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Abstract book

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Slemenda Award

The 2017 Charles Slemenda Award was awarded to Frank Rauch (Montreal, Canada) in recognition of his outstanding contribution to children’s bone research.

Previous winners:

2015 Zulf Mughal (Manchester, UK)
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1999 Francis Glorieux (Montreal, Canada)
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Roland Baron

Biographical details

Dr Roland Baron

Dr Roland Baron is Professor of Medicine at the Harvard Medical School, Endocrine Unit, Massachusetts General Hospital, Professor in the Division of Bone and Mineral Research and Chair of the Department of Oral Medicine, Infection and Immunity at the Harvard School of Dental Medicine since January 2008. From 1977 - 2007 Dr Roland Baron was a Professor in the departments of Medicine, Orthopedics and Cell Biology at Yale University School of Medicine. He received his DDS and PhD degrees from the Medical School, University of Paris, France. He is the founder and past Editor-in-Chief of BONE. Between 1994 and 2002, he also held the position of Vice President and Head of the Bone Diseases Group at Hoechst Marion Rousse and then at Aventis. In 2002 he founded ProSkelia, a small pharmaceutical company devoted to the discovery and development of new drugs for bone and hormonal dependent diseases, now part of Galapagos. He has held the positions of President and Chief Scientific Officer of ProSkelia and then ProStrakan, until April 2006. Dr Baron was the President of ECTS 2008-11 and the President of the American Society for Bone and Mineral Research (ASBMR) in 2014-15. Dr Baron received the William Neumann Award and the Avioli Founder Award from ASBMR, the Harold Copp Award from the International Bone and Mineral Society (IBMS), the Excellence in Research Award from the European Calcified Tissue Society (ECTS) and has published over 330 scientific papers in the field of bone biology, bone diseases and their treatment. He is currently the co-Chair of the International Federation of Musculoskeletal Research Societies.

DOI: 10.1530/boneabs.6.IS01
Highlights in clinical bone research

Frank Rauch
Shriners Hospital for Children and McGill University, Montreal, Canada

Pediatric bone health research is rapidly expanding. As many bone disorders in children are rare, the field benefits from the attention that rare disorders in general are currently receiving. Consequently, new approaches for treating bone diseases in children have been developed and are being studied in clinical trials. The treatment of hypophosphatasia with bone-targeted enzyme replacement therapy is one of the most advanced programs in this area. New studies on this approach show that it improves survival for perinatal and infantile hypophosphatasia and that school children with hypophosphatasia benefit from enzyme replacement long term. Novel genetic causes of rare pediatric bone diseases are being discovered, even though the large majority of patients with well-defined bone fragility phenotypes have mutations in known genes. New insights are also being generated on more traditional topics of the pediatric bone health field, such as vitamin D supplementation in infancy, where well-designed randomized trials have shown lasting benefits on bone mass and body composition. The pediatric bone health field greatly benefits from long-term studies on large cohorts. One study on healthy children investigated muscle-bone interactions and found that gross-motor skills at 18 months of age are related to measures of bone strength at the age of 17 years. Thus, considerable advances are being made in a wide range of pediatric bone health areas.

Disclosure
Receipt of grants/research support from Ultragenyx and Alexion.

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Biographical details

Frank Rauch
Frank Rauch, MD, is a Professor of Pediatrics and clinician-scientist at the Shriners Hospital for Children and at McGill University. His clinical activities and research program concentrate on improving bone health in children, with a special focus on genetic conditions leading to fractures and on the role of the muscle system in bone diseases. In his recent work, Dr Rauch has identified new genetic causes of brittle bone disorders and has assessed the long-term effects of bisphosphonate treatment in children with osteogenesis imperfecta. He is also collaborating with Statistics Canada in a study that assesses muscle and bone health in Canadians. Dr Rauch is currently serving as Editor-in-Chief of the Journal of Musculoskeletal and Neuronal Interactions. Dr Rauch has authored or co-authored more than 200 original publications.
**Bone cells in health and disease**

Roberta Besio, Roberta Gioia, Francesca Tonelli, Ilaria Ceppi, Laura Leoni, Linda Ofori Atta, Antonio Rossi & Antonella Forlino  
Department of Molecular Medicine, University of Pavia, Pavia, Italy

Bone is a complex tissue constituted by a mineral phase, hydroxyapatite, and an organic phase, mainly represented by collagen type I. Specialized cells are responsible for bone formation and remodeling. Osteoblasts represent the bone forming cells, osteocyt e are the orchestrator of bone remodeling through regulation of the other bone cells activity, by functioning as endocrine cells and by acting as mechanosensor, and osteoclasts, the bone resorbing cells. Mesenchymal osteoprogenitors and hematopoietic osteoclast precursor cells need also to be considered as active players in bone homeostasis. The bone cellular compartment is a dynamic environment and the cell crosstalk is regulating its activity. Abnormality in bone cell function causes various human diseases. Osteogenesis imperfecta (OI), also known as brittle bone disease, is a heritable skeletal dysplasia characterized by bone fragility and deformity, frequent fractures and short stature. Classical OI is caused by dominant mutations in the collagen type I coding genes, COL1A1 and COL1A2, but also defects in other proteins involved in collagen type I synthesis, posttranslational modification, maturation and secretion as well as in osteoblasts differentiation had been more recently described as causative for the disease. The bone phenotype of OI patients was traditionally attributed to the presence of altered collagen type I in the bone extracellular matrix. More recently, it became clear that that for OI, as for other skeletal dysplasia, a cellular function impairment, due to mutant protein retention, may have an effect on patients’ outcome and could be a target for the disease treatment. By using the OI murine model Brtl and the OI zebrafish model Chihuahua, carrying a typical glycine substitution in one α1 chain of collagen type I we demonstrated that the severity of the disease could be modulated by a different ability of bone to cope with the stress caused by mutant collagen retained in the endoplasmic reticulum. Many evidences suggest that intracellular events contribute to the OI phenotype and cellular stress seems to be an appealing new pharmacological target for OI.  

