood can be assayed[99,100]. Accumulation of TNSALP substrates is expected in hypophosphatasia, and is best marked by elevated levels of pyridoxal 5′-phosphate in the circulation[8]. The degree of hypophosphatasemia and substrate accumulation generally correlate with the severity of hypophosphatasia[22]. *TNSALP* mutation or deletion analysis is expected to reveal a defect (or defects) in all patients with hypophosphatasia[26].

Serum alkaline phosphatase activity

Blood must be collected correctly to assay serum alkaline phosphatase activity (BOX 1) because chelation of Mg^{2+} or Zn^{2+} will deactivate the enzyme[6,22,96,100]. Serum levels must be interpreted using reference ranges that are age-specific and sex-specific because puberty occurs earlier in girls than in boys[6]. Although the problem is waning, some clinical laboratories use only adult reference ranges and therefore infants or children with hypophosphatasia can go undiagnosed or be erroneously diagnosed with pseudohypophosphatasia[35]. Remarkably, some laboratories still provide no lower limit for serum alkaline phosphatase activity. Hypophosphatasaemia can also result from use of certain drugs (such as glucocorticoids, chemotherapy, clofibrate, tamoxifen or bone antiresorptives), massive transfusion of blood or plasma, milk–alkali syndrome, radioactive heavy metal poisoning or vitamin D toxicity[100,101] (BOX 1). Some disorders that can cause hypophosphatasaemia also feature bone disease, such as neonates with severe osteogenesis imperfecta[102] or quiescent osteoblast function in cleidocranial dysplasia[103]. Theoretically, conditions that increase serum alkaline phosphatase activity (for example, pregnancy, hepatobiliary disease, orthopaedic surgery and fracture) can obscure the diagnosis of hypophosphatasia[22]. Quantification of serum alkaline phosphatase isoenzymes and isoforms might be helpful in such circumstances[7]. Searching the patient's or family's medical records for hypophosphatasaemia could also be revealing.

TNSALP substrate accumulation

Documentation of an elevated phosphoethanolamine level in blood or urine supports a diagnosis of hypophosphatasia[88]. This test is offered by some commercial or 'inborn error' laboratories as a component of amino acid profiling. However, phosphoethanolamine excretion is conditioned by age, diet and circadian rhythm, and can be unremarkable in mild hypophosphatasia[61] and elevated in other metabolic bone diseases[104].

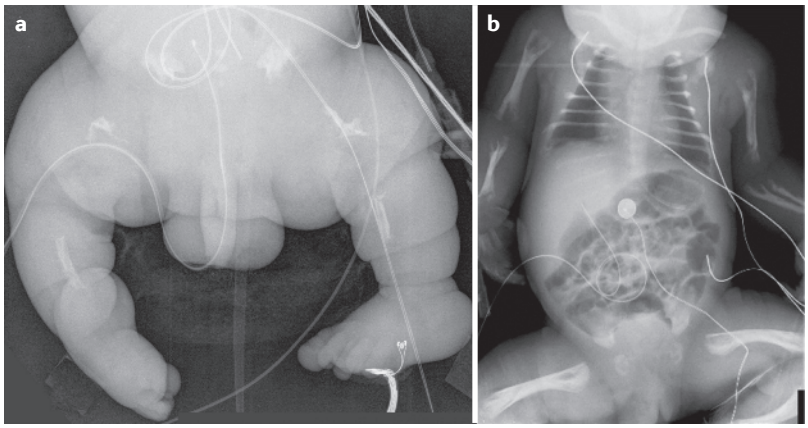

Figure 6 | **Radiographic features of perinatal hypophosphatasia. a** | Skeletal mineralization is nearly absent in this neonate. Only small bony areas are apparent in the pelvis, femurs and tibias. **b** | Noticeable rickets is present in this neonate. Pathognomonic metaphyseal 'tongues' of radiolucency, gracile ribs and long-bone deformities are apparent.

