



## Case Report

# Multigenerational case examples of hypophosphatasia: Challenges in genetic counseling and disease management



Erin Huggins<sup>a</sup>, Ricardo Ong<sup>a</sup>, Cheryl Rockman-Greenberg<sup>b</sup>, Lauren Bailey Flueckinger<sup>a</sup>, Kathryn M. Dahir<sup>c</sup>, Priya S. Kishnani<sup>a,\*</sup>

<sup>a</sup> Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC, USA

<sup>b</sup> Department of Pediatrics and Child Health, Max Rady College of Medicine, University of Manitoba, Winnipeg, MB, Canada

<sup>c</sup> Vanderbilt University Medical Center, Program for Metabolic Bone Disorders, Division of Endocrinology, Nashville, TN, USA

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## ABSTRACT

Hypophosphatasia (HPP) is an inherited metabolic condition caused by pathogenic mutations in the *ALPL* gene. This leads to deficiency of tissue non-specific alkaline phosphatase (TNSALP), resulting in decreased mineralization of the bones and/or teeth and multi-systemic complications. Inheritance may be autosomal dominant or recessive, and the phenotypic spectrum, including age of onset, varies widely. We present four families demonstrating both modes of inheritance of HPP and phenotypic variability and discuss the resultant challenges in disease management, genetic counseling, and risk assessment. Failure to consider different modes of inheritance in a family with HPP may lead to an inaccurate risk assessment upon which medical and reproductive decisions may be made. We highlight the essential role of high-quality genetic counseling and meaningful biochemical and molecular testing strategies in the evaluation and management of families with HPP.

## 1. Introduction

Hypophosphatasia (HPP) is an inherited metabolic disorder caused by pathogenic variants in the *ALPL* gene (NM\_000478.6), resulting in deficiency of tissue non-specific alkaline phosphatase (TNSALP), an isoenzyme that plays a vital role in normal bone and tooth mineralization [1]. Affected individuals demonstrate a wide phenotypic spectrum of skeletal, musculoskeletal, and dental anomalies generally categorized based on the age of symptom onset (perinatal, infantile, childhood, adult). Age of onset generally correlates with symptom severity, with a high mortality rate observed in perinatal and infantile onset patients (73% deceased at 5 years) [2]; death in these cases is often caused by vitamin B6-responsive seizures or respiratory complications [2,3]. Children and adults with HPP may present with a variety of symptoms including premature loss of primary teeth, multiple fractures, pain, muscle weakness, gait abnormalities, craniostylosis, and fatigue [4–6]. Diagnosis and risk assessment for HPP is complicated by lack of established genotype-phenotype correlations and the presence of over 400 unique, primarily missense, pathogenic *ALPL* variants [7–9]. Inheritance may be autosomal dominant (AD) or autosomal recessive (AR); those with AR disease often experience the most severe clinical features [9–12]. Heterozygous parents may be classified as

asymptomatic under a presumed classic AR inheritance pattern, yet it is known that the presence of heterozygous dominant negative variants can result in clinical manifestations of HPP [13]. However, there have been limited in vitro studies on the variants identified thus far, and it can be difficult to predict the effect of a single heterozygous variant without reported clinical or laboratory data. Without defined genotype-phenotype correlations, the distinction between a true “carrier” of AR HPP and an individual who may manifest clinical symptoms of disease remains a challenge to determine.

Although morbidity and mortality are highest in perinatal and infantile forms, significant disease burden may exist at any age, with symptom overlap across the subtypes and worsening over time [14]. There are attenuated forms of HPP including benign perinatal HPP (also known as transient prenatal HPP) and odontohypophosphatasia. Benign perinatal HPP is characterized by prenatal bowing of the long bones with spontaneous improvement or resolution, either prenatally or postnatally [15,16], while odontohypophosphatasia is characterized by the presence of dental abnormalities without skeletal involvement, which can present at any age [17,18]. However, recent evidence has shown that some patients classified as having odontohypophosphatasia due to initial presentation of early tooth loss may actually develop systemic manifestations over time including chronic pain, fractures,

\* Corresponding author at: Medical Genetics, Duke University Medical Center, DUMC, Box 103856, Durham, NC 27710, USA.

E-mail address: [priya.kishnani@duke.edu](mailto:priya.kishnani@duke.edu) (P.S. Kishnani).

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weakness, and gait abnormalities [19]. Individuals with AD HPP, previously described as “mild” cases, may manifest symptoms at any point throughout the lifespan that can result in significant disease burden. It has been reported that some adults with a mild clinical presentation (or only biochemical evidence of disease) may appear asymptomatic for many years and subsequently develop fractures later in life, including after exposure to bisphosphonate therapy [14,20], thus representing a continuum of disease severity throughout the lifespan. Continued monitoring of symptom development over time is vital to the growing understanding of the clinical spectrum, and ongoing management of individuals with HPP, including those with a suspected attenuated form.

While clinical evaluation of parents of children with AR HPP can reveal clinical features that may indicate AD disease, a single evaluation may not be sufficient to make this determination. The lack of ability to distinguish between asymptomatic carriers and those who are affected with AD disease can lead to the communication of inaccurate recurrence risks and lack of anticipatory guidance for at-risk family members. Failure to identify and evaluate family members at risk for HPP may result in a lengthened diagnostic odyssey, delayed treatment, and potentially inaccurate reproductive decision-making.

