The U.S. Hypophosphatasia Foundation

Soft Bones

Finding the Key to HPP

SECOND SCIENTIFIC MEETING

MEETING DIGEST SUMMARY

BETHESDA USA

JUNE 3-4 2023

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What We Learned

This year's scientific meeting was full of insights and information about the latest in HPP research. Key themes from the conference include:

Diagnosing HPP, including characterizing carriers, remains complicated

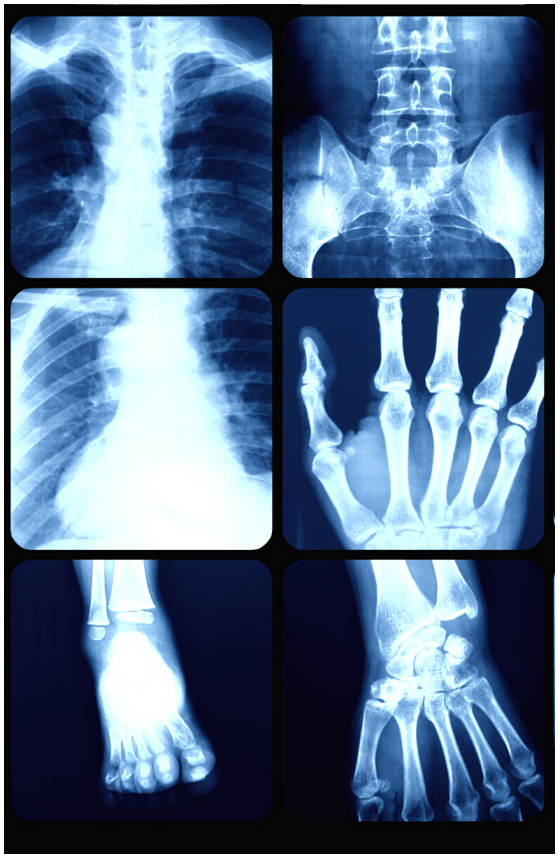
- Diagnosis of HPP is complex; no single clinical finding or result can confirm HPP
- Research showed that a combination of clinical, laboratory, imaging results and pedigree analysis is required to designate carrier parents as "affected" (showing clinical evidence of HPP)
- A wide range of conditions can be mistaken for HPP, making diagnosis difficult; HPP diagnosis are often delayed for years due to non-specific symptoms and varied rheumatological manifestations
- Individualized evaluation and treatment is critical given that HPP can masquerade as osteoporosis which has implications for appropriate treatment
- The *ALPL* Gene Variant Project maintains a public database that displays all *ALPL* variants and reclassifies variants to ultimately provide more clarity to families and healthcare providers
- Research evaluating the biochemical assessment of HPP suggests that the use of thresholds rather than reference limits may improve diagnosis rates
- The diagnosis of HPP in a young child can have multi-faceted implications throughout the family tree

The ability to predict the path of HPP remains elusive

- HPP symptoms are highly variable; there are likely many with HPP who have unrecognized symptoms, particularly parents of pediatric patients
- The variability of HPP within families suggests that HPP is not just about the genes but also about enzyme production and effects on different organs, substrates and otherwise; necessitates an individualized treatment approach
- Although the number and severity of the *ALPL* mutation(s) seems to have the greatest impact on the severity of HPP, many other factors may influence HPP severity including other genes, epigenome, microbiome, environment, other medical conditions, diet & more
- Individuals with HPP may experience all, some or none of the dental problems associated with defects in the tooth; early treatment intervention has been shown to help preserve tooth structure and function

What we know about the underlying biology of HPP continues to expand

- Pyrophosphate plays a critical role in numerous biological pathways, with disorders of pyrophosphate metabolism leading to conditions such as HPP among many others
- Research has shown that the process of mineralization forms a tessellation pattern resulting from the removal of inhibitors at specific sites in the body such as bones and teeth
- TNAP activity, an enzyme known for its mineralization effects, may be essential for mitochondrial dynamics and function in mesenchymal stem cells and adipocyte differentiation
- Initial research in mouse models suggests that HPP may have an impact on growth, size, fiber structure and force of the muscle; mitochondrial respiration and organization does not appear to be impacted
- Research in mouse models explored how TNAP may be implicated in bone and teeth defects in HPP, and how TNAP could affect muscle on a development or functional level
- Research showed that patients with HPP are not able to utilize phosphate for mineralization of bones in spite of having enough or even too much phosphate in the blood.
- Research aimed to identify a dental phenotype in HPP revealed that different parts of the teeth (enamel, dentin) are impacted differently depending on levels of ALP and severity of HPP
- HPP sheep were shown to have decreased musculoskeletal energetics at the cellular level that leads to compromised musculoskeletal structural development and function
- The most common dominant HPP mutations are ALPL D378V and N417S; counseling for families with these common mutations is important