An elevated serum pyridoxal 5′-phosphate level is a more sensitive and specific biochemical marker for hypophosphatasia[56,84,85] and the value generally reflects the severity of the disease[100]. Even odontohypophosphatasia features this finding[84]. Assaying serum pyridoxal 5′-phosphate seems to be particularly helpful when hypophosphatasaemia has an explanation other than hypophosphatasia, because an elevated level might be expected exclusively in hypophosphatasia as the activities of all TNSALP isoforms, not just from bone, are low (M. P. Whyte, unpublished work). The other causes of hypophosphatasaemia (BOX 1) seem to particularly suppress bone TNSALP activity. Serum pyridoxal 5′-phosphate quantification can be obtained from commercial laboratories by ordering an assay of 'vitamin B_6.' False-positive elevations are avoided if any vitamin B_6 supplementation can be stopped 1 week before blood is collected[85,105]. An especially elevated serum level after pyridoxine loading has been used to detect particularly well patients with hypophosphatasia and carriers of the disease[105].

Currently, assays for inorganic pyrophosphate are carried out only in research laboratories. Urinary levels of inorganic pyrophosphate are elevated in most patients with hypophosphatasia[91,100] and reportedly are a sensitive marker for detecting carriers of hypophosphatasia[106].

Nowadays, positive *TNSALP* structural mutation (rarely deletion) analysis in research and commercial laboratories can help recognize patients or carriers with hypophosphatasia, but establishing the diagnosis requires documentation of one or more of the disorder's complications.

Radiological findings

Skeletal radiographs reveal pathognomonic changes in perinatal, infantile and severe childhood hypophosphatasia[56,107] (FIGS 2,4,5b,6). Adult hypophosphatasia features osteopenia, poorly-healing metatarsal stress fractures, and pseudofractures that commonly involve the lateral subtrochanteric region of the femurs[37,38,40–43,45] (FIG. 1). CPPD deposition, pyrophosphate arthropathy and calcific periarthritis can also appear in affected adults[46]. Bone scanning will reveal fractures and pseudofractures, and can aid detection of craniosynostosis early in life[58]. MRI helps to identify the rare, painful, bone marrow oedema syndrome in children with hypophosphatasia[52]. Dual-energy X-ray absorptiometry (DXA) can be difficult to interpret, especially if there is significant bone deformity or short stature[26]. In 2012, we published simple equations for calculating 'height–age' values to 'correct' DXA results in prepubertal children, including those with hypophosphatasia[108]. In affected adults, assessments of BMD can be distorted by the underlying osteomalacia.

Histopathological findings

Hypophosphatasia causes hypomineralization of hard tissues[49,69,70,96]. Weak muscles appear normal on routine laboratory testing[22,51]. Abnormalities elsewhere result predominantly from skeletal deformities and fractures, craniosynostosis, inorganic pyrophosphate crystal deposition and, sometimes, hypercalcaemia and hypercalciuria. In perinatal hypophosphatasia featuring a profoundly distorted thorax, the lungs can be hypoplastic[63] and extramedullary haematopoiesis might reflect marrow space crowding by excessive osteoid[49,70].

Skeleton. Un-decalcified bone specimens, acquired after two courses of tetracycline are administered orally to the patient[49,70], show impaired mineralization, except in odontohypophosphatasia[37,45,49]. Excessive osteoid, sometimes in a patchy distribution, and diffuse or absent tetracycline fluorescence characterize the bone surfaces[49]. Features of hyperparathyroidism, such as peritrabecular fibrosis, typical of most rickets or osteomalacias are absent in hypophosphatasia[49]. Diminished skeletal alkaline phosphatase activity assessed histochemically correlates with the degree of osteoid accumulation and reflects the clinical nosology[49].

