

# Pediatric hypophosphatasia: a retrospective single-centre chart review of 50 children

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## Research article

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# Abstract

**Background:** Hypophosphatasia (HPP) is a rare, inherited metabolic disorder caused by loss-of-function mutations in the ALPL gene that encodes the tissue-nonspecific alkaline phosphatase TNAP (ORPHA 436). Its clinical presentation is highly heterogeneous with a remarkably wide-ranging severity. HPP affects patients of all ages. Therefore, diagnosis is often difficult and delayed. To improve the understanding of HPP in children and in order to shorten the diagnostic time span in the future we studied the natural history of the disease in our large cohort of pediatric patients. In light of the enzyme replacement therapy (Asfotase alfa, a recombinant mineral-targeted TNAP), HPP patients may benefit from early and patient severity orientated calculated treatment in the course of the disease.

**Methods:** This single centre retrospective chart review included longitudinal data from 50 patients with HPP diagnosed and followed at the University Children`s Hospital Wuerzburg, Germany over the last 25 years.

**Results:** The cohort comprises 4 (8%) perinatal, 17 (34%) infantile and 29 (58%) childhood onset HPP patients. Two patients were deceased at the time of data collection. Diagnosis was based on available characteristic clinical symptoms (in 88%), low alkaline phosphatase (AP) activity (in 96%), accumulating substrates of AP (in 58%) and X-ray findings (in 48%). Genetic analysis was performed in 48 patients (35 compound heterozygous, 11 heterozygous, 2 homozygous mutations per patient), allowing investigations on genotype-phenotype correlations. Based on anamnestic data, median age at first clinical symptoms was 3.5 months (min. 0, max. 107), while median time to diagnosis was 13 months (min. 0, max. 103). Common symptoms included: impairment of motor skills (78%), impairment of mineralization (72%), premature loss of teeth (64%), musculoskeletal pain and craniosynostosis (each 64%) and failure to thrive (62%). Up to now 20 patients started medical treatment with Asfotase alfa.

**Conclusions:** Reported findings support the clinical perception of HPP being a chronic multi-systemic disease with often delayed diagnosis. Our natural history information provides detailed insights into the prevalence of different symptoms, which can help to improve and shorten diagnostics and thereby lead to an optimised medical care, especially with promising therapeutic options like enzyme-replacement-therapy with Asfotase alfa in mind.

## Background

Hypophosphatasie (HPP) is a rare, inherited, systemic, metabolic disorder that was first described by J.C. Rathbun in 1948 (ORPHA 436) (1). It is caused by loss-of-function-mutations in the ALPL gene, encoding tissue-nonspecific alkaline phosphatase (TNAP). It results in reduced serum alkaline phosphatase (AP) activity and increased extracellular accumulation of TNAP substrates like inorganic pyrophosphate (PPi), pyridoxal-5'-phosphate (PLP, the circulating form of vitamin B6) or phosphoethanolamine (PEA) (2). PPi is a known inhibitor of hydroxyapatite crystal formation and propagation and therefore acts as a potent calcification inhibitor (3). Furthermore, it supposedly supports the deposition of pyrophosphate and

calcium outside the osteoblasts in a crystallised form, which - similar to PPI itself – may cause inflammatory processes like arthritis or chronic non-bacterial inflammation (3). PLP is an essential cofactor in many biochemical processes of the human body, e.g. in the synthesis of important neurotransmitters like gamma aminobutyric acid (GABA), serotonin or dopamine, and must be dephosphorylated to pyridoxal (PL) to be able to pass the blood-brain barrier as well as cell membranes. This process is catalysed by TNAP (4). There are only a few studies that address HPP incidence and prevalence. Life-threatening HPP occurs in approximately 1 per 100,000 and 300,000 births in Canada and in Europe, respectively (5). Due to low selective pressure and a large number of undiagnosed patients, the prevalence of mild forms of HPP is suspected to be much higher. Based on ALPL gene mutation analysis the prevalence in the European population was estimated to be 1/1,6370 (6).