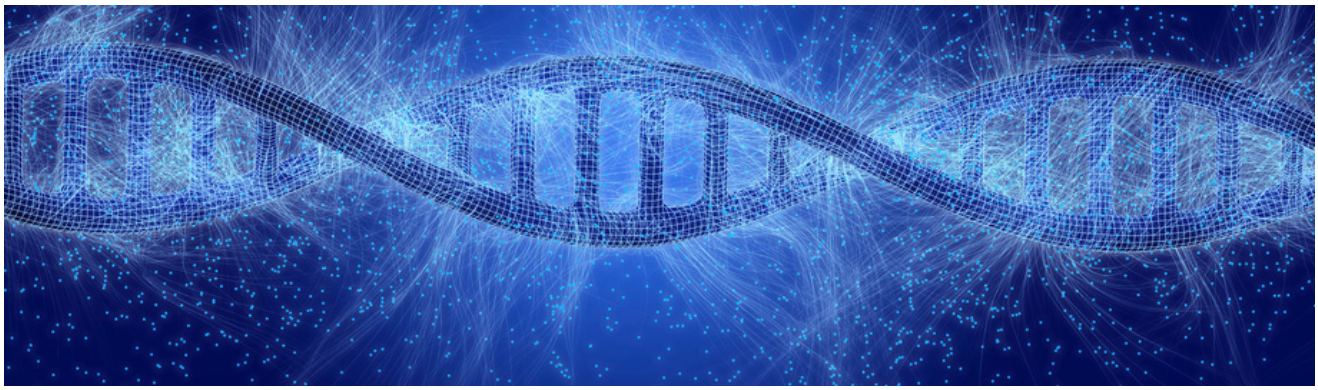
Insights into HPP symptoms, treatment effects and multidisciplinary care can help advance care for patients with HPP

- A new HPP phenotype called “treated HPP” represents patients who received early treatment intervention and have distinct needs; there are various challenges in the transition from pediatric to adult care for all patients with HPP
- Bone pain may not only be inflammatory but also, with time, may be neuropathic in nature
- HPP can cause ectopic calcification, however, the correlation between enzyme replacement therapy and ectopic calcification is unclear
- Discontinuing enzyme replacement therapy can lead to loss of gains achieved with treatment; demographics differed substantially for patients who discontinued treatment versus those that remained compliant
- Physical therapy, occupational therapy and speech-language pathology specialists can help establish baseline assessment of impairment in HPP and develop treatment plans
- There are various standardized tests and outcome measures around physical performance (more than just endurance) that are useful in clinical practice to identify functional deficits, inform clinical and treatment decision-making, and develop a baseline for comparison

The future is bright: Promising preclinical research and new research models are bringing us one step closer to transformative options, including genomic medicines

- Initial results in mice studies show the potential of gene therapy in treating perinatal, severe infantile HPP in animal models but still have hurdles to clinical application; unique aspects of HPP human pregnancies include an increase in maternal circulation of ALP
- A potential gene therapy used as a preventative treatment in the infantile HPP mouse model and a corrective treatment in the adult HPP mouse model; longer-term studies are underway
- Sheep are a valuable animal model for HPP research given they have two sets of teeth, bone remodeling that is similar to humans, and allow for repeated tissue sampling across a range of ages

Session 1: Genetics, Where Are We Now?



The ALPL Gene Variant Database: Current Construction, Validation and Variant Interpretation

Wolfgang Högler, MD FRCPCH; Johannes Kepler University Linz

Key takeaway: Receiving a result of "variant of uncertain significance" can delay a HPP diagnosis and decision making. The ALPL Gene Variant Project maintains a public database that displays all ALPL variants and reclassifies variants to ultimately provide more clarity to families and healthcare providers

The *ALPL* gene provides instructions for making an enzyme called tissue-nonspecific alkaline phosphatase (TNSALP). Loss-of-function mutations in *ALPL* cause HPP. Genetic variants range from benign (not harmful) to pathogenic (disease-causing), although often genetic results can characterize the variant as having "uncertain significance." Receiving genetic results noting a "variant of uncertain significance" (VUS) can delay diagnosis and therapeutic decision-making for patients and families. The ALPL Gene Variant Project aims to create more clarity around genetic results by classifying VUS, reclassifying variants and maintaining a public database on all *ALPL* variants. Prof. Högler detailed the six-step process for VUS re-/classification (process takes approximately three months) which includes retrieving detailed clinical case information, a deep literature check on the variant, full genetic assessment and functional testing of ALP activity, all of which culminates in a consortium coming together to make a decision on the VUS. The ultimate goal is assess all VUS and re-/classify them to being either pathogenic (or likely pathogenic) or benign (or likely benign), although sometimes there is not yet sufficient information and the VUS remains uncertain. Healthcare professionals are encouraged to submit VUS via the website for analysis.

Clinical, Biochemical, and Genetic Studies of Pediatric Hypophosphatasia "Carrier" Parents

Nilton Salles Rosa Neto, MD, PhD, RhMSUS;
Universidade Santo Amaro, São Paulo, Brazil

Key takeaway: Characterizing carrier parents as being "affected" (showing clinical evidence of HPP) can be complicated. Research showed that a combination of clinical, laboratory, imaging results and pedigree analysis is required to make this distinction

Research evaluated parents of children with HPP to better understand what is happening within families with respect to *ALPL* genetic variants. Dr. Salles Rosa Neto discussed a study involving retrospective chart reviews to evaluate data from 302 parents of children with HPP (169 mothers, mean age = 34; 133 fathers, mean age = 36). Medical charts were reviewed for a range of information including the presence of typical HPP clinical features (signs and symptoms consistent with HPP overt disease), biochemical results (blood work assessing alkaline phosphatase (ALP) levels and other aspects), imaging (to look for the presence of bone abnormalities/fractures/calcium deposition) and *ALPL* gene variant analysis.

