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MEETING REPORT

Calcification disorders: from hardened arteries to soft bones (RDDS 2013)

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Meeting Report from the Fourth Annual Rare Disease Day Symposium, Sanford-Burnham Medical Research Institute, La Jolla, CA, 28 February 2013

In 2010, the Sanford-Burnham Medical Research Institute (SBMRI) organized its first Rare Disease Day Symposium (RDDS) both to recognize Rare Disease Day and to present cutting-edge research within a uniquely collaborative framework. By inviting contributions from both research and family-advocate communities, the intent was to share perspectives on what scientists and advocacy groups can do to further our ultimate goal of curing diseases. The first three symposia, organized by Professors Hudson Freeze and Yu Yamaguchi from the Genetic Disease Program at the Sanford Children's Health Research Center at SBMRI, focused on diverse diseases with a heavy focus on Congenital Disorders of Glycosylation, reflecting the areas of interest of Drs Freeze and Yamaguchi. This year, Professor José Luis Millán, from the same Genetic Disease Program and whose major scientific interests include skeletal dysplasias and dystrophic calcification, organized the fourth symposium on the theme 'Calcification Disorders: From Hardened Arteries to Soft Bones.' Coinciding with Rare Disease Day, the meeting took place at the SBMRI La Jolla Campus. Around 130 participants attended the meeting, which included scientists from local institutions, disease advocates and patients. The event was also broadcast live and was accessible online to registered remote attendees around the world, allowing approximately 50 additional participants to attend.

The morning session focused on the Diagnosis and Treatment of Vascular Calcification. Keynote speaker William A Gahl (Clinical Director, NHGRI, Director, NIH Undiagnosed Diseases Program, National Institutes of Health, USA) informed the audience that the NIH Undiagnosed Diseases Program (UDP) seeks to determine the cause of disorders that have long escaped diagnosis and to discover new biochemical and cell biological mechanisms of disease. Over 60 rare diseases, along with a score of potentially new disorders, have been diagnosed to-date by the UDP. Among the new diseases currently being studied is Arterial Calcification due to Deficiency of CD73 (ACDC), a disorder of lower extremity arterial calcifications due to biallelic mutations in NT5E, encoding CD73. This enzyme converts AMP to adenosine and inorganic phosphate at the surface of vascular endothelial cells. In ACDC, however, paucity of adenosine causes increased levels of tissue-nonspecific alkaline phosphatase (TNAP) and, consequently, decreased inorganic pyrophosphate, which inhibits calcification.

Hervé Kempf (Université de Lorraine, Lorraine, France) followed with a developmental biologist perspective of vascular calcification, indicating that this represents a common complication of various pathologies and is a strong predictor of subsequent cardiovascular mortality. Dr Kempf reasoned that the fact that vascular beds are not created alike during development could impact their ability to respond to pathological calcifying signals in the adult. The aortic arch media made up of vascular smooth muscle cells (VSMCs) of neural crest origin calcifies significantly earlier than the descending aorta composed of VSMCs, which are mesoderm-derived. In mice deficient in Matrix Gla Protein, a potent calcification inhibitor, extensive and spontaneous medial calcifications initiate in the aortic arch very early after birth, probably due to the overexpression of TNAP in VSMCs, and subsequently progress outside this neural crest-derived aortic region to ultimately spread all over the entire arterial tree, including the descending aorta, which is of mesoderm origin.

Frank Rutsch (Children's Hospital, Muenster, Germany) described his experience with the management of two rare diseases characterized by arterial calcification. Generalized arterial calcification of infancy (GACI) is a rare autosomal recessive disorder characterized by calcification of the internal elastic lamina and proliferation of the intima of muscular arteries leading to arterial stenosis. The outcome is usually severe with congestive cardiac failure, hypertension and myocardial ischemia, and only few patients survive beyond the neonatal period. Mutations in ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), the molecule that generates the mineralization inhibitor inorganic pyrophosphate, have been identified as the genetic cause of 80% of GACI patients. Recently, Dr Rutsch detected mutations in ABCC6 (ATP-binding cassette subfamily C number 6), the gene associated with pseudoxanthoma elasticum (PXE), in a cohort of 18 patients with GACI without ENPP1 mutations. Based on the overlap in genotype and phenotype in GACI and PXE, it can be speculated

that the underlying disease genes, *ENPP1* and *ABCC6*, respectively, drive a cohesive molecular pathophysiology system modulated by ATP metabolism, PP_i, and P_i generation and functional activities.