We present four illustrative HPP families from three institutions demonstrating both AR and AD modes of inheritance within the same family. These families illustrate the importance of recognizing the possibility of AD inheritance in order to provide accurate genetic counseling, risk assessment, diagnosis, management, and treatment of HPP across the disease spectrum.

## 2. Subjects and methods

The following families (one family from Duke University Medical Center, two families from Vanderbilt University Medical Center, and one family from University of Manitoba) were seen for clinical evaluation at their respective institutions. Families were selected as illustrative examples based on misdiagnosis or misidentification of the correct mode of inheritance of HPP in the family that resulted in inaccurate or incomplete risk assessment and/or clinical evaluation.

## 3. Illustrative families

### 3.1. Family #1

The proband is a 46-year-old Caucasian female who presented to clinic for evaluation of HPP due to possible symptom development and positive family history (Fig. 1). In childhood and adolescence, she had poor dentition fully rooted adult tooth loss at age 14, tooth fracture at age 17, and multiple cavities despite appropriate dental hygiene. Over time, she also developed frequent popping and periodic dislocation of the jaw, joint pain, hand pain, pseudo-gout, chronic fatigue, memory difficulties and calcifications of the sacrum, shoulder, and elbow. She had no overt fractures, but did have a history of fractures in her rib and toe associated with minor trauma in childhood. Serum alkaline phosphatase (ALP) was low on two occasions at 18 and 19 U/L (reference range: 39–117 U/L).

The family history was positive for a daughter (III.3) diagnosed with perinatal HPP in the late 1990s, deceased at 31 days of life due to respiratory insufficiency. *ALPL* gene sequencing was performed on the daughter, which identified biallelic variants; c.331G > A (p.Ala111Thr) and c.1426G > A (p.Glu476Lys). Trans configuration was confirmed by targeted parental testing, and the proband was identified as a “carrier of c.331G > A”. It is unknown whether deletion/duplication analysis was pursued. Of note, sequencing and subsequent targeted testing were performed on a research basis. Genetic counseling was subsequently provided for autosomal recessive inheritance and a recurrence risk to future pregnancies of 25% was given to the parents (II.2 and II.3), who were each presumed to be unaffected carriers. The

proband subsequently conceived fraternal twins via egg donation and gave birth to a male and female child (III.1 and III.2) without signs or symptoms of HPP in utero or in the neonatal period. After several years, the proband then became pregnant with a fourth child (III.4) with a new partner (II.4) without the use of assistive reproductive technologies (ART). The decision was made not to utilize ART for that pregnancy based on previous risk assessment for AR HPP and < 1% risk for the new partner to be a carrier. The fourth child did not show signs or symptoms of HPP in utero or in the neonatal period.

At the time of the proband's evaluation, she reported experiencing severe and chronic muscle and joint pain and fatigue. She had low serum ALP of 16 U/L (reference range: 24–110 U/L) and elevated pyridoxine 5-pyrophosphate (PLP) of 61 µg/L (reference range: 5–50 µg/L). Complete skeletal survey revealed minimal degenerative changes, normal osseous mineralization, and no lytic lesions. CT scan of chest and abdomen revealed mild degenerative disc disease.

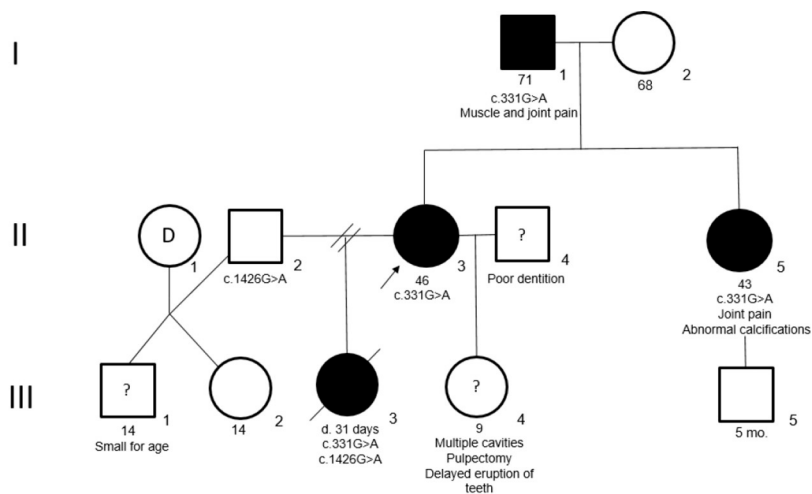
The family history was updated at the time of evaluation as well. The twins were 14 years of age and asymptomatic, though it was noted that the male twin was thought to be small for his age, measuring around the 11th percentile for height. The fourth child was 6 years old and had experienced multiple cavities, a pulpectomy, and delayed eruption of teeth without premature tooth loss; no skeletal abnormalities had been noted. Of note, the father of this child also reportedly had poor dentition, but no further workup for HPP. None of the children had undergone genetic testing. The proband's former partner reportedly had poor dentition, suffered from significant pain and was a chronic opioid user. Aside from targeted *ALPL* sequencing after the diagnosis of their first child, he had no additional workup for HPP. The proband reported her father (I.1) had muscle and joint pain and her sister (II.5) had a history of joint pain and abnormal calcifications in the ears.