Results so far showed that among the 302 parents, only 18.2% were considered affected, that is, showing clinical, laboratory and imaging evidence of overt HPP, while 46.7% were carriers (showing laboratory abnormalities but no clinical symptoms) and 35.1% were controls/unaffected (showing normal ALP levels and absence of *ALPL* gene pathogenic variants). Affected parents had lower serum ALP levels and higher plasma pyridoxal 5′-phosphate (PLP) compared to the other groups of parents, and the majority of the affected parents reflected autosomal dominant inheritance. The study is still ongoing (completion of *ALPL* gene assessments) but preliminary analyses show that low ALP, elevated PLP and having a pathogenic *ALPL* gene variant were insufficient to designate parents as "affected". Instead, detailed clinical evaluation, combined with laboratory, imaging results and pedigree analysis is required to achieve this designation.

Implications of Positive Findings for Families

Michael P. Whyte, MD; Washington University School of Medicine in St. Louis

Key takeaway: The diagnosis of HPP in a young child can have multi-faceted implications throughout the family tree

When a young child is diagnosed with HPP, that diagnosis can often have broad implications cascading throughout the family. From current and future siblings being at risk for disease, to young parents being carriers (and affected or unaffected themselves), up through grandparents who may be affected but misdiagnosed with ailments typically associated with aging. Dr. Whyte led a discussion about the multi-faceted impact on families as well as potential new advances when it comes to prenatal and newborn screening.

Session 2: Mineralization, Genetics, & Epigenetics

Pyrophosphate: Homeostasis and Aberrations

Carlos Ferreira, MD; National Human Genome Research Institute/NIH

Key takeaway: Pyrophosphate plays a critical role in numerous biological pathways, with disorders of pyrophosphate metabolism leading to conditions such as HPP among many others

The history of pyrophosphate (PPi) dates back to 1827, when it was discovered by heating a sodium phosphate salt to red heat (hence where the term “pyro” came from). PPi is involved in more than 200 biological pathways including the biosynthesis of protein, nucleic acids, carbohydrates, cholesterol and others. Dr. Ferreira delved into many aspects of PPi including its transport into cells and the effect of a number of factors on PPi (including circadian rhythm, exercise, fasting and age). PPi also plays critical roles in growth and mineralization. There are several disorders of PPi metabolism such as HPP which have elevated PPi levels. Other disorders include ENPP1 deficiency (associated with generalized arterial calcification of infancy or GACI), ABCC6 deficiency, ANKH-related disease, and PPA2 deficiency.

The Phenome and HPP

Deborah Wenkert, MD; Wenkert & Young LLC

Key takeaway: Although the number and severity of the ALPL mutation(s) seems to have the greatest impact on the severity of HPP, many other factors may influence HPP severity including other genes, epigenome, microbiome, environment, other medical conditions, diet and more

The phenome is defined as the set of all phenotypes expressed by cells, tissues, organs, individuals or species, including those due to both genetic and environmental influences. Dr. Wenkert explored the question - what impacts how mild or severe HPP is in children? Autosomal dominant versus recessive inheritance does not fully predict age of onset or severity of HPP. In addition, age of onset does not fully predict age of dental involvement, height, or bone mineral density (BMD). Identical mutations do not uniformly predict phenotype even within a family. So, what are the factors that can influence the phenome? Dr. Wenkert touched on many different aspects that may have an impact such as other genes, epigenome, microbiome, environment (fetal crowding, biochemical environment of a carrier mother, heavy metals in soil), ALP activity suppression (due to other medical conditions) and diet (effects of zinc, magnesium, phosphate and vitamin B6).

The *Stenciling Principle* for Extracellular Matrix Mineralization: What is it and What is its Importance?

Marc D. McKee, PhD; McGill University

Key takeaway: Research has shown that the process of mineralization forms a tessellation pattern resulting from the removal of inhibitors at specific sites in the body such as bones and teeth

The field of biomineralization has always been marked by a duality of induction versus inhibition. Dr. McKee reviewed various aspects of research about mineralization, focusing on inhibition by pyrophosphate and proteins, and suggesting that inhibition of mineralization is the body's default pathway that must be overcome for proper skeletal and dental development. He introduced a double-negative concept that "inhibiting the inhibitors" of mineralization – by enzymatic degradation of the inhibitors – will drive mineralization patterns in the extracellular matrix. Applying this concept to bone, he described the *Stenciling Principle* for extracellular matrix mineralization where physiologic mineralization occurs through the actions of enzyme substrate pairs, namely the TNAP-pyrophosphate pair and the PHEX-osteopontin pair. The enzymes of these two pairs (TNAP and PHEX) are highly expressed and function at the specific sites in the body where "stenciling" of mineralization is required – that being bones and teeth. The normal pattern that is formed from these actions is called *crossfibrillar mineral tessellation*, which he showed is defective in X-linked hypophosphatemia. Ongoing studies are now looking at how this applies to other osteomalacic diseases such as hypophosphatasia.

Session 3: HPP In Adults

Pain: Is it in Your Muscles and/or Bones? What Explains Bone Pain?

Patrick Mantyh, PhD; University of Arizona College of Medicine

Key takeaway: Bone pain may not only be inflammatory but also, with time, may be neuropathic in nature

Although Dr. Mantyh's initial research started with studying bone pain in bone cancer, he realized these learnings could be translated to other bone diseases, including in people whose bones don't heal well, such as the elderly or in people with genetic diseases. With HPP, there is a variety of skeletal pain including bone fracture pain with delayed bone healing, joint pain, muscle pain, etc. In general when bones don't heal rapidly, nerves can begin to sprout up in places they shouldn't and in a greater number. When looking at bone pain, he theorizes that it may not just be inflammatory pain but probably with time, also neuropathic pain which is much more difficult to control.