Patients with HPP show a wide range of different symptoms, even among individuals with the same genotype or in the same family, so elaborating HPP as the primary disease is a big challenge for attending physicians. Based on clinical characteristics and the age of onset HPP has been classified in five different subtypes (perinatal, infantile, childhood, adult, odonto) (7–10). The characteristic manifestations of HPP in infants may include failure to thrive, rickets-like deformities, pulmonary insufficiency, muscle weakness, nephrocalcinosis, premature craniosynostosis, and vitamin B6-responsive seizures; in toddlers, young children, and adolescents, premature tooth loss, bone deformities and rachitic changes in long bones, musculoskeletal pain and delayed motor development are characteristic (2, 11).

The natural history of HPP is poorly understood at this time, likely because of the rarity and wide clinical heterogeneity of the disease. Early onset forms (perinatal, infantile) of HPP show a high mortality, while other forms seem to go along with normal life expectancy but can be associated with significant burden of disease, including but not limited to functional disability due to musculoskeletal problems as well as reduced physical activity and quality of life.

Clinical management and treatment of HPP is based on supportive measures that address the symptoms of the disease (e.g., respiratory support, nutrition, orthopedic or neurosurgical intervention, pain relief) (12). Since 2015 Asfotase alfa (AA, Strensiq®, Alexion Pharmaceuticals, Inc., Boston, MA, USA), a human recombinant TNAP enzyme replacement therapy, is approved in many countries for long-term enzyme replacement therapy (ERT) in patients with pediatric-onset HPP to treat bone manifestations of the disease (13, 14). Treatment with AA was associated with skeletal, respiratory and functional improvement and was considered a milestone in the management of severe forms with improved overall survival in this cohort (15–18). But also young children, and adolescents affected by milder forms of HPP have been shown to benefit from AA by improving physical activity and reducing pain (19). No data is available on potentially therapeutic modification of neurological, gastrointestinal and cardiovascular problems of HPP patients treated with AA.

HPP has a very heterogeneous presentation, which, coupled with its rarity, often leads to missed or delayed diagnosis and an incomplete understanding of its natural history. To better understand the

epidemiology and clinical course of HPP, including first clinical symptoms, timing of diagnosis, genotype phenotype correlation of the disease we present data of our German referral centre retrospective chart review. Especially in the light of an effective bone targeted treatment it seems to be important to improve our understanding of pediatric HPP to shorten the diagnostic time span and thereby enable an optimised medical care and to avoid or postpone disease-related symptoms and complications.

## Methods

We performed a retrospective chart review, which included longitudinal demographic and clinical data from all pediatric patients who had been diagnosed and followed at the University Children's Hospital in Würzburg over the last 25 years. The center has a great history and experience with this disease and the German patients' organisation is also based in this city. Eligibility criteria were the presentation of characteristic clinical symptoms associated with HPP in addition to a reduced AP activity (according to age and gender adjusted normal values) in serum and/or at least one documented mutation in the ALPL gene. In total, we identified 50 patients with appropriate documentation to confirm these criteria. For each patient we collected data concerning age and gender, clinical subtype, age at onset, first HPP-related symptoms, age at diagnosis, findings leading to the HPP diagnosis, previous misdiagnoses or differential diagnoses, laboratory findings (AP, PLP, PEA, vitamin D, calcium, phosphate), genetic findings and treatment in a Microsoft Access® database.

## Results

**Demographics:** Our cohort of 50 HPP patients (26 male, 24 female) consists of 4 perinatal (8%), 17 infantile (34%) and 29 childhood (58%) onset HPP patients. Up to now, two patients (both of perinatal subtype) died at the age of 3 months, due to cardiac arrest and deleterious neurological outcome (15).

**HPP medical history:** Median age at first HPP symptoms was 3.5 (min. 0, max. 107) months, median age at HPP diagnosis was 24 (min. 0, max. 127) months, and median time to diagnosis was 13 (min. 0, max. 103) months (table 1). There was no delay in making the diagnosis in perinatal HPP. Median time from the first attributable symptom to diagnosis in infantile HPP was 12 months, in childhood HPP 22.5 months.

In 82.1% (32/39) of patients with sufficiently documented data, the diagnosis was made in hospital, 7.7% (3/39) of the patients were diagnosed by pediatric practitioners, 7.7% (3/39) by dentists and one patient by a general practitioner.