The proband subsequently had full sequencing of the *ALPL* gene in a clinical lab, as her previous targeting testing had been performed on a research basis. The c.331G > A variant was reported as a variant of uncertain significance (VUS) and no additional variants were found. This variant had been previously reported in the literature in 1998 (around the time of the birth of her daughter affected with perinatal HPP) as a heterozygous variant in a 4-year-old Japanese boy with odontohypophosphatasia without skeletal manifestations [21]. It was later reported again in a child with perinatal lethal HPP in compound heterozygosity with a known severe variant; of note, the father of the child in this case was found to carry the c.331G > A variant and had a low-normal ALP, but no clinical signs of a mild form of HPP [22]. Despite some supporting evidence, there was insufficient information to classify this variant as pathogenic; therefore, it was reported as a VUS based on the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) standards for variant interpretation [23].

Based on the information available, the proband was diagnosed with AD HPP. Genetic counseling was subsequently provided after the proband's diagnosis, which altered the recurrence risk for her child III.4 from a < 1% chance to be affected with perinatal HPP to a 50% chance to be affected with a milder, autosomal dominant form. The risk to her twins remained uncertain, as her former partner's variant has not been reported in AD HPP to our knowledge, and he did not pursue any additional workup for HPP. The proband elected not to pursue genetic testing for any of her children at this time and none have had further workup for HPP. Testing of the proband's parents and sister identified the same c.331G > A variant in her father and sister.

### 3.2. Family #2

The proband is a 67-year-old female who presented for evaluation of HPP due to a history of osteoporosis, multiple fractures, and a positive family history (Fig. 2). Her fracture history included a right leg fracture at age 2, right proximal fibula and right tibia fractures at age 16, and right femoral neck and right shoulder fractures at age 65 after a ground-



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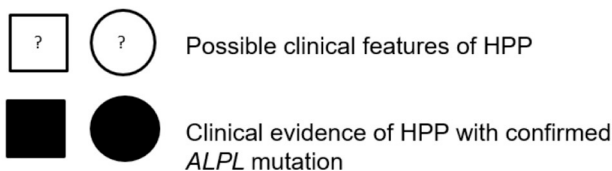


Fig. 1. Family #1 pedigree demonstrating AD inheritance in generations I and II and AR inheritance in generation III.

level fall. After the latter fractures, she was treated weekly with alendronate for two years and was briefly on denosumab, a drug often used to treat osteoporosis. She had two low ALP values of 32 and 39 U/L (reference range: 40–150 U/L), but this had not been clinically appreciated. She experienced frequent cavities in childhood despite appropriate dental hygiene, malformed incisors, and spontaneous loss of two adult teeth at age 13 requiring a bridge.

Family history was significant for the death of a grandson (IV.1) at one day of life affected with hypoplastic lungs. The child was found to be a compound heterozygote for two variants in the *ALPL* gene: c. 648 + 1G > A, and an approximately 1.4 kb deletion in exon 2. The proband's son (III.1) and daughter-in-law (III.2) underwent *ALPL* gene sequencing which confirmed trans configuration of the variants. At that time, genetic counseling was provided and the family was told this was a case of autosomal recessive HPP and the parents were presumed to be unaffected carriers. Subsequent familial testing led to discovery of the c.648 + 1G > A variant in the proband.

At the time of her evaluation, she reported bilateral foot and hand pain, arthritis of the right ankle and knee, and one cracked tooth. She underwent X-rays which revealed the aforementioned fractures of the femoral neck, fibula, tibia, as well as severe right tibial osteoarthritis and moderate left midfoot osteoarthritis. Degenerative changes of the spine and overall demineralization were also noted. She reported experiencing sleep disturbances including light sleep and poor overall sleep quality resulting in fatigue. Her ALP was found to be 39 U/L (reference range: 40–150 U/L) and her Vitamin B6 was 1367 nmol/L (reference range: 12–20 nmol/L).

The family history was updated and positive for HPP-like symptoms in several family members. The proband's son (III.1) did not undergo clinical evaluation but was reported to have a history of degenerative spine disease and foot pain. The proband reported two sisters (II.1 and

II.2) with osteoporosis treated with bisphosphonates without improvement, including new fractures while on treatment. The proband's mother (I.2) also had a history of osteoporosis treated with bisphosphonates; she broke her hip while on treatment. It is not known at this time whether any of the proband's family members have undergone clinical evaluation for HPP.

### 3.3. Family #3

The proband is a 39-year-old female who presented for evaluation of HPP after a bone density scan revealed osteoporosis and positive family history (Fig. 3). Past medical history was positive for a fracture as a teenager of her right ankle after a minor motor vehicle accident with limited trauma. This required physical therapy and she exhibited unremarkable bone healing without residual pain. Pediatric history was positive for early loss of primary teeth prior to the typical age of five, significant lower leg pain deemed “shin splints,” and difficulty running.

Family history was positive for a spontaneous pregnancy loss of unknown gestational age followed by the birth of a son (III.8) when the proband was 32 years of age. HPP was suspected in her son based on hypomineralization detected on ultrasound prenatally and multiple broken bones at birth. A genetics consultation was performed after delivery, and osteogenesis imperfecta was suspected initially but testing was negative. Further genetic testing revealed compound heterozygosity for two pathogenic variants in the *ALPL* gene, c.119C > T (p.Ala40Val) and c.1231A > G (p.Thr411Ala), confirming the diagnosis of HPP. Parental testing was not performed. Treatment with asfotase alfa was initiated at the time of diagnosis. The child was hospitalized until age 7 months and was discharged home with mechanical ventilation. He passed away at age 8 months secondary to respiratory infection.