Challenges of Diagnosing HPP: Molecular Diagnosis is Never Enough

Gary Gottesman, MD, FAAP, FACMG;
Washington University School of Medicine in St. Louis

Key takeaway: Diagnosis of HPP is complex; no single clinical finding or result can confirm HPP

Gottesman discussed the complexities of HPP diagnosis, noting that it remains a clinical exercise of identifying early tooth loss, using radiological evidence of skeletal changes of HPP, observing elevated levels of substrates (which can be elevated in carriers as well), noting the family history, and carrying out histopathologic and genetic testing. He emphasized the importance of understanding that no single clinical finding or result can confirm the diagnosis of HPP. Causes of hypophophatasemia can range from medical disorders to nutritional changes to medications to inappropriate laboratory reference ranges and environmental exposures. HPP family pedigrees demonstrate autosomal dominant and autosomal recessive patterns. He presented a variety of medical cases of hypophosphatasemia including a one-week boy with signs and symptoms of perinatal HPP but normal biochemical substrates, two brothers with variations in ALP expression, a 10-year-old boy with Duchenne muscular dystrophy, and an asymptomatic woman with low ALP activity.

Hypophosphatasia or Osteoporosis: A Clinical Conundrum

Peter Tebben, MD; Mayo Clinic

Key takeaway: Individualized evaluation and treatment is critical given that HPP can masquerade as osteoporosis which has implications for appropriate treatment

Dr. Tebben discussed the scope of patients with HPP masquerading as osteoporosis, looking at individuals who overlap between being a silent carrier/having minimal HPP symptoms and having low bone mass. Importantly, when diagnosing HPP, it is critical to remember that low ALP does not automatically equal HPP. He went on to discuss potential hazards and strategies for treating low bone density in adults with low ALP/HPP. Potential predictors of response to therapy include clinical features, biochemical studies, the specific genotype and bone biopsies. Overall, there can be a wide range of severity in adults with HPP in a population with low bone mass, and those adults who are minimally symptomatic may be mistaken for having osteoporosis. Regarding treatment, antiresorptive therapy may lead to bone pain/fractures, while anabolic treatment increases bone density and heals fractures in some but not all patients. He reinforces the importance of individualized evaluation and treatment.

Rheumatologic Manifestations of Hypophosphatasia

Chad Deal MD; Cleveland Clinic

Key takeaway: HPP diagnosis are often delayed for years due to non-specific symptoms and varied rheumatological manifestations

Dr. Deal provided a unique perspective, having both metabolic bone and rheumatology expertise. He reviewed various databases and studies, and drew from his own practice, noting that rheumatologic presentations (chrondrocalcinosis, calcific periarthritis, enthesopathy, Diffuse Idiopathic Skeletal Hyperostosis (DISH) and exostosis) are rarely the basis for an HPP diagnosis although they certainly are an important part of clinical manifestations of HPP. Instead, the most common reason an HPP diagnosis is made in his clinic is due to diffuse, widespread pain, as well as the low bone mass fracture type. Overall, rheumatological manifestations are varied, many symptoms are nonspecific and as a result, HPP diagnoses are often delayed for years.

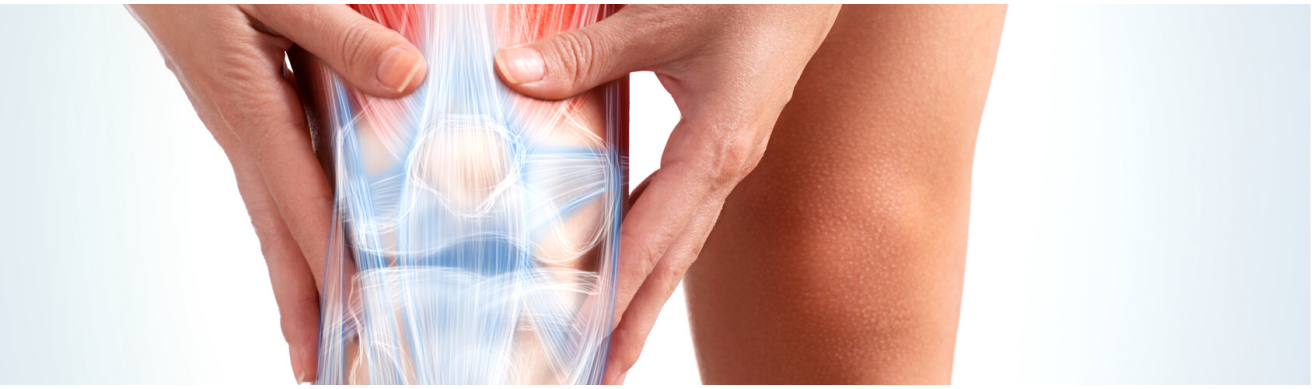
Hypophosphatasia: What Have We Learned? Indications for Treatment?

Lothar Seefried, MD; University of Würzburg

Key takeaway: The variability of HPP within families suggests that HPP is not just about the genes but also about enzyme production and effects on different organs, substrates and otherwise; necessitates an individualized treatment approach

Dr. Seefried posed the idea that we should treat HPP like other metabolic conditions such as hypothyroidism or diabetes, and importantly that anyone affected deserves an individual treatment approach. He delved into the high intrafamilial phenotypic variability in HPP, which is evidence that the disease is not just about the genes but also potentially in the transcription and translation affecting enzyme production, as well as different organs, substrates and otherwise. He provided an overview of HPP in adults, including the use of anti-sclerostin, pharmacodynamics of asfotase alfa treatment as well as specific case studies. He described the EmPATHY study (which studied enzyme replacement therapy in adults) as well as NuSTEPs (examining nutritional aspects around HPP). He emphasized that there is still much more to learn about HPP.