The cellular sources of bone TNSALP (that is, chondrocytes and osteoblasts) are typically present, but osteoblast numbers and morphology vary, as does the amount of osteoid[49]. Some affected adults show very few osteoblasts[37]. Rachitic changes can include disruption of the columnar arrangement of physeal chondrocytes, widening of the zone of provisional calcification, and failure of primary spongiosa to calcify[49,70]. Unmineralized cartilage can project into metaphyses from physes and be encased in unmineralized bone. Electron microscopy of the perinatal and infantile hypophosphatasia skeleton reveals normally distributed collagen, proteoglycan granules and matrix vesicles[49,69,70]. The matrix vesicles lack alkaline phosphatase activity, but contain hydroxyapatite crystals[70]. By contrast, only isolated or tiny groups of hydroxyapatite crystals (calcospherites) are seen nearby[70]. Thus, phase 1 intravesicular skeletal mineralization is intact, but phase 2 extravesicular mineralization, which features growth of hydroxyapatite crystals, is compromised in hypophosphatasia[8,22].

Dentition. Deciduous teeth from patients with hypophosphatasia, even if shed and desiccated, are useful to examine. Despite the presence of cells that appear like

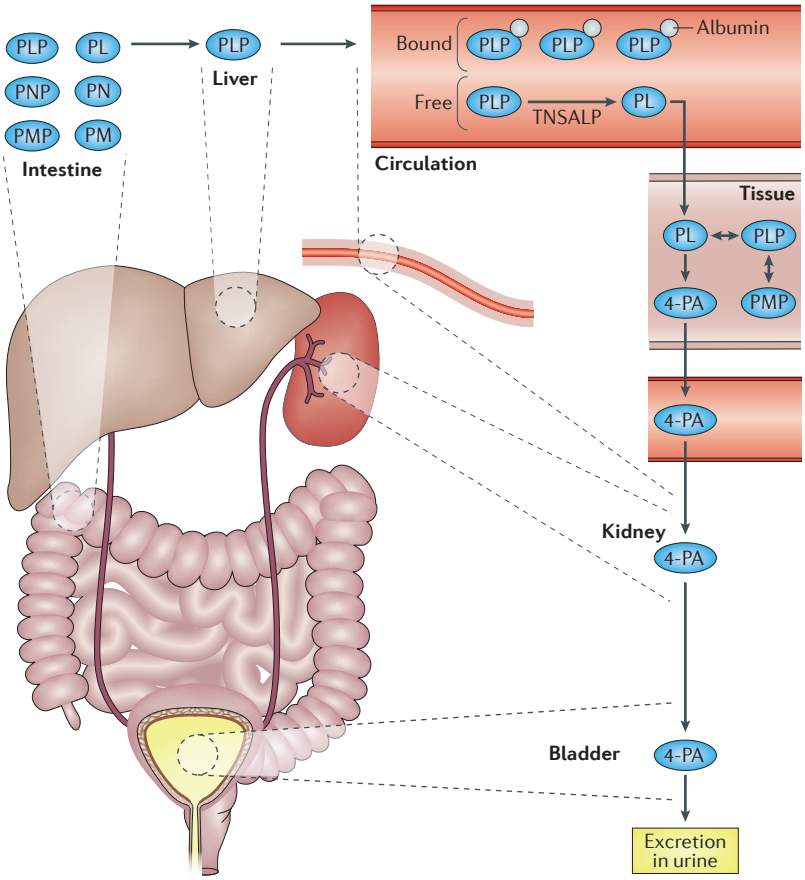

Figure 7 | **Role of TNSALP in vitamin B_6 metabolism.** The phosphorylated forms of vitamin B_6 in the diet, pyridoxal, pyridoxine and pyridoxamine phosphate (PLP, PNP and PMP, respectively) are dephosphorylated in the intestine and then absorbed into the hepatic portal circulation. In the liver, unphosphorylated PL, PN, and PM are converted to PLP, which is secreted into the plasma largely bound to albumin. Before entering tissues, free PLP in the plasma must be dephosphorylated to PL, which can traverse membranes and be phosphorylated to PLP and PMP to act intracellularly as cofactors. 4-pyridoxic acid (4-PA), the major degradation product of vitamin B_6, is excreted in the urine. Elevated plasma levels of PLP in hypophosphatasia, yet normal plasma concentrations of PL, are consistent with a cell-surface role for the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) in the extracellular dephosphorylation of PLP to PL.

cementoblasts, the cementum is afibrillar and variably deficient from tooth to tooth[109]. Dentin tubules can be few and enlarged. Big pulp chambers suggest retarded dentinogenesis. Excessive width of predentin, increased amounts of interglobular dentin, and impaired calcification of cementum seem analogous to the osteoidosis in bone. Enamel is also compromised[75]. Changes in the permanent teeth are milder than those in the deciduous teeth[55,109].