The most frequent first symptoms in our cohort were failure to thrive in 34% (17/50) followed by an abnormally shaped head / prominent fontanel in 22% (11/50) and a premature loss of teeth in 20% (10/50) of the patients. Less frequent first symptoms were: nutritional problems (14%, 7/50), respiratory problems (12%, 5/50), muscle weakness (12%, 6/50), bone deformities (8%, 4/50), abnormally shaped thorax (8%, 4/50), impairment of motor skills (4%, 2/50), parodontitis (2%, 1/50), musculoskeletal pain

(2%, 1/50), cerebral seizures (2%, 1/50). In some patients a composite of symptoms was noticed from the beginning.

In 21 patients (42%), at least one differential diagnosis was documented before diagnosing HPP as the underlying cause of the presented symptoms. Ten patients were diagnosed with various forms of rickets and based on a failure to thrive, two patients were each suspected with cystic fibrosis and a lack of growth hormone. Further documented differential diagnoses were hypo-/achondroplasia, different forms of food intolerance, neurofibromatosis, polyarthritis, tethered-chord syndrome, chronic non-bacterial osteomyelitis, Langerhans cell histiocytosis or extradermal dysplasia (one each).

During the course of disease, the patients in our cohort presented a wide range of different symptoms. We divided these symptoms into various categories (table 2). All patients with perinatal HPP showed pulmonary abnormalities, cerebral seizures and impairment of mineralization. In addition, 3 out of 4 patients with perinatal HPP had early impairment of motor skills, gastrointestinal problems and craniosynostosis. In infantile HPP most frequently reported findings were: impairment of motor skills (94%), impairment of mineralization (88%), nephrocalcinosis and craniosynostosis (76% each), failure to thrive (71%), pulmonary abnormalities (65%) and premature loss of deciduous teeth (59%). Patients with childhood HPP presented with impairment of motor skills, premature loss of deciduous teeth (69% each), impairment of mineralization (62%) and failure to thrive (59%). Looking at our cohort of 50 patients in total impairment of motor skills (78%), impairment of mineralization (72%) and premature loss of deciduous teeth (64%) were most commonly reported in the HPP-related disease history. Pathologic fractures were found in 14% of the patients.

Laboratory findings: All patients showed a reduced activity of AP in laboratory blood testing at least once. In patients with perinatal HPP there was almost no detectable residual AP activity (median 0.5 U/l). Elevated calcium levels (median 2.82 mmol/l, [normal range: 2.0-2.7]) were solely found in the perinatal subtype, while phosphate levels were noted in the upper normal range of all subtypes. Median vitamin D (25(OH)D) was reduced in patients with childhood HPP, but in the lower normal range for perinatal and infantile HPP. Parathyroid hormone (PTH) was found to be in the lower normal range. PLP was considerably elevated (median 142.5 ng/ml [5–30]). All our patients with perinatal HPP were substituted with pyridoxin due to cerebral seizures, thus measuring PLP was not diagnostically meaningful since lab test for pyridoxal phosphate do not discriminate these two forms properly (table 3).

Genetics: Genetic testing was performed and sufficiently documented in 48 patients. In our cohort, 35 (72.9%) patients are compound-heterozygous, 11 (22.9%) are heterozygous (with 2 being listed as dominant negative mutations (20)) and 2 are homozygous for mutations on the ALPL gene. There are 85 documented mutations in total, of which 78 mutations are located on exons and 7 mutations on introns of the ALPL-gene. As some mutations occur more frequently than others, we found 40 different mutations in our cohort, of which 35 are listed in the online ALPL gene mutation database by E. Mornet (20). 5 mutations have not been published yet (Table 4). 77 mutations were missense mutations, 6 small deletions/insertions and two were stop-mutations.

The most common mutation in our cohort is p.Glu191Lys, which was found in 18/48 patients and p.Gly334Asp in 8 patients. 7 patients share the combination of these two frequent ALPL mutations (p.Glu191Lys/p.Gly334Asp, compound heterozygous) but differ in their clinical presentation (2 infantile, 5 childhood HPP, (21)).

Core diagnostic findings leading to HPP diagnosis: At the time of established HPP diagnosis the following supportive diagnostic findings have been documented: 96% (48/50) showed a documented low AP activity, 88% (44/50) typical HPP-associated symptoms, 58% (29/50) elevated levels of AP substrates (PLP and PEA), 48% (24/50) radiological abnormalities, 18% (9/50) genetic testing of the ALPL gene as performed at the time of diagnosis and in 12% (6/50) an HPP positive family history was known.