Session 4: HPP Beyond Bones and Joints



Insights into Dentoalveolar Defects Associated with Hypophosphatasia from Novel Mouse Models

Brian L. Foster, PhD; The Ohio State University, College of Dentistry

Key takeaway: Individuals with HPP may experience all, some or none of the dental problems associated with defects in the tooth; early treatment intervention has been shown to help preserve tooth structure and function

The tooth and its supporting connective tissues is composed of the four main components: enamel, dentin and cementum and alveolar bone. Shortly after HPP was defined in 1948, premature tooth loss was reported in affected individuals attributed to the lack of cementum (which attaches the tooth to the bone). Individuals affected by HPP may experience all, some or none of dental problems associated with defects in the tooth components, with the most prevalent being issues with cementum. This results in teeth not being well attached which can lead to mobility and easy tooth loss with the full root intact. Early studies with asfotase alfa showed that early intervention prevents loss of cementum and preserves the tooth structure and function. Dr. Foster described various models that have been useful in research including knock-out, knock-in, and several conditional knockout mice models, as well as a case report on dental effects of HPP and use of a sheep model of HPP. Overall, animal models in HPP have expanded significantly in the last five years, with a variety of mouse models available as well as a sheep model which has benefits from being a larger animal model.

Compromises in Skeletal Muscle and Myoblast Energetics are Associated with Compromised Kinematics in Sheep with Hypophosphatasia

Dana Gaddy, PhD; Texas A&M University

Key takeaway: Sheep are a valuable animal model for HPP research given they have two sets of teeth, bone remodeling that is similar to humans, and allow for repeated tissue sampling across a range of ages

Dr. Gaddy discussed her development of an animal model for HPP that could better mimic the human HPP condition than mice. As mice don't lose baby teeth and no muscle weakness had been described, Dr. Gaddy and team chose the sheep because of its advantages of having two sets of teeth, osteonal bone remodeling to insert a mutation in the ALP gene and create HPP sheep. The HPP sheep model has advantages such as having two sets of teeth, osteonal bone remodeling like humans, and repeated tissue sampling of muscle and bone biopsies across a range of ages. She detailed how HPP lambs with reduced ALP activity were created and shared various aspects of the sheep model and its value in better understanding premature tooth loss, muscle weakness and respiratory conditions in HPP.

Fat Metabolism in Hypophosphatasia: Clinical and Basic Implications

Victoria E. DeMambro, PhD Candidate; Maine Medical Center Research Institute

Key takeaway: The fat/bone connection: TNAP activity, an enzyme known for its mineralization effects, may be essential for mitochondrial dynamics and function in mesenchymal stem cells and adipocyte differentiation

Dr. DeMambro has been studying the connection between fat and bone for the past 20 years alongside Dr. Clifford Rosen. Several years ago, after seeing photos of ectopic fat mass accumulation at asfostase alfa injection sites in HPP patients, the question arose: How is Tissue Non-specific Alkaline Phosphatase (TNAP), an enzyme known for its mineralization effects, stimulating fat accumulation in these patients? Dr. DeMambro started by looking at literature which showed that in vitro, when TNAP was suppressed there was decreased lipid accumulation. She provided an overview of related research in mouse and sheep models which lead to the central hypothesis that proper TNAP activity is essential for mitochondrial dynamics and function in mesenchymal stem cells and adipocyte differentiation. She details TNAP and mitochondrial dynamics, as well as related research and what is to come in human studies around body composition and energy expenditure.

Hypophosphatasia and Ecotopic Calcification: Ocular Findings from the Enobia Study

Mark S. Rallo, OD; Washington University in St. Louis

Key takeaway: HPP can cause ectopic calcification, however, the correlation between enzyme replacement therapy and ectopic calcification is unclear

Dr. Rallo provided results from around the Enobia Study, which included 13 patients (ages 6 to 16 years) at the St. Louis site. All patients were on asfotase alfa and underwent eye exams every six months. In the study, nine children showed signs of ectopic calcification although four of those patients showed evidence of calcium deposits before the start of enzyme replacement therapy. Two patients in the study were diagnosed with optic atrophy due to hydrocephalus and no patients were diagnosed with angioid streaks. Calcification was mild and vision was not impacted. Overall, the study, as well as additional patients seen since that time, showed that HPP can cause ectopic calcification, however, what is less evident is the correlation between enzyme replacement therapy and ectopic calcification.

Are There Phenocopies of HPP? (Yes!)

Eric T. Rush, MD, FAAP, CCD; University of Missouri-Kansas City School of Medicine

Key takeaway: A wide range of conditions can be mistaken for HPP, making diagnosis difficult

There are a number of conditions that can be partial phenocopies to HPP and generally fall into one of three categories: other skeletal dysplasias, connective tissue disorders or acquired disorders. Dr. Rush discussed the “goodness of fit” for these disorders and the challenges with diagnosis. He delved into how different diseases are similar (or not) to HPP and covers a range of diseases including osteogenesis imperfecta, metaphyseal dysplasias, Jansen metaphyseal chondrodysplasia, X-linked hypophosphatemia, Ehlers-Danlos syndromes, nutritional rickets, metabolic bone disease of prematurity, fibromyalgia, as well as other acquired phenocopies.