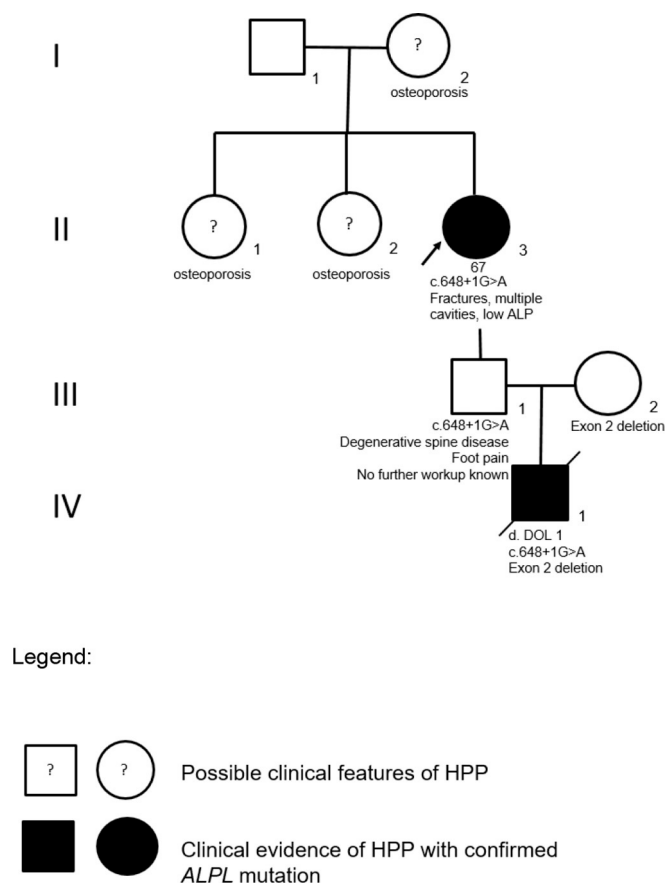


Fig. 2. Family #2 pedigree demonstrating suspected AD inheritance in generations II and III and AR inheritance in generation IV.

At the time of the proband's evaluation, seven years after the death of her son (III.8), she had a decreased ALP of 29 u/L (reference range: 41–82 u/L), an elevated PLP of 264.9 nmol/L (reference range: 20–120 nmol/L), and an elevated urine PEA of 114 nmol/mgCr (reference range: 0–27 nmol/mgCr). She reported frequent headaches worsening with age, balance problems, “brain fog,” and elevated intra-ocular pressure on ophthalmologic exam. She experienced multiple cavities despite appropriate dental hygiene and one cracked tooth. She suffered from chronic pain in her left hip and bilateral ankles and reported worsening bone pain over time. She experienced a left metatarsal fracture in at age 20 (unknown if related to trauma). *ALPL* sequencing subsequently revealed heterozygosity for the c.1231A > G variant, classified as likely pathogenic.

The family history was positive for HPP-like symptoms in several family members. The proband's father experienced bilateral foot deformities described as lateral pronation, dental cracking in his 40s, a fractured clavicle after a fall, and was described as having short stature. He was deceased at the time of evaluation and never pursued additional testing for HPP. Her six full siblings all pursued molecular testing of the *ALPL* gene. Four of the six (II.2–4, II.7) had the c.1231A > G variant detected in the proband and were subsequently diagnosed with AD HPP after clinical evaluation. The proband's eldest sister (II.2) had biochemical workup revealing low ALP of 26 u/L (reference range: 37–150 u/L), elevated PLP of 121.6 ng/mL (reference range: 2.1–21.7 ng/mL). Clinically, she had experienced early loss of primary teeth, poor secondary dentition, history of “growing pains,” osteoporosis noted on dual-energy X-ray absorptiometry (DEXA) scan, a metatarsal fracture, scoliosis, bilateral hallux valgus, bilateral heel enthesopathy, and calcium pyrophosphate deposition (CPPD) arthropathy (pseudogout). More recently, she had developed gait abnormalities which were described as broad-based and “wobbly.” The proband's

eldest brother (II.3) experienced early loss of primary teeth and bone pain and had a low ALP value. One affected sister (II.4) experienced foot fractures, early loss of primary teeth, CPPD arthropathy, and had a low ALP value. Another affected sister (II.7) experienced early loss of primary teeth and bone pain; she had both a low ALP value and elevated PLP value.

Symptoms have also been reported in several of the proband's nieces and nephews, though none have had genetic testing to confirm the diagnosis to our knowledge. Individual II.2's daughters have both low ALP values and early loss of primary teeth. Individual II.7's children have not had biochemical workup or genetic testing, but clinically, she has one son with malformed teeth, one son with a fractured arm at age 3 (unknown if related to trauma), and a daughter with scoliosis. The proband had two additional sons without clinical symptoms suggestive of HPP at the time of their initial evaluation (ages 4 and 5), which occurred seven years after the death of her first son.

Family history was thus suggestive of AD HPP in generations I and II, with a case of AR inheritance in generation III.

### 3.4. Family #4

The proband is a 16-month-old female who initially presented for clinical genetics evaluation four decades ago with brachycephaly, premature synostosis of the coronal and sagittal sutures, and rachitic changes at the ends of her long bones and at the costochondral junctions. She underwent craniectomy and throughout childhood was well with normal cognitive development except for gait disturbance with leg bowing.