TNSALP mutation analysis

Although hypophosphatasia can typically be diagnosed without *TNSALP* mutation analysis[26], this information is crucial for documenting the inheritance pattern and recurrence risk, and for prenatal assessment when requested[26,34]. In my experience, all patients with unequivocal hypophosphatasia carry one or two mutations in *TNSALP*[26,56]. For first occurrences of hypophosphatasia, mutation analysis must examine all splice sites and coding exons of *TNSALP*; infrequently, large deletions need to be searched for. Sporadic cases and those from uniparental isodisomy are quite rare[25,110].

Prenatal diagnosis

Cordocentesis to quantitate fetal circulating alkaline phosphatase and pyridoxal 5′-phosphate is untested for the prenatal diagnosis of hypophosphatasia. Amniotic fluid contains predominantly fetal intestinal alkaline phosphatase, and therefore assaying its alkaline phosphatase is unsuitable[111,112]. Chorionic villus samples studied with a monoclonal antibody to TNSALP proved unreliable[113].

During the second trimester, perinatal hypophosphatasia has been diagnosed using ultrasonography (focusing on the limbs and skull) and radiography[34]. Ultrasonographs could, however, appear normal at 16–19 weeks gestation, yet near-term radiographs reveal no skeleton[114]. Although several early assessments reported that skeletal abnormalities detected *in utero* predicted a fatal outcome, an investigation in 2011 of benign prenatal hypophosphatasia showed that even autosomal recessive inheritance of hypophosphatasia or skeletal abnormalities on first trimester ultrasonography do not reliably predict lethality[34]. Delineation of benign prenatal hypophosphatasia has revealed important uncertainties about outcomes, especially when two defective *TNSALP* alleles are present[34]. Since 1995, *TNSALP* mutation analysis has helped assess pregnancies at risk of hypophosphatasia[115,116].

Treatment

As reviewed below, enzyme-replacement therapy (asfotase alfa) was approved in 2015 in Japan to treat hypophosphatasia, and soon after in Canada, the European Union and the USA to treat paediatric-onset hypophosphatasia. Otherwise, patient care is typically supportive[8].

Prognosis

In 2013, a retrospective review of 15 untreated patients (Mennonites from Manitoba, Canada) with perinatal hypophosphatasia confirmed rapid fatality in all[117]. Before the early successes to treat life-threatening hypophosphatasia reported in 2003 (REF. 118) and 2007 (REF. 119) using marrow cell transplantation and then in 2012 (REF. 56) using asfotase alfa, skeletal deterioration[57] with respiratory complications or vitamin B_6-dependent seizures[58] indicated a fatal outcome within the first year of life for the majority of patients with infantile hypophosphatasia[8,100]. Seizures of this type occurred because TNSALP deficiency was especially profound[60,84]. In survivors of infantile hypophosphatasia, rachitic disease often persists, but sometimes improves spontaneously[100]. In 1986, a preliminary report from Canada indicated that some patients with infantile hypophosphatasia fare well and achieve normal adult height[120]. Perhaps any endogenous TNSALP activity becomes more effective after the particularly rapid increase in body size that characterizes infancy passes. When infantile hypophosphatasia is first encountered,

Box 1 | **Causes of hypophosphatasaemia**

- Cardiac bypass surgery
- Coeliac disease
- Clofibrate therapy
- Cleidocranial dysplasia
- Cushing syndrome
- Hypophosphatasia
- Hypothyroidism
- Improperly collected blood (oxalate, EDTA*)
- Inappropriate reference range
- Massive transfusion
- Milk-alkali syndrome
- Multiple myeloma
- Osteogenesis imperfecta, type II
- Pernicious or profound anaemia
- Radioactive heavy metals
- Starvation
- Vitamin C deficiency
- Vitamin D intoxication
- Wilson disease
- Zn^{2+} or Mg^{2+} deficiency

