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CLINICAL CASES

Asfotase alfa treatment of an African-American infant with perinatal hypophosphatasia and homozygous hemoglobin SC disease

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Hypophosphatasia (HPP, OMIM #241500) is characterized by defective bone mineralization, associated with decreased activity of the tissue non-specific alkaline phosphatase (TNSALP), due to mutations in the *ALPL* gene. Asfotase alfa is a bone-targeted recombinant alkaline phosphatase, currently Food and Drug Administration approved for infantile and pediatric onset HPP. We report a 7-month-old African-American girl with prenatal HPP. On day 4 of life, she was started on asfotase alfa. Skeletal survey obtained at 6 weeks of age, revealed improved calcification of all osseous structures. *ALPL* gene sequencing revealed a novel heterozygous compound c.46_49delAACT/c.360_361 delGG mutation. Tracheostomy tube was placed at 15 weeks of age and she was maintained on long-term ventilator settings until 7 months of age when she died secondary to overwhelming culture-negative sepsis and multi-organ failure. Perinatal HPP is associated with high mortality rate due to respiratory failure, poor ventilation and need for aggressive respiratory management. We report a case of a novel compound heterozygous *ALPL* gene sequence variant c.46_49delAACT/c.360_361 delGG in an African-American girl with HPP and homozygous HbSC disease who survived up to 7 months of age after early diagnosis and treatment with asfotase alfa, and high-dose supplementation with cholecalciferol and calcium.

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Introduction

Hypophosphatasia (HPP, OMIM #241500) is a rare genetic disorder, with variable clinical presentation.[1] HPP is characterized by defective bone mineralization associated with decreased activity of the tissue non-specific alkaline phosphatase (TNSALP), due to mutations in the *ALPL* gene.[2–4] HPP has been divided into six clinical subtypes based on the age of manifestation and severity of symptoms: perinatal, prenatal benign, infantile, childhood, adult and odontohypophosphatasia.[1,2] The clinical presentation varies depending on the type of HPP, bone deformities and pulmonary involvement.[5] The most severe form, perinatal HPP, presents with poorly mineralized bones and pulmonary hypoplasia often detected *in utero*.[4] These severely affected neonates often die shortly after birth from respiratory insufficiency due to difficulty with adequate ventilation.[6] Asfotase alfa is an alkaline phosphatase enzyme, currently Food and Drug Administration (FDA) approved for infantile and pediatric-onset HPP, which results in improved bone mineralization and prolonged survival.[7]

HPP appears to be rare in the Black population. Whyte *et al.*[8] in 2006 reviewed 350 cases, which revealed that $<1\%$ of described patients with HPP are Black. The finding of HPP and homozygous HbSC disease may be a coincidental finding; however given that HPP appears to be rare in the Black population, which has a high prevalence of HbSC, it is unclear how the presences of both diseases may have affected the prognosis.[9] Here we report a case of a novel compound heterozygous *ALPL* gene sequence variant c.46_49delAACT/c.360_361 delGG in an African-American infant with HPP and homozygous HbSC. This infant survived up to 7 months of age after early diagnosis and treatment with asfotase alfa, and high dose supplementation with cholecalciferol and calcium

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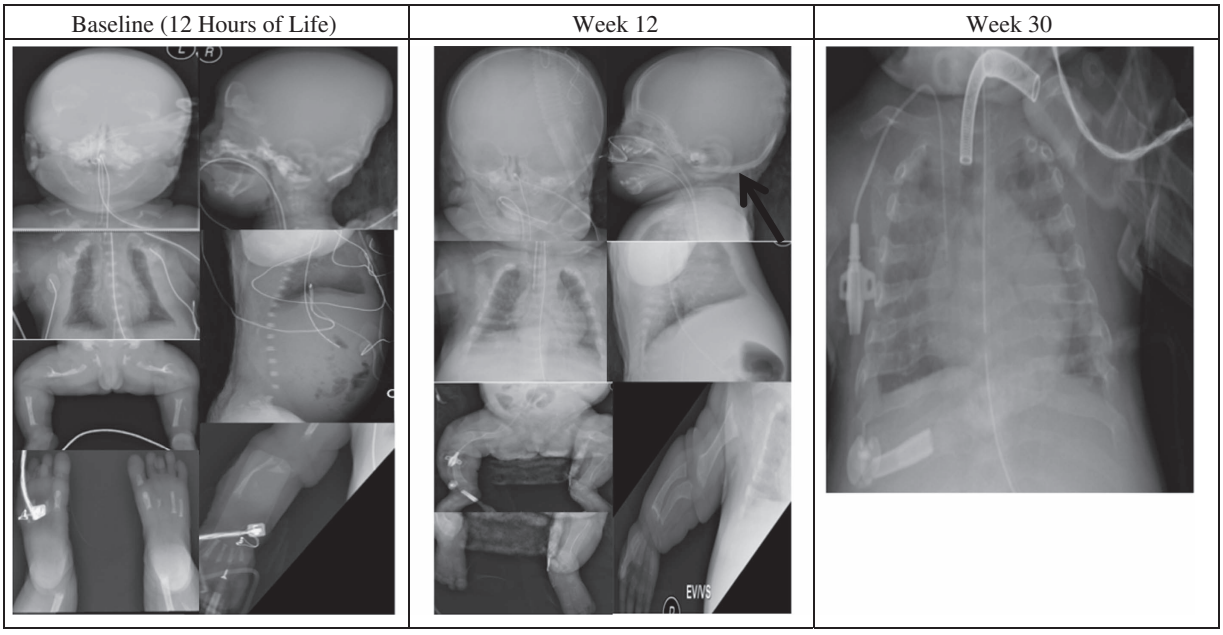

