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# Hypophosphatasia: Biochemical hallmarks validate the expanded pediatric clinical nosology☆,☆☆

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ABSTRACT

Hypophosphatasia (HPP) is the inborn-error-of-metabolism due to loss-of-function mutation(s) of the ALPL (TNSALP) gene that encodes the tissue non-specific isoenzyme of alkaline phosphatase (TNSALP). TNSALP represents a family of cell-surface phosphohydrolases differing by post-translational modification that is expressed especially in the skeleton, liver, kidney, and developing teeth. Thus, the natural substrates of TNSALP accumulate extracellularly in HPP including inorganic pyrophosphate (PPi), a potent inhibitor of mineralization, and pyridoxal 5′-phosphate (PLP), the principal circulating form of vitamin $B_6$. The superabundance of extracellular PPi regularly causes tooth loss, and when sufficiently great can lead to rickets or osteomalacia. Sometimes diminished hydrolysis of PLP engenders vitamin $B_6$-dependent seizures in profoundly affected babies. Autosomal dominant and autosomal recessive inheritance from among >340 *ALPL* mutations identified to date, typically missense and located throughout the gene, largely explains the remarkably wide-ranging severity of HPP, greatest of all skeletal diseases.

In 2015, our demographic, clinical, and DXA findings acquired over 25 years from 173 children and adolescents with HPP validated and expanded the clinical nosology for pediatric patients to include according to increasing severity "odonto" HPP, "mild childhood" HPP, "severe childhood" HPP, "infantile" HPP, and "perinatal" HPP. Herein, we assessed this expanded nosology using biochemical hallmarks of HPP. We evaluated exclusively data from the 165 preteenage HPP patients in this cohort to exclude potential effects from physiological changes in TNSALP levels across puberty.

All patients had subnormal serum total and bone-specific ALP and elevated plasma PLP, and nearly all had excessive urinary PPi excretion. Only the PLP levels were unchanged across puberty. Mean levels of all four biomarkers correlated with HPP severity ranked according to the HPP nosology, but the data overlapped among all four patient groups.

Hence, these four biochemical hallmarks represent both a sensitive and reliable tool for diagnosing children with HPP. Furthermore, the hallmarks validate our expanded clinical nosology for pediatric HPP that, with limitations, is an improved framework for conceptualizing and working with this disorder's remarkably broad-ranging severity.



## 1. Introduction

*Hypophosphatasia* (HPP) is the inborn-error-of-metabolism caused by loss-of-function mutation(s) of the ALPL (TNSALP) gene that encodes the "tissue-nonspecific" isoenzyme of alkaline phosphatase (TNSALP) [1,2]. TNSALP comprises a family of cell-surface phosphohydrolases differing by post-translational modification and is expressed ubiquitously but especially in the skeleton, liver, kidney, and developing teeth [3,4]. Thus, the natural substrates of TNSALP accumulate extracellularly in HPP; i.e., inorganic pyrophosphate (PPi) [5,6], a potent inhibitor of hydroxyapatite crystal formation and growth and thereby biomineralization [7]; pyridoxal 5′-phosphate (PLP) [8], the principal circulating

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form of vitamin $B_6$; and phosphoethanolamine (PEA) [9], a component of the phosphatidylinositol-glycan linkage apparatus that couples TNSALP and other ectoproteins to plasma membranes [10]. Therefore, the biochemical hallmarks of HPP are subnormal serum ALP activity (hypophosphatasemia), including bone-specific ALP (BAP), and elevated plasma PLP and blood and urine levels of PPi and PEA [1–4].

When HPP manifests clinically during the first years-of-life, the endogenous excess of PPi nearly always leads to premature loss of primary teeth [11], explained by insufficiently calcified cementum anchoring the tooth root to the periodontal ligament [1–4]. When HPP is severe during growth, rickets and muscle weakness are additional complications [12,13]. In profoundly affected newborns and infants, impaired dephosphorylation of PLP can add pyridoxine-dependent seizures, a harbinger of eventual fatal outcome [14]. Consequences from the PEA accumulation have not been identified [1–3]. When HPP presents during adult life, loss of secondary teeth often accompanies osteomalacia that can cause debilitating bone pain and fractures [1,2,15,16], and sometimes the PPi excess results in various arthropathies [17]. Notably, HPP has the broadest range of severity of all dento-osseous diseases [1,2], explained largely by autosomal dominant or autosomal recessive inheritance from among >340 identified mutations [18] located throughout *ALPL* that can disrupt the cellular processing and various functional domains of the homodimeric or homotetrameric TNSALP molecule [3].