Session 5: Treatment

Biochemical Assessment of Adults in the Metabolic Bone Disease Clinic

Richard Eastell, MD; University of Sheffield Medical School

Key takeaway: Research evaluating the biochemical assessment of HPP suggests that the use of thresholds rather than reference limits may improve diagnosis rates

In Dr. Eastell's clinic, he most often sees people with osteoporosis but notes the importance of correctly identifying patients with HPP to ensure that these patients are not treated with bisphosphonate treatment. For the past few years, he has been contemplating how to fine tune diagnoses and do a better job of identifying HPP. He discussed biochemical testing for HPP, including ALP activity and pyridoxal 5'-phosphate, and if we can improve diagnosis of HPP by using "thresholds" versus reference limits to analyze test results.

Hypophosphatasia and the Pediatric Orthopedic Surgeon: Changing Perspectives

Laura L. Tosi, MD; Children's National Hospital

Key takeaway: HPP symptoms are highly variable; there are likely many with HPP who have unrecognized symptoms, particularly parents of pediatric patients

Dr. Tosi noted that she has treated only a few children with HPP and shared insights around some of those experiences. In her hospital, children are being diagnosed in utero, leading to earlier treatment, and in turn, lessening (or eliminating) the need for an orthopedic surgeon. She shared a few cases of young children who presented with different symptoms yet all had HPP, as well as some experiences with teenagers, where she noted the psychological impact of HPP and enzyme replacement treatment in these patients. Dr. Tosi concluded by saying that there are likely more patients than most think, including the parents of pediatric patients who have unrecognized symptoms. She also noted challenges with medical coding and insurance denials

Transition of Pediatric to Adult Care: Challenge and Opportunity

Mark Nunes, MD; Valley Children's Healthcare and Hospital

Key takeaway: A new HPP phenotype called "treated HPP" represents patients who received early treatment intervention and have distinct needs; there are various challenges in the transition from pediatric to adult care for all patients with HPP

This year marks the 75th anniversary of HPP since being first described in 1948 and since that time, much progress has been made to better understand and treat the disease, although there is still much to learn. Dr. Nunes outlined the fundamental differences between adult and pediatric HPP, and the emergence of a new phenotype which is "treated HPP" as a result of early diagnosis and treatment. He talked about the different kinds of healthcare systems supporting HPP care and introduced the concept of the care wheel, with patients at the center and specialists along the spokes. For adult HPP, the care wheel would have the individual at the center surrounded by an endocrinologist or rheumatologist, physical medicine rehabilitation specialists, dental support, nutrition, orthopedics, pain management and more. The pediatric HPP care wheel is infinitely more complex and includes the patient and parent at the center, surrounded by a greater number of specialists to support the patient's needs. The care wheel for the new phenotype of treated HPP is difficult to decipher. He also raised the question of the impact of long-term use of asfotase alfa and concluded by outlining the four major issues with transitioning from pediatric to adult including maintaining the same level of health care, preserving or improving level of function, assuring continuity of care and structuring psychosocial and work-related systems.

Enzyme Replacement Therapy Cessation in Adults with Hypophosphatasia

Cheryl Rockman-Greenberg, MD; University of Manitoba Max Rady College of Medicine and Kathryn Dahir, MD; Vanderbilt University Medical Center

Key takeaway: Discontinuing enzyme replacement therapy can lead to loss of gains achieved with treatment; demographics differed substantially for patients who discontinued treatment versus those that remained compliant

Looking at both clinical trial data and real-world evidence, Dr. Rockman-Greenberg and Dr. Dahir presented research around the impact of discontinuing enzyme replacement therapy with asfotase alfa in adults with HPP. Findings in a clinical trial setting (six patients) showed that discontinuation of treatment was accompanied by the loss of the gains achieved during the clinical trials with varying degrees of clinical, radiologic and biochemical deterioration. With re-initiation of treatment, clinical improvement was observed. In real-world patients (17 patients who discontinued, 32 patients who continued on treatment), findings showed that patients who tend to remain compliant on treatment were males with a higher degree of musculoskeletal disease burden and who started enzyme replacement therapy at an older age. Those patients that discontinued treatment due to a lack of perceived benefits were younger females who started enzyme replacement therapy earlier, have better mobility, less musculoskeletal features, but a high disease burden of neurologic features. Patients that discontinued due to intolerable side effects such as injection site reactions were typically male with hypermobility, fractures, stiffness and other symptoms while those patients with no access to enzyme replacement therapy had the highest disease burden. Although the number of patients studied was relatively small, the results indicate that there is a need for better clinical treatment guidelines and other, more sustainable therapies.

Monitoring of Physical Performance in Patients with Hypophosphatasia

Donna Griffin, PT, DPT, PCS, Shriners Hospitals for Children

Key takeaway: There are various standardized tests and outcome measures around physical performance (more than just endurance) that are useful in clinical practice to identify functional deficits, inform clinical and treatment decision-making, and develop a baseline for comparison

Dr. Griffin discussed models and measures of physical endurance including applying the International Classification of Functioning, Disability and Health (ICF) model as a way to focus on the components of health rather than the consequence of disease. She noted the goals of standardized test and outcome measures (STOM) is to quantify observations and identify functional deficits, when present (sometimes there are no functional deficits found and that also aides in determining the need, or not, for pharmaceutical treatment). Additional goals include informing clinical and treatment decision-making (including monitoring the effectiveness of treatment), as well as developing a baseline for comparison which can help facilitate reimbursement for treatment. In trying to determine which STOM to use, the recommended process is to first identify the body structure or function items and determine the activity and participation (e.g. sit to stand, floor to stand, balance, stairs, etc), and then choose the appropriate standardized test or outcome measure based on patient's age, function, environment and other factors. She recommended the use of a data collection template for consistency and prioritization of testing to ensure patient tolerance. Dr. Griffin reviewed a number of different assessments and measures, as well as presenting several case studies and practical advice for clinical implementation.