Medication and treatment history: None of our patients received bisphosphonates or PTH analogues. Two patients were treated with growth hormone. One girl was treated for 6 months before making the diagnosis and stopped afterwards (22) and the other one started treatment after making the diagnosis of mild HPP (heterozygous) and small for gestational age constellation without catch up growth. 54% (27/50) received vitamin D supplementation (dose range 500 to 1000 IU per day), 48% (24/50) NSAIDs for pain management and treatment of inflammation (ibuprofen, naproxen), 16% (8/50) phosphate binding agents and 4 patients pyridoxine/vitamin B6 due to cerebral seizures. Half of the patients diagnosed with craniosynostosis (12/24) developed raised intracranial pressure with need for neurosurgical interventions (skull remodelling). Fractures, if prevalent, could be conservatively treated, in none of the cases surgery was required. Physiotherapy and/or occupational therapy were/was recommended for almost all patients. 7 patients (3 perinatal, 3 infantile 1 childhood) needed mechanical ventilation; 3 patients experienced resuscitation during course of the disease.

At the time of assessment 20/50 patients were receiving asfotase alfa: 4/4 of perinatal HPP, 11/17 of infantile HPP, 3/29 of childhood HPP (all were homozygous or compound heterozygous). In 14 patients asfotase alfa was started before the European approval 2015 within a phase 2 clinical trial (ENB010-10, ClinicalTrials.gov NCT01176266, (15)).

## Discussion

The aim of this investigation was to better understand the epidemiology and clinical course of disease in pediatric HPP. Here we report characteristics, medical history, laboratory findings including genotypes and treatment history of 50 pediatric patients with HPP that have been followed at the University Children's Hospital of Wuerzburg over the last 25 years.

Medical history and diagnostic delay: In our cohort more than half of the patients had a so called childhood form with first clinical symptoms after the first 6 months of life. 34% had an infantile form and only 4 out of 50 patients were diagnosed as a perinatal form. Two of the latter died at the age of 3 months. There was no delay in making the diagnosis in perinatal HPP probably due to the severity of the disease in this small cohort with all of them being treated in a neonatal intensive care ward. Interestingly median age at first clinical symptoms in infantile HPP was 2 months and in childhood HPP 9 months.

Substantial diagnostic delays between median age at first clinical symptoms and age at diagnosis of HPP was noted in infantile (12 months) and even prolonged in childhood HPP (22.5 months). Unfortunately more differentiated information is lacking: number of doctor contacts, number of diagnostic procedures (laboratory or imaging), exact time from the first clinical symptom until referral to a center, e.g.. Similar findings have been published by Högler et al. with a diagnostic delay of 20.4 months in children (n = 90) and 47.5 months in adults (n = 52) documented in the Global HPP Registry (23). There may be several reasons for the diagnostic delay, including low awareness, heterogeneity of disease manifestations especially in clinically “milder” forms and lack of diagnostic guidelines or routine testing of HPP.

Medical history documented systemic manifestations of HPP in perinatal, infantile and childhood HPP, generally consistent with HPP-related clinical symptoms described in the literature (7–9). Interestingly, the most frequent first symptom in our cohort was failure to thrive and thus, musculoskeletal manifestations were less frequent. Therefore testing for AP activity should be definitely part of a routine diagnostic workup in infants and children with problems in gaining weight and growth. While suggesting this one has to keep in mind, that children with feeding and growth disorders due to other means than HPP, often have reduced AP levels due to general lack of nutrients, including zinc as the catalytic ion of TNAP. As a consequence, careful analysis of AP levels and, in case of suspiciously low levels, subsequent diagnostic measures including the analysis of TNAP levels in leukocytes, substrate analysis and genetic analysis may have to be considered.

Over time impairment of motor skills, impairment of mineralization, premature loss of teeth and failure to thrive were most commonly reported in our cohort. Impairment of mineralisation in infantile and childhood HPP may also be underestimated due to the necessity of radiation exposure. In our cohort we were reluctant to use X-ray diagnostics as they were not performed routinely just in case of local pain problems, suspicion of fractures, e.g. ultrasound and MRI diagnostics were used instead (24).