*EDTA, ethylenediaminetetraacetic acid

clinical and radiographic assessments (perhaps monthly) have been crucial for prognostication[56,100]. Perhaps 50% of these patients die in infancy, typically from respiratory complications[8,100]. In 2016, a retrospective study confirmed a high mortality for perinatal and infantile hypophosphatasia if chest deformity, respiratory difficulties, or vitamin B_6-dependent seizures manifest before 6 months of age[60]. In childhood hypophosphatasia, signs and symptoms typically persist, but sometimes improve when growth plates fuse[2]. However, complications from osteomalacia can eventually emerge[2,48]. Adult hypophosphatasia is a lingering condition that sometimes becomes debilitating[37,41,48].

Supportive

Mechanical ventilation can be especially challenging for the most severely affected babies with hypophosphatasia owing to thoracic deformity, weakness, fractures, tracheomalacia, and perhaps pulmonary hypoplasia[121]. Vitamin B_6-dependent seizures can respond, but only temporarily, to pyridoxine administration[59]. Hypercalcaemia may improve with reduction of dietary calcium and then, if needed, hydration, loop diuretics or glucocorticoid therapy[53,56,57]. Synthetic salmon calcitonin[122] has little benefit, perhaps because bone resorption is already blocked by the excessive osteoid covering bone surfaces. Bisphosphonates are analogues of inorganic pyrophosphate and could further impair mineralization, slow bone turnover, or bind Zn^{2+} or Mg^{2+}, and thus compromise any residual TNSALP activity[38,80].

Patients with infantile hypophosphatasia must be followed carefully in order to detect neurological complications from 'functional' craniosynostosis[50] that could require craniotomy[53]. Severely affected young children with hypophosphatasia might need craniectomy for neurological complications resulting from bony craniosynostosis[50]. Expert dental care is important. Loss of many teeth can impair speech and nutrition. Dentures are sometimes necessary[39]. Also, bacteria on teeth can accelerate tooth loss. Fractures often mend, but delayed healing has been observed following osteotomy. Naproxen might diminish skeletal pain, including that from bone marrow oedema[52,123].

In adults with hypophosphatasia, pseudofractures or completed fractures of the femur heal best with load-sharing intramedullary fixation, whereas load-sparing plates can be problematic[41]. Ankle-foot orthoses are often applied for metatarsal stress fractures. Symptoms resulting from CPPD or hydroxyapatite crystal deposition sometimes respond to nonsteroidal anti-inflammatory medication[44,46].

Medical

Hypophosphatasia was the last form of rickets or osteomalacia to await a medical treatment[100]. In fact, conventional regimens for impaired skeletal mineralization (for example, vitamin D and mineral supplements) seem best avoided in hypophosphatasia unless specific deficiencies are identified[56,100]. Circulating levels of calcium, inorganic phosphate and vitamin D are usually not low, and excessive supplementation could provoke or exacerbate any hypercalcaemia, hypercalciuria or hyperphosphataemia encountered especially in patients with infantile hypophosphatasia[56]. However, restriction of vitamin D intake or sunshine exposure should be avoided[78].

Several treatment approaches have been attempted for hypophosphatasia. Decades ago, normalization of serum alkaline phosphatase activity and radiographic improvement was briefly described for cortisone given for severe paediatric hypophosphatasia[2,124]. Then, alkaline phosphatase replacement was tested in the 1980s using intravenous infusions of various soluble alkaline phosphatases[57,125,126]. However, plasma rich in bone TNSALP from patients with Paget's bone disease had no significant clinical or radiographic benefit for four infants who subsequently succumbed to hypophosphatasia[125]. In 1992, alkaline phosphatase purified from a human placenta and administered intravenously to a boy with infantile hypophosphatasia achieved hyperphosphatasaemia, but without clinical or radiographic improvement before a fatal outcome[126]. Thus, correction of the TNSALP deficiency within the skeleton itself seemed necessary to effectively reduce inorganic pyrophosphate levels at bone surfaces[125,126].