Figure 1 Skeletal survey just after birth, at weeks 12 and 30. At birth, skeletal survey revealed dysplastic skull with only frontal bones visualized, hypoplastic ribs, ulna, humerus, femur and fibula with diffuse metaphyseal fraying. The metacarpals were the only bones visualized in the hand. Note the general improvement in mineralization, reduction in bone deformities and improvement in shape of the skull base and chest cavity with continuous treatment with asfotase alfa.

(http://www.sesep.uvsq.fr/03_hypo_mutations.php.com). This is the second case report to describe a patient with HPP started on asfotase alfa within the first week of life.

Patient Characteristics

We report a 7-month-old African-American girl with prenatal history of polyhydramnios and skeletal anomalies. Fetal ultrasound was notable for polyhydramnios, disorganized ribs, hypoplastic chest cavity and angulation of the femurs, and the initial diagnosis of skeletal dysplasia was made. There was no family history of bone disease. Shortly after birth she could not sustain her respirations requiring intubation and initiation of mechanical ventilation with high-frequency oscillator ventilation. Echocardiography determined pulmonary hypertension at 24 h of life.

The birth weight was 2565 g (-1.49 s.d.) and length was 42 cm (-4.46 s.d.). Initial work up revealed hypercalcemia, hyperphosphatemia and undetectable alkaline phosphatase (normal reference range 530–1610 IU l^{-1}), high serum pyridoxal 5′ phosphate (PLP): >250 ng ml^{-1} (<100 ng ml^{-1}) and urine phosphoethanolamine (PEA): 11 133 mcmol GCr^{-1} (<50 mcmol GCr^{-1}). The initial skeletal survey, at 12 h of life, showed radiographic findings consistent with perinatal hypophosphatasia including: severe shortening of all limbs, metaphyseal flaring, hypoplasia of both ulnas and fibulas, dysplastic skull, narrow ribcage with short hypoplastic ribs, flattened vertebral bodies and dysplasia of the scapulae and clavicles bilaterally (**Figure 1**). The metacarpals were the only visualized bones within the hands (**Figure 1**). The diagnosis of perinatal hypophosphatasia was made. Although the patient was diagnosed with homozygous HbSC disease, none of the radiographic changes were consistent with HbSC disease at presentation.[9,10] In patients with homozygous HbSC disease, effects of chronic anemia, including bone abnormalities, are often not likely to be present before childhood.[10]

ALPL gene sequencing using genomic DNA identified a novel heterozygous compound c.46_49delAACT/c.360_361 delGG which has not previously been described in HPP (http://www.sesep.uvsq.fr/03_hypo_mutations.php.com). The first sequence variant c.46_49 del AACT, is predicted to result in premature protein termination (p.Ash16 Profs*2). This variant has been reported to be pathogenic for infantile hypophosphatasia in one patient by de Roo *et al.*[11] The second heterozygous sequence variant was defined as c.360_361 del GG, which is predicted to result in premature protein termination (p.Val 121 Glufs*4), thought to be pathogenic in HPP as well. Genetic analysis of both parents is warranted to provide appropriate genetic counseling.

A family meeting was held to discuss the prognosis and available treatment options. It was made clear to the family that without medical intervention the mortality rate was certain. The family desired to trial any treatment option available with the care goal of extending their time with the infant. The parents provided informed consent for the treatment with asfotase alfa. The treatment was approved by the institutional review board at Children's Hospital of Los Angeles for compassionate use, prior to FDA approval in the United States in October of 2015. The medication was provided by Alexion Pharmaceuticals. On day 4 of life, she was started on asfotase alfa, 2 mg kg^{-1} per dose subcutaneously, treatment three times a week. The results of the phase two clinical trial of enzyme replacement therapy, using recombinant TNSALP and asfotase alfa (ENB-0040; Alexion Pharmaceuticals, Cheshire, CT, USA), recommended utilizing a dose of 2 mg kg^{-1} per dose three times a week for infantile hypophosphatasia.[2,12]