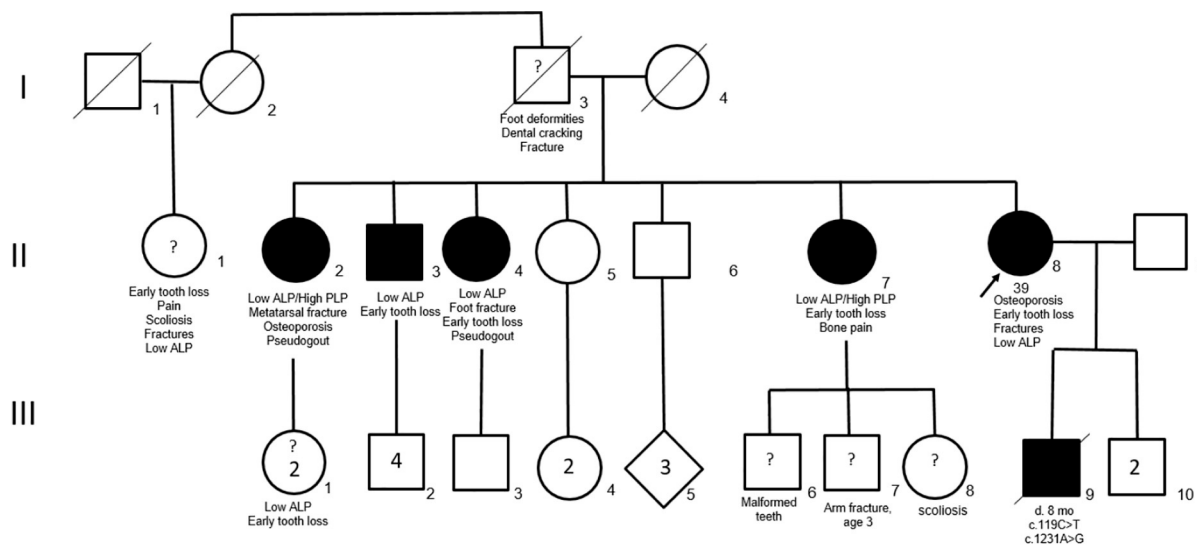
Family history (Fig. 4) was positive for neonatal death of a male sibling (III.1) with severe “skeletal deformities” five years prior to the birth of the proband and juvenile HPP in a maternal second cousin (III.3), confirmed biochemically and molecularly. The parents of the proband (II.3 and II.4) were asymptomatic at the time of her evaluation. They were non-consanguineous and of Mennonite descent.

The proband was subsequently diagnosed with juvenile HPP based on her clinical history, radiologic findings, family history, and persistently low ALP ranging from 30 to 45 U/L (reference range [13 mo–10 years]: 70–258 U/L). As a young adult, she had persistently low ALP (17 U/L; reference range: 30–120 U/L), normal serum calcium, elevated serum phosphate of 1.61 mmol/L (reference range: 0.81–1.45 mmol/L), and elevated urine phosphoethanolamine (PEA) of 68 uM/mmolCr (reference range: < 10 uM/mmolCr). She was subsequently lost to follow up.

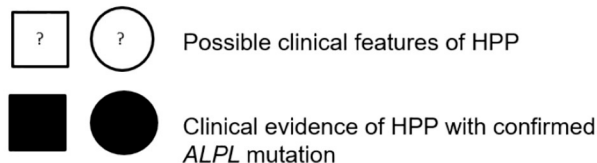
The proband re-established care approximately 15 years after her initial evaluation. She had remained generally asymptomatic except for a stable scoliosis. At that time, molecular studies were performed and revealed compound heterozygosity for two *ALPL* variants, c.1001G > A (p.Gly334Asp), and c.571G > A (p.Glu191Lys). The c.1001G > A variant was identified as a founder variant in the Mennonite population in Manitoba and other parts of Canada [24]. The c.571G > A variant had been identified in autosomal recessive HPP [9], but was not considered a founder variant.

Upon review of the family history, it was found that the proband's father (II.3) presented at 47 years of age with severe headaches and vomiting secondary to acute increased intracranial pressure due to longstanding, previously undetected craniosynostosis. Further review of the father's history revealed a long-standing history of unexplained trigeminal neuralgia and headaches. He had undergone 3 craniectomies with complete resolution of the neurologic symptoms. Pediatric history was positive for “rickets” that improved over time and difficulty running and keeping up with his peers.

Extensive family studies were initiated and targeted *ALPL* sequencing of the father identified him as a compound heterozygote for the same variants as the proband. He also had a decreased serum ALP of 15 U/L (reference range 30–120 U/L), elevated serum phosphate of 1.59 mmol/L (reference range: 0.81–1.45 mmol/L), and urine PEA of



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**Fig. 3.** Family #3 Pedigree demonstrating suspected AD inheritance in generation II and AR inheritance in generation III.

43 uM/mmolCr (reference range: < 10 uM/mmolCr). The paternal extended family history was negative; all three of the father's siblings had normal ALP values, normal serum phosphate, and normal urine PEA. Molecular studies for each of these siblings did not identify either of these pathogenic *ALPL* variants. The paternal grandparents were deceased and never underwent evaluation for HPP. The proband's mother (II.4) remained asymptomatic, but had a low ALP of 14 U/L (reference range: 30–120 U/L), high-normal serum phosphate of 1.26 mmol/L (reference range: 0.81–1.45 mmol/L), and normal urine PEA. She was heterozygous for one *ALPL* variant (c.1001G > A). The proband's maternal grandmother (I.3), was also heterozygous for the c.1001G > A variant. The maternal grandfather (I.4) had normal biochemistry and neither *ALPL* variant. The proband's maternal second cousin (III.3), previously clinically diagnosed with HPP, had the same two variants as the proband and followed a more typical course of juvenile-onset HPP with craniosynostosis, premature loss of deciduous teeth, rickets, and fractures. At present, the proband's father is in his 70s and has recently experienced bilateral fractured femurs following a fall, with one requiring rodding. This has led to chronic pain and limited ambulation requiring the use of a walker. Her mother has remained asymptomatic.