Our data also support the fact that HPP in children, regardless of subtype, is not characterized by genuine pathologic fractures, not regarding the generalized mineralization deficit in the perinatal form. In other pediatric bone diseases like osteogenesis imperfecta or in adults HPP patients may suffer from recurrent or poor healing fractures (25). Musculoskeletal pain has been documented for almost half of the patients being aware that it is difficult to diagnose in small infants probably resulting in an underestimation at least in this age group. On the other hand one cannot completely exclude the possibility of chronically developing myopathies caused by chronically elevated proinflammatory substrate accumulation, which might contribute to explain the higher incidence (55%) of such conditions in the group of childhood HPP, where patients (and their tissues) suffered already for some years from this inborn error of metabolism. For what reason ever we find here, that in childhood HPP musculoskeletal pain moves towards one of the lead symptoms in comparison and may also be one cause of diagnostic delay if not adequately assigned. In general it appears that a (not exactly quantifiable) threshold of minimum requirement AP activity exists, beyond which important facets of tissue formation and maturation are impaired concerning e.g. the CNS, the lung and bone mineralization, while in milder forms of the disease

accumulating substrates can still produce chronic symptoms of dysfunction such as musculoskeletal pain.

Dental manifestations especially premature loss of teeth are common HPP-related symptoms that can be found in all ages and should always prompt a dentist to advise patients and their families for further diagnostic procedures including AP testing. Cerebral seizures were solely and pulmonary and gastrointestinal abnormalities and nephrocalcinosis predominantly affecting perinatal and infantile forms. HPP is associated with an increased incidence of craniosynostosis, hydrocephalus, syringomyelia and Chiari malformation (26). In our cohort craniosynostosis has been diagnosed in almost half of the patients predominantly in perinatal and infantile forms. Half of them developed raised intracranial pressure (diagnosed by cranial X-rays, eye examination - papilledema, lumbar puncture with measurement of cerebrospinal fluid opening pressure) and followed by neurosurgical intervention (skull remodelling). This highlights the importance to carefully look for craniosynostosis in patients with pediatric HPP in particular in more severely affected individuals with early disease manifestations and to follow them very closely in an experienced center for pediatric neurosurgery. The more sutures fuse prematurely the more likely it results in raised pressure due to imbalance between the space requirement of the growing brain and the limited intracranial space. This is also true in our cohort of HPP patients. But interestingly radiological signs of raised intracranial pressure can also be observed in patients with only one single suture being involved; e. g. in a girl (infantile HPP) with premature fusion of the left coronal suture (Fig. 1). This girl did not show any additional features like Arnold Chiari malformation or syringomyelia etc. in the MRI and interestingly no papilledema or clinical symptoms were present.

As craniosynostosis usually is a very slow and chronic process it mostly does not result in obvious acute neurological symptoms based on elevated intracranial pressure, like headache, nausea, vomiting or altered consciousness. It must also be taken into account that after a neurosurgical therapy the affected suture may fuse again prematurely possibly leading to another event of still premature craniosynostosis. The reason for rapid desmal reossification or sclerosis is not understood. In addition, premature closing of the sutures may come along with ossification/calcification of the meningeal membranes, a particular aggravation of the complexity of the neurosurgical procedure. In our cohort 3 patients needed a second operation. Therefore, patients should be monitored for intracranial pressure at least until the end of brain growth which is around 12 years of age. As the reported incidence of craniosynostosis and even craniosynostosis in pediatric HPP seems to be remarkably lower this fact adds an important feature to the protocol/algorithm that we have to be aware of in diagnosis and guidance of these patients.

Laboratory findings and genotypes: AP activity is almost absent in our perinatal forms, very low in infantile HPP (30, reference level 110–590 U/l) and low in childhood HPP (45, reference level 110–550 U/l). From the literature it is known for pediatric HPP that AP levels seem to correlate with age of onset and negatively correlate with disease severity (7–9).

In two cases HPP suggestive symptoms were present during the course of the disease including a positive family history. In both patients AP measurement was in the lower normal range at time of

diagnosis (both heterozygous genotype). In one case AP activity fell below the lower limit later on, in the other patient it remained in the lower normal range. Clinical diagnosis was supported by genetic testing results, two heterozygous mutations, one in each patient, were noted. These findings highlight the need for careful clinical assessment, which includes measurement of AP activity also over time (several times if applicable) in children with a positive family history or/and HPP related symptoms. Especially in heterozygous carriers harbouring dominant negative mutations and who often show a very mild phenotype it is a great challenge to make the diagnosis. The “carrier” status of a single mutation should eventually be clinically unremarkable, almost. Mild, but clinically overt HPP might be caused by a dominant negative effect of one particular mutation in this patient. The physician has to be aware of the lower limits of age-, and sex adjusted AP reference intervals and clinical conditions also resulting in reduced levels. PLP values were increased in our infantile and childhood patients and may be helpful in establishing the diagnosis of HPP especially if clinical findings and AP measurements are somehow not clear or borderline. One must also be aware that PLP measurement may not be helpful in case of vitamin B6 or multivitamin intake (treatment of seizures, dietary supplement e.g.).