To conclude this session, José Luis Millán (SBMRI, La Jolla, CA) described his efforts to target TNAP pharmacologically as a means of preventing and treating medial vascular calcification (MVC), such as manifested in patients with ENPP1 deficiency but also prevalent in chronic kidney disease, obesity, type II diabetes and even aging. Dr Millán's laboratory developed a conditional knock-in mouse model that overexpresses human TNAP under the control of the VSMC-specific Tagln promoter, in an X-linked manner and showed that TNAP overexpression is sufficient to cause calcification, hypertension, cardiac hypertrophy and sudden death. Using the resources of the Conrad Prebys Center for Chemical Genomics at the SBMRI, Dr Millan and collaborators developed a specific drug-like small-molecule inhibitor of TNAP (SBI-425) with suitable pharmacokinetic and pharmacodynamic properties for *in vivo* use. Preliminary preclinical studies indicate that SBI-425 can effectively reach and inhibit TNAP in the vasculature, indicating that it may be an effective treatment for MVC, as seen in GACI and CKD.

The afternoon session focused on the pathogenesis and treatment of soft bones. Michael P Whyte, MD (Shriners Hospital for Children, St Louis, MO) presented what must be considered the most successful enzyme-replacement therapy to-date and the first one ever to involve a skeletal dysplasia. Hypophosphatasia (HPP) is the inborn-error-of-metabolism that is caused by loss-of-function mutation(s) within ALPL, the gene that encodes the TNAP isozyme of alkaline phosphatase (ALP), often leading to rickets in children and osteomalacia in adults. Without treatment, perinatal HPP is usually soon fatal from profound skeletal hypomineralization. In HPP, excess PP_i extracellularly inhibits the growth of hydroxyapatite crystals causing rickets or osteomalacia. Enzyme-replacement therapy for HPP using injections of recombinant, mineral-targeting, TNAP (asfotase alfa) is being intensively investigated (www.ClinicalTrials.gov) with favorable results detailed in severely affected infants and young children treated for 1 year. Currently, 61 patients with HPP, spanning all ages, are enrolled in clinical trials of asfotase alfa treatment.

Sometimes the most severe forms of HPP can resemble the most severe forms of osteogenesis imperfecta (OI). Professor Peter H Byers (University of Washington, Seattle, WA) summarized current efforts to uncover the genetic bases of rare forms of OI, caused by mutations in genes other than the collagen genes themselves. While more than 90% of OI patients have heterozygous mutations in one of the two type I collagen genes, COL1A1 and COL1A2, recent efforts have identified genes that affect regulatory pathways (SP7/Osterix, Wnt1), modify the assembly of the Type I collagen triple helix (CRTAP, LEPRE1, PPIB, PLOD2, FKBP10), bone homeostasis (TMEM38B), chaperone function (SERPINH1) and affect matrix and its processing (BMP1, SERPINF1), as causing OI.

Kenneth White (Indiana University, Indianapolis, IN) discussed his functional studies on Fibroblast growth factor-23 (FGF23), a phosphaturic hormone produced in osteoblasts and osteocytes that acts in the kidneys to regulate phosphate and vitamin D metabolism. Syndromes such as autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemic rickets (XLH), tumor-induced osteomalacia (TIO) and autosomal recessive hypophosphatemic rickets are caused by gain-of-function mutations in FGF23 (ADHR) or by FGF23 over production. The syndrome Osteoglophonic Dysplasia (OGD) is caused by mutations in FGFR1 and leads to dwarfism and craniosynostosis. Some patients with OGD mutations have elevated FGF23 and hypophosphatemia, indicating the FGFR1 activity is upstream of FGF23 expression in bone. The acquired disorder TIO has a similar biochemical phenotype to ADHR and OGD, and it was found that FGF23 is highly expressed in these tumors.

XLH can be considered the prototype disorder of renal phosphate wasting, demonstrating the clinical biochemical profile associated with elevated circulating FGF23 levels. Professor Thomas O Carpenter (Yale University, New Haven, CT) reflected that the natural history of XLH encompasses a wide spectrum of severity and is manifest primarily by bow deformities of the lower extremities in childhood. Even with optimal phosphate replacement therapy, dental disease occurs and is progressively problematic through adult years. In adult life further complications ensue including the development of arthritis, osteophytes and calcified entheses. Cardiac (left ventricular hypertrophy) abnormalities occur and hyperparathyroidism is frequent. These abnormalities remain difficult to explain on the basis of the ambient hypophosphatemia, suggesting that loss of function of PHEX (mutated in XLH) results in heterotopic mineralization of soft-tissue structures such as tendons and ligaments. Novel approaches to therapy include targeting inhibition of FGF23 action (via neutralizing antibodies to FGF23, and via the inactive, receptor binding C terminus of FGF23), decreasing FGF23 secretion (via calcitonin) and stimulation of FGF23 catabolism (via enhancement of subtilisin protein convertase activity).