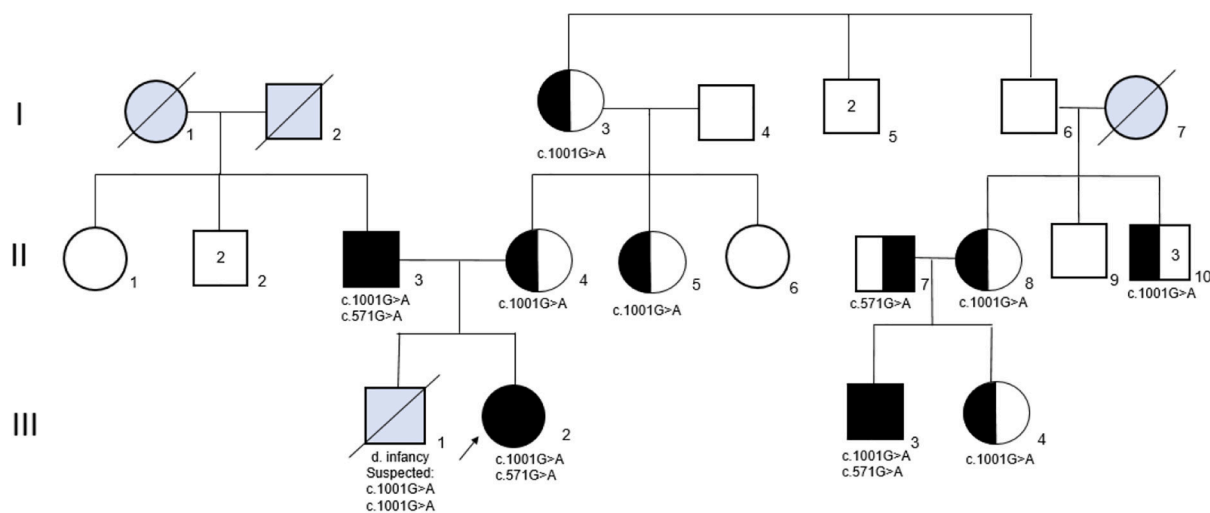
**4. Discussion**

We present four families in which diagnosis of and genetic counseling for HPP was complicated by multiple modes of inheritance and a wide phenotypic spectrum. These complex cases highlight the importance of careful clinical evaluation combined with a comprehensive and detailed family history analysis in order to provide the most accurate risk assessment and genetic counseling. Until recently, HPP has

been perceived as a disorder with distinct dominant and recessive forms, wherein parents of children with neonatal or infantile disease are considered asymptomatic carriers, similar to other AR metabolic disorders. There are indeed cases of true carriers that do not manifest clinical symptoms at any point in the lifespan; however, without evidence of genotype-phenotype correlation, case reports, or in vitro studies to determine the effect a specific variant in heterozygosity, these cases can be challenging to identify. Failure to thoroughly assess presumed carriers can lead to inaccurate recurrence risk, missed diagnosis of parents and extended family members, and potentially missed opportunities to treat an individual with significant disease burden. Biochemical evidence alone (low ALP value) is not sufficient to make this distinction. Lessons learned from these cases highlight the gap in our understanding of the disease burden for individuals with a single heterozygous *ALPL* variant in the setting of presumed autosomal recessive inheritance.

In the first three cases, the precipitating event that led to the diagnosis of HPP in the proband was the birth and subsequent death of a child (or grandchild, as in family #2) with a severe form of HPP caused by two pathogenic *ALPL* variants. These were presumed to result from autosomal recessive inheritance, and the respective probands did not undergo comprehensive clinical evaluation until many years after the death of the child in their respective families. The evaluations were subsequently based on clinical symptoms. This delay in diagnosis led to improper recurrence risk assessment in all cases, impacting medical and reproductive decision making. This is highlighted in particular in family #1, in which family planning was altered and the decision to utilize ART (and subsequently to decline ART with a new partner) was made. However, at the time of the initial genetic counseling provided to the proband in family #1, information on the phenotypic spectrum of HPP





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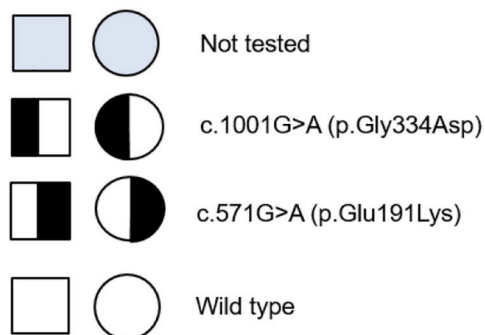


Fig. 4. Family #4 pedigree demonstrating AR inheritance in generations II and III with variable expressivity among affected individuals with the same genotype.

was limited, and she was counseled with the information that was available at the time. There is now a comprehensive database (The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database: [http://www.sesep.uvsq.fr/03\\_hypo\\_mutations.php](http://www.sesep.uvsq.fr/03_hypo_mutations.php)) that does report the c.331G > A variant found in proband #1 in symptomatic heterozygotes characterized as having odontohypophosphatasia [7]. In vitro studies, however, suggest this variant in heterozygosity does not result in a dominant negative effect, with an estimated 2–4% residual wild type activity in a homozygous state [13,35]. While the proband's musculoskeletal symptoms are not easily explained based on what is known about her variant, it is clear that she has a low ALP, elevated PLP, and clinical manifestations reported in patients with HPP. As such, she has been managed as a patient with AD HPP. The second variant in this family, c.1426G > A, has not been reported as a single heterozygous variant. It remains unclear whether this variant can result in autosomal dominant HPP, thus complicating risk assessment for the proband's twin children.