Our findings support the importance to check calcium phosphate metabolism in all patients in general over time (with or without ERT). Especially in more severe forms a low calcium diet may be necessary, at least before ERT. During ERT calcium levels tend to go down, presumably caused by an uptake into bone (“hungry bones”) and need to be monitored even more closely.

In our cohort for all but two patients results of genetic testing were available and a high number of different mutations could be found. Most of our patients (35/48) had a compound heterozygous genotype, 2 had a homozygous genotype. All patients with a heterozygous mutation (two of them are known to have a dominant negative effect) were not severely affected and characterized as childhood HPP. Interestingly p.Glu191Lys has been found in 18, p.Gly334Asp in 8 and the combination of both most common mutations in 7 patients (2 infantile, 5 childhood). P.Glu191Lys is known to occur with a high frequency (up to 55%) in HPP patients with European ancestry and tested moderate in our in vitro testing (68% wild type AP enzyme activity) and it has no dominant-negative effect (21). P.Gly334Asp has been found previously in homozygous Mennonite patients (founder effect) and in vitro testing showed very low residual activity of this severe mutation and a clear dominant negative effect (21).

The very high number of different mutations in the ALPL gene with various effects on the enzymatic activity in in vitro studies has been correlated with the high variability of phenotypes observed in patients with HPP (27). According to our clinical observations, however, intra- and interfamilial variability of phenotypes can also be observed in patients with identical genotypes suggesting that additional genetic confounders, as well as epigenetic or environmental factors, may also be involved individually or even at a tissue level (21).

Diagnosis of the disease: In our cohort in most cases typical HPP-associated symptoms and subsequently a low AP activity level led to the diagnosis of HPP. To a minor extend high substrate levels were available at time of diagnosis in addition. Substrate analysis was performed only in AP borderline

cases or within a clinical trial. Furthermore, a positive family history may lead to further diagnostic procedures. Radiological abnormalities at the time of diagnosis were only documented in less than half of our patients. This may be due to restrictive usage of X rays with regard to radiation exposure for children. X ray diagnostics were performed in case of suspicion of fracture or elevated intracranial pressure. Currently X rays are more common due to low exposure doses in modern X-ray equipments and to assess the individual bone phenotype in the light of an approved bone-targeted therapy

Further on it still remains a challenge to establish diagnosis of HPP in heterozygous carriers (with or without mutations showing a dominant negative effect) who might present with non-typical, likewise mild symptoms, nevertheless of relevance for the patient (“carrier” status versus HPP patient, as discussed previously).

Treatment of pediatric HPP: In our cohort nobody was treated with bisphosphonates as these drugs should be avoided because of their similarity with inorganic pyrophosphate and because they limit bone turnover resulting in reduced activity levels of bone-specific alkaline phosphatase. Supplementation with vitamin D seems reasonable due to low serum levels (Table 3). Treatment with NSAIDs was performed in almost half of our patients mainly during childhood when musculoskeletal pain and /or inflammation has been documented (on demand or 4 to 8 weeks).

The high percentage of patients receiving Asfotase alfa (AA) may be explained as our center was a study site in clinical studies of AA before the approval. All of them showed compound heterozygous or homozygous mutations in the ALPL gene.

Conclusion: Reported findings support our clinical impression of HPP as a chronic multi-systemic disease with often delayed diagnosis in particular in not severely affected children. Our natural history information provides detailed insights into the prevalence of different symptoms, which can help to improve and to shorten the time to diagnosis. Simple laboratory tests (AP activity in the serum, PLP in the plasma) can substantiate the clinical differential diagnosis. Genetic testing for mutations in the ALPL gene may add additional information for making the diagnosis of HPP. The very mild end of the spectrum of even unspecific symptoms poses a particular problem, not to “overdiagnose” HPP. Especially in case of mild or unspecific complaints and a moderately reduced AP, diagnosis should be evaluated very carefully if only one single mutation is found in the TNSALP gene. Promising therapeutic options like ERT might help to optimise medical care when an early diagnosis is made.