Then, in 2003 and 2007, two infant girls with rapidly deteriorating hypophosphatasia seemed to benefit from marrow and bone cell transplantation aimed at enhancing bone TNSALP activity within their skeletons[118,119]. Also, beginning in 2007 (REF. 43), some adults with hypophosphatasia given teriparatide (PTH fragment 1–34) 'off label' to stimulate their osteoblasts to make more bone TNSALP reported diminished pain and showed healing of pseudofractures or stress fractures, with benefit seemingly more likely if a healthy *TNSALP* allele was present to transcribe[43,127,128].

Then, in 2008 in *Alpl* knockout mice, recombinant, bone-targeted, human TNSALP (asfotase alfa) administered subcutaneously from birth was shown to prevent their infantile hypophosphatasia[96,129,130], including its associated seizures and dental abnormalities[96,129]. Asfotase alfa is a mineral-targeted biologic that incorporates TNSALP, the Fc fragment of IgG_1 and a deca-aspartate motif for binding to hydroxyapatite[56,96,129]. In 2008, patient trials began and in 2012 clinical, radiographic and biochemical improvements were detailed for infants or young children with life-threatening complications from perinatal or infantile hypophosphatasia given asfotase alfa for 1 year[56]. Muscle strength and bone mineralization improved, sometimes within several weeks (FIG. 8), and preceded better pulmonary, cognitive and motor function[55]. In 2016, attainment of good health was delineated for children severely affected by hypophosphatasia who were administered asfotase alfa treatment for 5 years[107]. Their bone health assessed by radiography, stature, muscle strength and physical function improved and there was resolution of pain and disability without resistance to this biologic with a good safety profile[107]. Clinical trials involving asfotase alfa have included patients with hypophosphatasia of wide-ranging ages[131–140]. In 2015, asfotase alfa (Strensiq™; Alexion Pharmaceuticals, USA) became available in Japan for hypophosphatasia, and then in Canada, the European Union and the USA for paediatric-onset hypophosphatasia.

In 2015, recombinant, chimeric, soluble, human intestinal alkaline phosphatase prevented hypophosphatasia in *Alpl* knockout mice[141]. Investigations of mouse models of hypophosphatasia suggest that gene therapy using marrow cell transplantation or viral vectors for delivering alkaline phosphatase may someday cure hypophosphatasia[142].

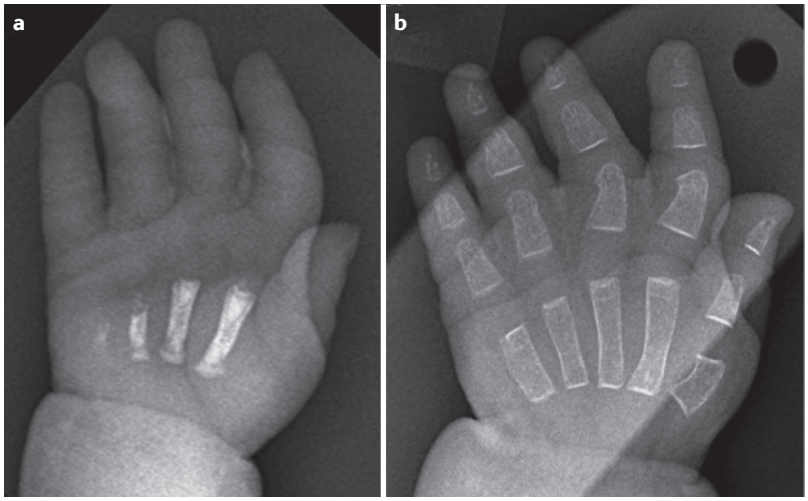

Figure 8 | **Perinatal hypophosphatasia: response to asfotase alfa treatment.** **a** | Before asfotase alfa treatment, this 20-day-old boy has such extreme skeletal hypomineralization that only a few bones are apparent in his left hand. **b** | At week 24 of treatment, substantial mineralization is apparent. Reproduced from Whyte, M. P. *et al.* Enzyme-replacement therapy in life-threatening hypophosphatasia. *N. Engl. J. Med.* **366**, 904–913 (2012), copyright Massachusetts Medical Society.