In family #2 and family #3, we highlight two additional examples of presumed autosomal recessive HPP with neonatal lethal outcomes. However, after further workup and familial testing, the “carrier” parents are found to be symptomatic in both cases. The c.648 + 1G > A variant in family #2 has been reported in compound heterozygosity with a missense mutation in a fetus with perinatal lethal HPP, whose parents both had low ALP values [25]. Although further clinical workup on the father of the child (III.1) was not performed to our knowledge,

the diagnosis in the child led to the subsequent diagnosis of his grandmother, the proband (II.3), and potentially additional family members. This variant has been reported in several other patients with perinatal HPP in the ALPL mutation database; however, there have been no additional case reports of this variant as a single heterozygous variant causing clinical symptoms of HPP to our knowledge. Of note, the variable expressivity of presumed AD HPP is apparent in these two families. Family #2 demonstrated significant fracture history (II.3) and unexplained osteoporosis that appeared unresponsive to treatment (I.1, II.1, II.2) as these individuals sustained additional fractures while on treatment. In family #2, it is likely that the diagnosis of AD HPP would have eventually been made in the affected family members after experiencing fractures on bisphosphonate therapy, even without the precipitating diagnosis of AR HPP in the proband's grandson. There were metatarsal fractures in family #3, and symptoms of chronic pain and poor dentition in the proband (II.8). Further, family #3 highlights the utility of a continually updated, detailed family history and subsequent targeted familial testing, with an additional four family members diagnosed with AD HPP.

Family #4 is an example of the importance of thorough genetic testing and clinical evaluation of parents of an affected proband. In this case, the proband was found to be compound heterozygous for two known pathogenic ALPL variants, c.1001G > A (the common Mennonite founder mutation) and c.571C > T. Her parents (II.3 and II.4) were asymptomatic at the time of her initial evaluation, and

autosomal recessive inheritance was presumed and the family received genetic counseling. A possible relationship between the proband's diagnosis of juvenile HPP and the history of the unexplained prior neonatal death of a sibling with "limb deformities" was not appreciated at the time. After her father presented with symptoms of HPP in later age, it might have been concluded that this was in fact a case of autosomal dominant inheritance; however, molecular testing identified the same two pathogenic variants in *ALPL* in the father and daughter, indicating both were affected with autosomal recessive HPP and presenting with markedly different phenotypes. The mother of the proband carried only the c.1001G > A variant. While not confirmed, it was presumed the case of neonatal death in this nuclear family is a result of inheriting one c.1001G > A allele from each parent, given the known lethality of the variant in homozygosity in the Mennonite population at that time [26]. However, it is also possible the different phenotypes in the father and daughter could be explained by another *ALPL* variant not detected by the targeted DNA testing that was performed in this case. This highlights the need for full gene sequencing and potentially deletion/duplication analysis, rather than targeted testing, for parents of an affected child with known HPP.

While initial suspicion of HPP may occur in the setting of decreased serum ALP, there are several challenges in ensuring an accurate diagnosis of HPP is confirmed in an individual with clinical and/or biochemical suspicion of the condition. While serum ALP analysis is generally an accessible and affordable test and useful for HPP screening, proper interpretation of ALP levels can present a challenge. Close attention should be given to age and gender-adjusted reference ranges, particularly in the pediatric population. When reviewing ALP results, it is important for the clinician to note whether the appropriate reference ranges have been used, as results interpreted incorrectly may not have an obvious "abnormal" or "flagged" status. Such ranges have been published based on data from the CALIPER (Canadian Laboratory Initiative in Pediatric Reference Intervals) database [27]. However, for adults of either gender, the reference range is generally standardized, though it may vary slightly between performing laboratories. Low ALP values should be interpreted in the context of clinical presentation and other known HPP biomarkers, including serum pyridoxal 5'-phosphate (the primary circulating form of Vitamin B6), urine phosphoethanolamine (PEA), and plasma inorganic pyrophosphate (PPI), all of which are natural substrates for ALP that accumulate and are often increased on biochemical analysis of HPP patients [28]; however, analysis of PPI levels is not currently a clinically available test. Additionally, it must be noted there are other non-HPP causes of decreased ALP that must be considered such as including zinc deficiency, Wilson disease, and other causes [29,30]; thus, evaluation of physical manifestations and additional biomarkers are critical to determining the significance of decreased ALP. When an individual is found to have persistently low ALP and there is clinical suspicion for HPP, ALP analysis should be repeated periodically to screen for transient elevations caused by one of several factors, including both overt and stress fractures, or other bodily stress such as illness or surgery. Finally, all individuals with unexplained persistently low ALP should be offered sequencing of the *ALPL* gene with reflex to deletion/duplication analysis if indicated; however, making a formal diagnosis remains a challenge in the absence of obvious clinical manifestations at the time of mutation testing.