Possibility of Prenatal Gene Therapy for Lethal HPP Model Mice/Pregnancy? Prenatal Treatment for Baby?

Tae Matsumoto, MD, PhD; Nippon Medical School and Michael P. Whyte, MD; Washington University School of Medicine in St. Louis

Key takeaway: Initial results in mice studies show the potential of gene therapy in treating perinatal, severe infantile HPP in animal models but still have hurdles to clinical application; unique aspects of HPP human pregnancies include an increase in maternal circulation of ALP

Dr. Matsumoto examined the potential to prevent severe HPP through prenatal gene therapy. In the study, type 9 adeno-associated viral vector AAV vector (AAV9) expressing bone-targeted TNALP (TNALP-D10) was transferred into the mice on Day 15 of gestation. *ALPL* knockout mice have no TNALP activity and are a good model for perinatal/severe infantile HPP. Treated HPP mice (7 of 9 survived) achieved seizure-free survival and normal weight gain (up to 56 days of observation). ALP activity also increased in the treated mice and histochemical examination confirmed ALP activity in the bone. These initial results show the potential of gene therapy in lethal perinatal HPP, however in general, fetal gene therapy still has its hurdles to clinical application. Dr. Whyte provided additional context around unique aspects of HPP human pregnancies, noting that in the third trimester, maternal circulation of ALP has substantially increased due to placental ALP (PALP) in the bloodstream. He reviewed previous research which showed that substrate accumulation in women with HPP corrects in pregnancy. The study showed that ALP entered the normal range in pregnancy until the woman delivered the placenta and then it decreased. After performing isoenzyme identification, the increase of circulating ALP was attributed to the PALP isoenzyme (which does not appear to benefit the baby).

Gene Therapy for Hypophosphatasia

José Luis Millán, PhD; Sanford Children's Health Research Center

Key takeaway: Pre-clinical research shows the potential of genomic medicine approaches in HPP

There are ex vivo and in vivo approaches to gene therapy. In the ex vivo approaches, cells that have been isolated from peripheral blood or bone marrow and are expressing a functional copy of the missing/defective gene (ALP) are expanded in vitro and then re-introduced into the patient. The in vivo approaches take a functional copy of the gene or cDNA packed into a viral vector for delivery to the patient, either systemically or locally into individual tissues. Dr. Millán reviewed various studies examining the potential of gene therapy in HPP including different mouse models, viral vectors, and promoters. He also discussed the pros and cons of cell therapy, which is a therapy to replace or complement endogenous defective cells with new functional cells in mineralizing tissues. In addition, he reviews the viral vector delivery of TNAP-D10 for HPP, which is enzyme replacement where the viral vector delivers the TNAP to the patient's own cells to produce mineral-targeted TNAP.

Session 6: Research Roundup From Soft Bones Grant Recipients

Hypophosphatasia: *ALPL* c.1133A>T, p.D378V and c.1250A>G, p.N417S are the Most Prevalent Dominant American Mutations

Steve Mumm, PhD; Washington University School of Medicine in St. Louis

Key takeaway: The most common dominant HPP mutations are ALPL D378V and N417S; counseling for families with these common mutations is important

Dr. Mumm started sequencing *ALPL* for HPP in 1999 in collaboration with Dr. Michael Whyte, and has since sequenced more than 300 HPP patients and family members. He was the first recipient of a Soft Bones grant, with his initial work in 2012 focused on sequencing 144 HPP probands and family members to understand inheritance patterns and genotype/phenotype correlations. He discussed a study examining common dominant mutations, with initial data presented at the 2022 ASBMR meeting. His findings showed that *ALPL* D378V is the most common dominant American HPP mutation, followed by N417S. Both generally result in relatively mild disease when inherited alone. However, heterozygous D378V causes a more severe clinical phenotype than N417S and when inherited with a second *ALPL* mutation, both cause severe HPP. Counseling for families with these common mutations is important.

Muscle Weakness in the Severe Murine Hypophosphatasia Model

Luke Mortensen, PhD; University of Georgia College of Engineering

Key takeaway: Initial research in mouse models suggests that HPP may have an impact on growth, size, fiber structure and force of the muscle; mitochondrial respiration and organization does not appear to be impacted

Mesenchymal Stromal Cells (MSCs) can differentiate into cartilage, fat or bone. Proper MSC function is critical, and impaired MSC differentiation into bone is associated with diseases such as HPP. Dr. Mortensen discussed establishing a mouse model for HPP which was used to analyze HPP muscle, HPP bone collagen and MSC therapies for HPP. His presentation focused on the effect of HPP on muscle and found that HPP impairs growth, size, fiber structure and force of the muscle, whereas mitochondrial respiration and organization did not seem to be impacted. Additional research in humans is needed.