To organize the above remarkable expressivity of HPP, initially for prognostication and genetic recurrence risk counseling, but now useful for recognizing candidates for various treatment approaches [1,2], a clinical nosology has evolved since 1957 [19] based upon whether only dental problems are present (i.e., "odonto HPP") or patient age when further complications become manifest and HPP is diagnosed [1,2]. Traditionally, five principal forms of HPP were ranked by increasing severity: odonto HPP, adult HPP, childhood HPP, infantile HPP, and perinatal HPP [1–4]. However, in 2015, we reported [11] that cross-sectional demographic, clinical, and dual-energy x-ray absorptiometry (DXA) data selectively from our first encounters with 173 affected children validated this nosology and further supported distinguishing "severe" versus "mild" childhood HPP to better portray pediatric HPP. Childhood HPP had referred to presentation between 6 months-of-age and adulthood. Now, severe childhood HPP captured significant complications compromising physical function together with obvious skeletal changes radiographically, whereas mild childhood HPP described minor or no symptoms, good physical function, and relatively little skeletal change apparent radiographically [11]. Soon after we reported this expanded nosology for pediatric HPP [11], an enzyme replacement therapy (asfotase alfa) featuring a hydroxyapatite-targeted TNSALP was approved multinationally typically for pediatric-onset HPP [20].

Herein, we investigated biochemical data from this same cohort of pediatric patients to determine: i) if TNSALP levels change physiologically across puberty and thereby alter the levels of the biochemical hallmarks of HPP, ii) the reliability of the hallmarks for diagnosing HPP, and iii) whether the hallmarks support our expanded nosology for HPP in children.

## 2. Materials and methods

### 2.1. Study design

In 2008, we compiled our 25-year experience concerning 173 children with HPP, including our assessments of four biochemical hallmarks of their disorder; serum ALP activity, serum bone-specific ALP (BAP) level, plasma PLP concentration, and urine PPi excretion adjusted to creatinine content (hereafter "ALP", "BAP", "PLP", and "PPi"). Herein, we evaluated each of these biomarkers cross-sectionally to determine their sensitivity for diagnosing HPP and if the levels supported our expanded nosology for pediatric HPP [11]. We did not measure blood or urine levels of PEA because of the expense and reportedly poorer diagnostic specificity for HPP [21], and no assay was available for us to quantitate plasma PPi. After first considering the 18 patients studied "across" puberty for any impact the transition to adulthood might have on their PLP or PPi levels by physiologically decreasing circulating (endogenous) ALP and BAP (see Results) [3], we evaluated selectively our first-encounters with those 165 children who were preteenage. The patients had been referred randomly to the Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children; St. Louis, MO, USA (Research Center), and thus our assay instrumentation and methodologies, etc., were applied without ascertainment bias toward any clinical form of HPP.

### 2.2. Patients

Each of the 173 patients had been admitted to the Research Center for four-five days one or more times during September 1983 – December 2008 [11]. Informed written consent was obtained each occasion as approved by the Human Studies Committee, subsequently the Human Research Protection Office, Washington University School of Medicine; St. Louis, MO, USA. Patient referral was required before 18 years-of-age with follow-up possible until 21 years-of-age. None was acutely ill, and only two were non-ambulatory. The cohort of 173 patients represented 139 seemingly unrelated families. At the time, no pharmacologic treatment for HPP was available or tested.

Each of the 173 patients had been diagnosed with HPP ranging in severity from odonto HPP to infantile HPP [11] based on all of the following at first admission: i) medical history or physical examination indicative of one or more pediatric complications of HPP, ii) serum ALP activity below the pediatric reference range designated for our instruments by consulting clinical pathologists, iii) elevated plasma PLP level (i.e., all 168 patients tested beginning 1985) based upon our published reference range [8], iv) changes consistent with HPP if radiographic skeletal abnormalities were present (William H. McAlister, MD), and v) no evidence for another disorder that could cause hypophosphatasemia, skeletal disease, or premature (i.e., <age 5 years) loss of primary teeth [11]. Also, we had subsequently confirmed mono-allelic or bi-allelic *ALPL* mutation(s) in all 105 probands from whom genomic DNA became available [11]. Therefore, none of the study subjects was a clinically unaffected "carrier" of HPP [11,20].

After reviewing the referral medical information and our clinical and radiographic findings at their first admission, we had routinely classified each patient as odonto HPP, mild childhood HPP, severe childhood HPP, or infantile HPP (see Results) [11,22]. None had perinatal HPP, as these patients are too ill to be cared for at our facility [12]. Childhood HPP was designated 'mild' versus 'severe' by our consideration of their related signs, symptoms, and complications at referral, and after examining their bony changes of HPP upon review of their skeletal radiographs [23].

At the close of 2008, we locked the database and in 2015 published this methodology and selectively the cross-sectional first-admission results of the demographic, tooth loss, grip strength, and DXA data that each validated our expanded classification scheme.[11] In 2016, these same parameters studied longitudinally in the 101 patients with more than one admission during childhood revealed typically a stable clinical course for their HPP, including for the 18 patients who "crossed" puberty [22].

### 2.3. Biochemical assays

#### 2.3.1. Specimen collection

Blood had been collected after the patient fasted at least since midnight. In 1985, when we began routinely to assay plasma PLP [8], any supplement containing vitamin $B_6$ was stopped one week earlier. In 1994, when we began routinely to assay urinary PPi, typically the last of three consecutive 24-h collections was tested. No biochemical assay followed protracted storage of the specimen.