## List Of Abbreviations

AA Asfotase alfa

HPP Hypophosphatasia

PEA Phosphoethanolamine

PL Pyridoxal

PLP Pyridoxal-5'-phosphate

PPi Inorganic pyrophosphate

TNAP Tissue-nonspecific alkaline phosphatase

## Declarations

**Ethics approval and consent to participate:** The study was approved by the local ethics committee, University of Wuerzburg, Germany.

**Consent for publication:** not applicable

**Availability of data and material:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

**Competing interests:** The authors declare that they have no competing interests

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**Authors' contributions:** All authors made substantial contributions to the conception **or** design of the work, data acquisition, analysis or interpretation of data. All authors read and approve the final manuscript.

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## Tables

Due to technical limitations, Tables 1-4 are provided in the Supplementary Files section.

Captions

Table 1: Median age at first symptoms, diagnosis and median time to diagnosis

Table 2: HPP-related disease history in different subtypes of HPP

Table 3: Laboratory findings

AP=alkaline phosphatase, 25(OH)D= 25-OH-Vitamin D, PTH=parathyroid hormone, PLP=pyridoxal phosphate; \* reference levels of AP (37 C°, IFCC method): infants 110-590 IU/l, toddler 110-550 IU/l, pupil 130-700 IU/l according to local laboratory standards)

Table 4: Unpublished mutations of the ALPL gene found in our cohort<sup>1</sup>

<sup>1</sup>According to the online database of ALPL-mutations by E. Mornet (20)

## Figures

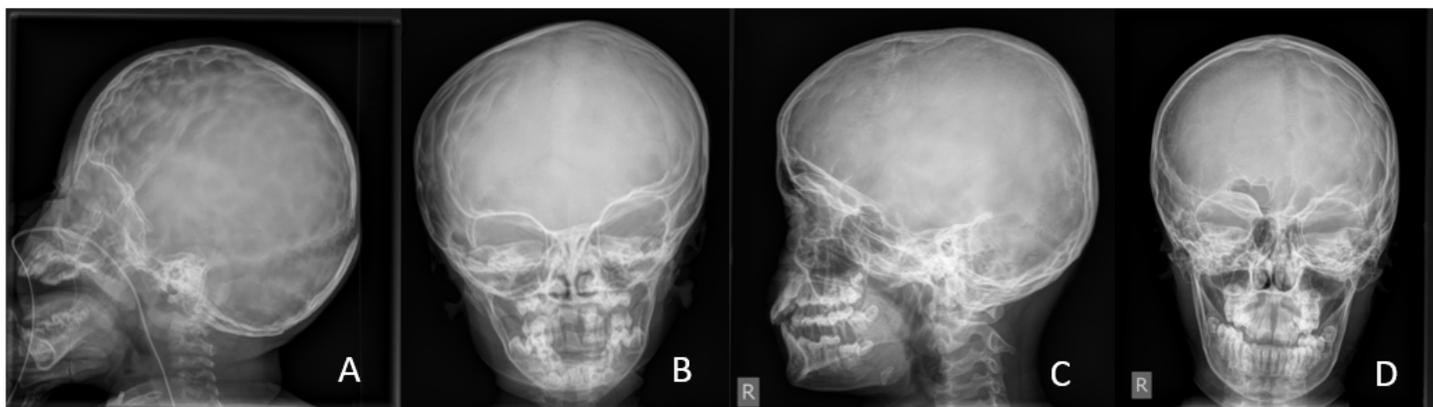


Figure 1

Craniosynostosis in HPP: A: Lateral skull X-ray of a 2 year old girl with premature fusion of both coronal sutures and signs of raised intracranial pressure, copper beaten skull. B: A.p. skull X-ray of a 5 year old girl with premature fusion of the left coronal suture with signs of raised intracranial pressure – impressiones temporal, right side. C and D: lateral and a.p. skull X-ray of a 12 year old boy with premature fusion of the left coronal suture and impressiones digitatae predominant on the left side.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [Table4.xlsx](#)
- [Table3.xlsx](#)
- [Table2.xlsx](#)