The last scientific session focused on uncontrolled soft-tissue calcifications, other than medial vascular calcification. Eileen M Shore (University of Pennsylvania, Philadelphia, PA) discussed heterotopic ossification—the formation of bone outside of the normal skeleton. Heterotopic ossification is most frequently associated with severe tissue trauma, and is a relatively common complication of central nervous system injury, total hip arthroplasty and deep tissue burns. Dr Shore's laboratory studies two inherited forms of heterotopic ossification: Fibrodysplasia Ossificans Progressiva (FOP) and Progressive Osseous Heteroplasia (POH). In each of these rare disorders, extensive heterotopic ossification begins during childhood and bone formation continues throughout life and can become severely debilitating. Dr Shore and collaborators have identified the underlying gene mutations in both FOP and POH and are investigating the roles of these genes and signaling pathways in the regulation of bone formation.

Maurizio Pacifici (The Children's Hospital of Philadelphia, Philadelphia, PA) referred to Multiple Hereditary Exostoses (MHE, also called Multiple Osteochondroma), a pediatric autosomal dominant disorder during which benign cartilage tumors called exostoses form next to the growth plate of several skeletal elements and cause deformities, chronic pain, early-onset osteoarthritis and other health problems. MHE affects 1 in 50 000 children, and most cases are caused by loss-of-function mutations in the heparan sulfate (HS)-synthesizing enzymes EXT1 and EXT2, resulting in HS deficiency throughout the body. Using *in vivo* and *in vitro* mouse models of MHE, Dr Pacifici has

found that the HS deficiency does in fact lead to wider distribution and ectopic activation of pro-chondrogenic factors within perichondrium, followed by the formation of ectopic exostosis-like cartilaginous tissue with perichondrium itself.

Matthew Warman (Boston Children's Hospital, Boston, MA) concluded the scientific session with a discussion of massive parallel sequencing efforts similar to those described in the keynote lecture by Professor Gahl, but focusing on non-hereditary genetic skeletal diseases, such as Gorham-Stout Disease (G-SD), Generalized Lymphatic Anomaly (GLA), Isolated microdactyly and CLOVES syndrome. These rare conditions all manifest lymphatic malformations in addition to progressive osteolysis affecting skeletal sites in different proportions or a single finger as in the case of isolated macrodactyly and omatic mosaicism of *de novo* embryonic mutations are a likely pathogenic mechanism. Dr Warman described his whole exome targeted capture sequencing efforts to compare the genetic make up of affected compared to unaffected tissue obtained from CLOVES Syndrome patients that led to the identification of an activating mutation in the PIK3CA kinase as the underlying genetic defect in this condition and identification of the same activating mutation in patients with the related Klippel-Trenaunay syndrome. Dr Warman's current efforts focus on engineering a mouse model to be able to activate the PIK3CA kinase in a specific cell type in a mosaic manner. These studies should facilitate the examination of the genetic mechanisms underlying the more complex GLA and G-SD.

The conference concluded with a panel of representatives of patient advocacy organizations in the rare bone field, including: The XLH Network (Becky Mock), the MHE Research Foundation (Sarah Ziegler), the Osteogenesis Imperfecta Foundation (Tracy Hart), The Lymphatic Malformation Institute (Tiffany Ferry), the Lymphangiomatosis & Gorham's Disease Alliance (Jack Kelly) and two organizations that focus on the disease HPP, the Soft Bones Foundation (Deborah Sittig) and HPP-Choose Hope (David A. Heaps). Charlene Waldman, former Executive Director of The Paget Foundation and currently a consultant to several rare bone organizations, was the panel moderator. The panel members discussed the mission and needs of their organizations, including expanded research, patient and professional education. The panel members emphasized the benefits of collaboration among their organizations and with the scientific community praising this conference as an excellent opportunity for such collaboration. Strategies for advancing advocacy for federally funded research and for enhanced interaction with the National Institutes of Health (NIH) were also discussed. The panel discussion added a very human element and patient perspective to what turned out to be a very vibrant and stimulating 1-day symposium.

Videos of some of the presentations are available at http://www.sanfordburnham.org/research/childrenshealth/genetic/symposium/Pages/2013.aspx

Conflict of Interest

The author declares no conflict of interest.

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