HPP is likely underdiagnosed, and a proper estimate of prevalence in the general population is not known. "Moderate" forms of HPP are estimated to be up to 50 times more prevalent than severe forms (1/6370 vs. 1/300,000, respectively) in European populations [31]. In addition, penetrance of *ALPL* mutations is not well-established; for these reasons, full gene sequencing and potentially deletion/duplication analysis of the *ALPL* gene may be considered for anyone with suspected HPP and their relatives. In the setting of presumed AD HPP, it is possible a parent carries an additional pathogenic variant not present in the child, especially in populations that have a higher incidence of *ALPL* variants (such as Canadian Mennonites, as in family #4). If only

one variant is detected, full clinical evaluation should still be performed given that those with AD HPP may still manifest the full range of symptoms at any point in the lifespan, including fractures, as evidenced in the literature [32,33] and in the case examples discussed herein.

The variable expression of the HPP phenotype in those with a single variant supports the role of a dominant negative effect that contributes to the manifestation of autosomal dominant HPP<sup>11</sup>. However, while this form has been previously described as "mild," or the individual considered to be a carrier, the cases presented here and in other reports support the high variability in symptom severity in those with disease caused by a single variant, highlighted by the variability between family #2 and family #3, each with one single *ALPL* variant identified. This effect has been observed in other genetic conditions, such as *COL1A1/2* osteogenesis imperfecta (OI) in which a dominant negative mechanism can lead to a range of phenotypes from perinatal lethal OI to the intermediate, common variable form. This has also been observed in other connective tissue conditions, such as Marfan syndrome, where single pathogenic variants in *FBN1* are responsible for the variable expressivity of the disease, even within the same family. In rare cases, if two individuals with Marfan syndrome and a single *FBN1* pathogenic variant have a child together, there is a chance for the child to inherit both variants and present with neonatal Marfan syndrome. In all four families discussed herein, the parents of children diagnosed with HPP were all initially presumed asymptomatic carriers of the condition. Information on specific variants may have been limited at the time of each respective assessment, however, and risk assessment was likely provided with the most up-to-date information available at the time. This highlights the importance of continued efforts to characterize the many *ALPL* variants detected in those with HPP in order to provide accurate genetic counseling and disease management.

Part of a complete genetics evaluation for an individual or family often involves genetic counseling. Genetic counselors should be well-versed on the various forms of inheritance and penetrance of HPP. Counseling on inheritance should be tailored appropriately, and a thorough explanation of the potential for "carriers" to exhibit symptoms of the disease should be provided. Discovery of vertical transmission of disease via dominant inheritance in a family results in a higher number of at-risk family members who may require education on the disease and the opportunity to pursue testing. In family #1, two additional family members were diagnosed with AD HPP after the proband's diagnosis, and in family #3, four of the proband's siblings sought molecular testing and were subsequently diagnosed with AD HPP. This approach may be considered similar to genetic counseling models of neurodegenerative disease and cancer predisposition syndromes, where the identification of a single affected individual can lead to identification of many others. In some cases, as in cancer predisposition, steps to reduce or delay symptom onset may be available.

Genetic counselors should also consider detailed and targeted family history collection to be imperative regardless of mode of inheritance, as family history may be under-collected in cases of presumed AR disorders. In cases of confirmed or suspected HPP, the family history should be screened specifically for symptoms such as chronic pain, pseudo-gout, dental problems, osteoarthritis, fractures, and other orthopedic concerns in both childhood and adulthood. The HPP pedigree should be treated as a living document and updated at each clinical visit in detail. Further, special attention should be given to other diagnoses and symptoms commonly overlooked in genetic family histories such as fibromyalgia, rheumatoid arthritis, and osteoporosis; these may indicate possible manifestations or misdiagnoses of HPP or potentially other bone disorders [34]. Genetic counselors should also inquire about ancestral backgrounds and presence of consanguinity to screen for potential founder mutations.

High-quality genetic counseling is focused on providing complex information in terms understandable by all patients. This includes careful use of language related to genetic disease. In discussing HPP and its various presentations, the term "carrier" should be used with caution

when describing the parents of severely affected children. This term implies a healthy individual with no personal risk for disease and may lead to confusion for patients and families. To avoid this confusion, the difference in presentation between individuals with one pathogenic variant and individuals with two pathogenic variants should be emphasized. In particular, anticipatory guidance for the severity of symptoms is indicated; if parents do have manifestations of HPP, these will not be as overt as those seen in their child. However, even if they are not experiencing symptoms at the time, this does not ensure a non-disease state. This aspect of counseling may be challenging for those who are symptomatic (including those previously misdiagnosed or unreported) and feelings of guilt and shame may be invoked if a parent feels he/she is “responsible” for passing on the disease to the child, even if the parent was unaware of actually having a genetic disease themselves. Careful consideration of both clinical and emotional manifestations of heritable disease must be taken when providing care to a family affected with a highly variable and complex condition such as HPP.

## 5. Conclusion

We present four family case studies that illustrate the evolving knowledge of the disease spectrum of HPP and highlight variable expressivity, variable penetrance, and multiple modes of inheritance; specifically, the presence of both AD and AR inheritance in a single family. These cases also highlight the gap in our understanding of genotype-phenotype correlations in HPP. We emphasize the repercussions of incorrect risk assessment leading to inaccurate genetic counseling, potential alterations in family planning decisions, and missed or delayed diagnoses and treatment of affected individuals. Both genetics and non-genetics clinicians should make themselves aware of the potential for vertical transmission of HPP in families and tailor their risk assessment, counseling, and testing strategies in order to improve outcomes for HPP patients and their families.

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