Conclusions

Hypophosphatasia is the highly informative inborn error of metabolism characterized enzymatically by deficient activity of alkaline phosphatase. Its biochemical hallmark, hypophosphatasaemia, reflects selective loss of the phosphohydrolase activity of the tissue nonspecific isoenzyme of alkaline phosphatase (TNSALP). All affected individuals harbour one or two defective *TNSALP* (also known as *ALPL*) alleles. TNSALP is highly expressed in hard tissues and is a key constituent of matrix vesicles formed by chondrocytes and osteoblasts for extravesicular growth of hydroxyapatite crystals during skeletal mineralization. The consequent impaired mineralization of the skeleton and teeth in hypophosphatasia is explained by extracellular accumulation of the TNSALP substrate inorganic pyrophosphate, an inhibitor of mineralization. The hypophosphatasaemia seems paradoxical for a type of rickets or osteomalacia. Delineation of the skeletal disease caused by hypophosphatasia verified the hypothesis of Robert Robison, who discovered alkaline phosphatase in 1923 (REF. 5), that this enzyme is important for skeletal mineralization. Further studies of hypophosphatasia revealed that alkaline phosphatase is necessary for mineralization of teeth.

Three natural substrates of TNSALP accumulate extracellularly in hypophosphatasia: phosphoethanolamine, which seems to have no clinical consequence; pyridoxal 5′-phosphate, the major circulating form of vitamin B_6, a finding that explains the vitamin B_6-dependent seizures; and inorganic pyrophosphate, the parent molecule of bisphosphonates and a potent inhibitor of mineralization. The aberrant metabolism of vitamin B_6 in hypophosphatasia correctly predicted that TNSALP is a cell-surface protein. The extracellular accumulation of inorganic pyrophosphate blocks hydroxyapatite crystal growth after matrix vesicles rupture. This engenders rickets in infants and children, and osteomalacia in adults. Premature loss of deciduous teeth reflects too little mineralized cementum covering the tooth root, and seems to be the most prevalent complication of hypophosphatasia. Muscle weakness can be severe and, perhaps, reflects the accumulation of inorganic pyrophosphate. In fact, toxicity from the first-generation bisphosphonate, etidronate, mimics hypophosphatasia by causing rickets or osteomalacia, muscle weakness with a waddling gait and hyperphosphataemia due to enhanced renal reclamation of filtered inorganic phosphate. In severe hypophosphatasia, blocked entry of minerals into the skeleton explains the frequent co-occurence of hypercalcaemia and hypercalciuria, and helps account for the associated hyperphosphataemia. In the most severe instances of hypophosphatasia, diminished hydrolysis of pyridoxal 5′-phosphate leads to pyridoxine-dependent seizures. Endogenous levels of these TNSALP substrates correlate inversely with residual serum alkaline phosphatase activity in hypophosphatasia, and directly with severity of the disease. Preliminary evidence indicates that additional natural substrates for TNSALP await discovery.

The extraordinarily broad-ranging severity of hypophosphatasia is largely, but not completely, explained by *TNSALP* mutant allele dosage with autosomal dominant

and autosomal recessive inheritance of any one of several hundred predominantly missense mutations. These mutations compromise the catalytic activity of the TNSALP homodimer in various ways.

In order of increasing severity, hypophosphatasia is classified as odonto, adult, mild childhood, severe childhood, infantile and perinatal hypophosphatasia. Diagnosis of severe paediatric hypophosphatasia is facilitated by pathognomic radiographic skeletal changes. An increased serum level of pyridoxal 5′-phosphate is a sensitive and apparently specific biochemical marker for hypophosphatasia, perhaps because it occurs only when the catalytic activities of all TNSALP isoforms are diminished endogenously. Assay of pyridoxal 5′-phosphate and mutation analysis of *TNSALP* are available in commercial laboratories.

In 2015, recombinant, bone-targeted, human TNSALP (asfotase alfa) was approved for paediatric-onset patients with this last form of rickets/osteomalacia awaiting a medical treatment.

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