Correlation of the Dental Phenotype in Hypophosphatasia with Clinical Subtype

Brian Foster, PhD; The Ohio State University, College of Dentistry

Key takeaway: Research aimed to identify a dental phenotype in HPP revealed that different parts of the teeth (enamel, dentin) are impacted differently depending on levels of ALP and severity of HPP

Although there is a range of severity of HPP, dental issues are spread across those subtypes. Dr. Foster asked the questions: Are dental tissues more sensitive than other tissues? Are there some mutations that cause worse dental phenotypes? His latest research explored these questions as he focused on correlating a dental phenotype in HPP. The dental effects of HPP include long roots due to premature exfoliation, lack of cementum and altered outermost mantle dentin region. Findings from 60 teeth (a sample that will expand to 200+ teeth) is that enamel is not affected until you reach the most severe HPP, crown and root dentin thickness may be affected in parallel to ALP levels, and overall dentin density is not affected by HPP. Looking more closely at dentin, the outer mantle dentin shows substantial effects on mineral density whereas other dentin regions appear less affected or unaffected by HPP. Dr. Foster also noted that a registry has been created at The Ohio State to collect oral health-associated information from individuals with HPP.

Assessment of Functional Performance in Adolescents and Adults with Hypophosphatasia

Kathryn Dahir, MD and Michael de Riesthal, PhD, CCC-SLP;
Vanderbilt University Medical Center

Key takeaway: Physical therapy, occupational therapy and speech-language pathology specialists can help establish baseline assessment of impairment in HPP and to develop treatment plans

There are general guidelines for physical therapy in children with HPP but not in adults, nor are there occupational therapy or speech-language pathology guidelines in HPP patients of any age. Dr. de Riesthal discussed research assessing the functional performance in individuals with HPP (15 patients, age 18+) using physical and occupational therapy, as well as speech-language pathology and patient-reported outcome tools. Regarding physical therapy results, the study showed that HPP participants traveled shorter distances, had slower gait and were slower to rise from a seated position. The occupational therapy results showed that HPP participants had decreased upper extremity dexterity, pinch strength and reaction times. HPP patients also reported worse energy/fatigue, social functioning, pain and general health, as well as higher scores for depression, anxiety and stress. The findings support that physical therapy, occupational therapy and speech-language pathology specialists can help establish baseline assessment of impairment and in developing treatment plans.

AAV8-TNAP-D_{10} Treatment in Mouse Models of HPP

Flavia Amadeu de Oliveira, PhD.; Sanford
Burnham Prebys Medical Discovery Institute

Key takeaway: A potential gene therapy used as a preventative treatment in the infantile HPP mouse model and a corrective treatment in the adult HPP mouse model; longer-term studies are underway

Dr. Amadeu de Oliveira discussed the potential of gene therapy using AAV8-TNAP-D_{10} in HPP mouse models. Two mouse models of HPP were used, early (infantile) and late-onset (adult) HPP. A single dose of AAV8-TNAP-D_{10} was injected intramuscularly. Short-term results showed improvements in both models – in early onset, improvements were seen in lifespan, epileptic seizures and skeletal/dental phenotypes whereas in the late onset, improvement was seen in the bone phenotype. She discussed details of the biochemical and radiographic results across models. Findings suggest that treatment with AAV8-TNAP-D_{10} acted as a preventive treatment in the infantile HPP mouse model, whereas it acted as a corrective treatment in the adult HPP model. Longer term studies are underway.

Role of Neural TNAP in Dental and Skeletal Defects Associated with HPP

Fatma F. Mohamed, PhD; The Ohio State University, College of Dentistry

Key takeaway: Research in mouse models explored how TNAP may be implicated in bone and teeth defects in HPP

Sensory nerves not only submit pain but they also secrete neuropeptides and signaling molecules for bone formation and repair. Several lines of evidence suggest that neural regulation of skeletal formation, remodeling and repair is accomplished through mechanisms including trophic signals, sympathetic control of vascular tone and protection from pain. The tooth is richly innervated by sensory and sympathetic nerves, which is essential for the function and protection of the tooth. TNAP is expressed in the nervous system during development and is important for vitamin B6 metabolism, myelin formation and regulating neural differentiation and axonal growth. Dr. Mohamed discussed primary research in mouse models exploring if neural TNAP is implicated in bone and teeth defects in HPP. A number of related experiments are underway to learn more about this area.

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Establishing Roles for TNAP in Muscle Health

Nan Hatch, DMD; University of Michigan School of Dentistry

Key takeaway: Research in mouse models examined how TNAP could affect muscle on a development or functional level

There are various substrates and functions of TNAP which can influence many processes in the body. Dr. Hatch delved into the aspects of TNAP and muscle. There is a high incidence of muscle symptoms in HPP. Using a knockout mouse model, HPP caused muscle weakness and impaired motor coordination. How does TNAP deficiency cause muscle weakness? Theories include it being due to a development defect (lack of progenitor cells developing), increased ATP synthesis, decreased ATP hydrolysis and changes in other metabolic pathways. TNAP may have an important role in progenitor cells, with research showing that TNAP stimulates signaling which is required for progenitor cells to develop. Research in mouse models examined if TNAP could affect muscle on a developmental or functional level, with future studies planned to look further into this aspect as well as delving more into the phosphocreatine/creatine energy system.

Meeting Summary & Considerations for the Future

Michael P. Whyte, MD; Washington University School of Medicine in St. Louis

Dr. Whyte summarizes key topics discussed throughout the meeting, as well as thanking speakers and meeting organizers. He expresses his joy at seeing the great progress that has been made in HPP in the nearly 50 years he has been involved in the field.

Soft Bones
Finding the Key to HPP

Thank you

To our presenters, we are grateful for your dedication and hard work that is needed to advance the understanding and care for those impacted by HPP. Thank you for your continued work in this field.

To our sponsors, without your assistance, important forums such as this scientific meeting would not be possible. Thank you for supporting the HPP community.

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