Within 2 weeks of initiating treatment, her calcium and phosphate levels normalized. Initial 25-hydroxyvitamin D level

was 15 ng ml^{-1} (20–100 ng ml^{-1}). Vitamin D supplementation was started at 400 IU per day, on day 4 of life.[13] As expected, with increasing in alkaline phosphatase, 25-hydroxyvitamin D levels trended down to <10 ng ml^{-1}, supplementation was then increased, at 4 weeks of age, to 5000 IU daily. Twenty-five hydroxyvitamin D level remained <20 ng ml^{-1} and so at 4 months of age the dose was increased to 7000 IU daily. Repeated 25-hydroxyvitamin D level obtained at 7 months of age was 35 ng dl^{-1} with a normal calcium level. At no point during the treatment course was there any evidence of hypercalciuria. She remained on full total parental nutrition until 2.5 months of age when she reached full-volume enteral feeds.

Chest radiograph obtained on day 2 of life and after 12 weeks of treatment with asfotase alfa showed increase in thoracic cage dimensions and rib size secondary to the increase in mineralization. At 3.5 months of age, a tracheostomy was placed. Respiratory status continued to improve and she was weaned from conventional ventilator settings to long-term ventilation assist control/pressure control with the following settings: 30 breaths per minute, 32 cm H_2O PIP, 6 mc H_20 PEEP, 40% FiO_2 and 0.6 s inspiratory time. Her growth was relatively slow but clearly progressing along the second percentile with a weight of 4.9 kg (-1.2 s.d.) and length of 55 cm (-3.6 s.d.). No formal neuropsychiatric testing was undertaken during her hospitalizations, but she was noted for her improvement in obtaining developmental milestones.

She was scheduled to be discharged home at the age of 7.5 months when she developed persistent fever and tachycardia with the development of metabolic acidosis associated with a severe drop in her hemoglobin, hematocrit and hypoxia non responsive to ventilator changes. Her fever persisted and she developed leukocytosis and then went into cardiac arrest and she was pronounced dead at 7.5 months of age, family declined autopsy and final cause of death was determined as overwhelming culture negative sepsis with DIC and multi-organ failure.[2,6]

Discussion

HPP if often misdiagnosed prenatally as skeletal dysplasia, campomelic dysplasia, chondrodysplasias, osteogenesis imperfecta, osteopenia or hypophosphatemia.[3] Infantile HPP can be differentiated from other etiologies based on radiographic findings of hypomineralization, elevated calcium and phosphorus levels and persistently low alkaline phosphatase levels associated with elevation of its substrate (phosphoethanolamine, inorganic pyrophosphate and pyridoxal-5-phosphate).[3,4] Early, accurate diagnosis is essential to ensure initiation of treatment is not delayed.[1] Infants with prenatal HPP show early respiratory failure due to poorly mineralized bones and pulmonary hypoplasia.[1] This combination often results in high mortality rates.[2]

Okazaki *et al.*[4] emphasized the need for emergent treatment at birth, with specially skilled respiratory support, to improve the prognosis for these infants. A recent publication by Whyte *et al.*[12] described a cohort of 68 patients with perinatal HPP receiving asfotase alfa. In those patients treated with asfotase alfa, the survival rate at 3 years was 91% with 85% of those patients ventilator free. In 48 historical controls, survival rate was 27% with 25% of those patients ventilator free at 3 years.[12] Perhaps this is the second case report to describe the effects of early treatment of asfotase alfa for perinatal HPP within the first week of life. From our experience and the current published literature it would seem apparent that early treatment with asfotase alfa and high-dose supplementation with cholecalciferol and calcium resulted in improved mineralization of the skeleton, respiratory function and prolonged survival to 7 months of age. However, as occurred in our patient the risk of morbidity from alternative causes such as sepsis remains elevated in patient with perinatal HPP.

In conclusion, Perinatal lethal HPP is associated with high mortality rate due to poorly mineralized osseous structures resulting in respiratory failure, poor ventilation and need for aggressive respiratory management. We report a case of a novel compound heterozygous *ALPL* gene sequence variant c.46_49delAACT/c.360_361 delGG in an African-American infant with HPP and homozygous HbSC who survived up to 7 months of age after early diagnosis and treatment with asfotase alfa, and high-dose supplementation with cholecalciferol and calcium.

Conflict of Interest

Dr PP is a consultant for Alexion Pharmaceuticals. The remaining authors declare no conflict of interest.

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Author contributions: Dr APV reviewed the medical literature, drafted the manuscript, and approved the final manuscript as submitted. Drs PP, AG and CN were the primary treating subspecialty and neonatology providers for the patient, revised the manuscript and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Ethical Approval

All procedures involving human participants were in accordance with the ethical standards of the Children's Hospital of Los Angeles research committee. Informed consent was obtained from the parents. Note Enzyme replacement therapy was supported by Alexion Pharmaceuticals as a compassionate use in the Case Report and followed by an investigator-initiated clinical trial.

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