**Disclosure**  
The authors declared no competing interests.

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**Biographical details**

**Dr Antonella Forlino**  
Dr Antonella Forlino obtained her Degree in Biological Science in 1991 at the University of Pavia, Italy; her PhD in Biochemistry in 1994 at the University of Pavia and her Speciality Degree in Genetic in 1997. From 1995 to 1999 Dr Forlino had a fellowship at NIH, Bethesda, MD, USA. She is now Associate Professor of Biochemistry at the Department of Molecular Medicine, Unit of Biochemistry, University of Pavia. Her research activity is focused on the molecular, biochemical, and functional study of genetic diseases of the connective tissue, in particular Osteogenesis Imperfecta (OI). Her present research interests are the investigation of the intracellular retained mutant collagen fate in OI using *in vitro* and *in vivo* models, the development of a cell/gene therapy approach using OI murine models and she also recently started a *D.Rerio* facility to generate zebrafish models of skeletal dysplasias and to start drug screening approach.
Cortical bone structure and material properties

Björn Busse

Head, Emmy Noether Research Group, Department of Osteology and Biomechanics (IOBM), Universitätsklinikum Hamburg-Eppendorf, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

While a strong emphasis has been put on the characteristics of trabecular bone due to its' high metabolic rate, the human skeleton actually consists of approximately 80% cortical bone. Furthermore, the cortical bone supports a major proportion of the mechanical load (i.e., up to 96%) at common fracture sites, such as the base of the femoral neck and the intertrochanteric region. In these regions of the hip, large compressive stresses concentrate during walking as well as during falls and may result in bone fracture. Bone’s ability to resist fracture originates from the quality of the trabecular and cortical compartments. Bone quality encompasses many aspects of the bone’s state including the multi-length-scale composition, mass, architecture, microdamage, bone turnover and osteocytic mechanosensitivity. When aspects of the bone quality are altered due to disease-specific disorders, the risk of fracture may be attributed to changes in bone quality endangering the bone’s normal organization and is particularly relevant in cases with diseased bone and its subsequent treatment. Here, our data shows how osseous and cellular characteristics of the cortical compartment vary with age, disease and treatment strategies (e.g. osteoporosis, vitamin D-deficiency, osteogenesis imperfecta, Paget’s disease of bone, bisphosphonate treatment, etc.) and may influence the risk of bone fracture. To assess changes in bone quality across bone’s hierarchical structure, a combination of techniques was carried out at the nano- to micron-level including 2D histomorphometry, 3D-microcomputed tomography, scanning electron microscopy/backscattered electron microscopy, Raman Spectroscopy, Fourier Transform Infrared Microscopy, micro-mechanical and materials testing. In this presentation, summarized findings emphasize how the structure of mineralized bone including the underlying osteocytic network, bone’s composition, as well as the mineral and collagen distribution may influence changes in the bone quality framework and the risk of fracture in a cohort of subjects suffering from skeletal aging, major bone diseases and/or pharmacologic treatment.

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.IS04

Biographical details

Björn Busse

Björn Busse is currently head of a ‘Emmy Noether Research Group’ (eq. Assistant Professor) which is a prestigious 5 year-program run by the German Research Foundation (DFG). The group of Björn is hosted by the Department of Osteology and Biomechanics at the University Medical Center Hamburg-Eppendorf. Björn has finished his PhD work (Free and Humboldt University Berlin, 2006-2009) with honors, where he has focused on research regarding bone biomechanics and bone mineralization. In particular, he has developed strong skills in scanning and backscattered electron microscopy, microanalysis, image analysis, materials testing and bone histomorphometry. Björn’s work provides a contribution to our understanding on the fracture of bone, specifically by focusing on aspects of bone quality, such as structural and compositional osseous changes with aging, osteoporosis, osteoporosis treatment, Paget’s disease of bone, etc. from both a medical and engineering perspective.
Skeletal mineralization – enzymes and animal models

José Luis Millán
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA

Increased understanding of the biochemical pathways leading to MV-mediated initiation of skeletal, dental and vascular calcification has been attained through the extensive use of single and double knockout mouse models as well as via overexpression of genes by transgenesis. Our current integrated model of these pathways is compatible with the following sequence of events: MVs initiate mineral deposition by accumulation of Pi generated intravesicularly by the action of PHOSPHO1 on phosphocholine derived from sphingomyelin by the action of SMPD3, and also via the Pi transporter PiT-1-mediated incorporation of extracellular Pi generated extravesicularly by TNAP and/or NPP1 on ATP. The extravesicular propagation of mineral onto the collagenous matrix is mainly controlled by the pyrophosphatase activity of TNAP that restricts the concentration of this potent mineralization inhibitor to establish a PPi/Pi ratio conducive to controlled calcification. Additionally osteopontin, another potent mineralization inhibitor that binds to mineral as soon as it is exposed to the extracellular fluid, further restricts the degree of extracellular matrix ECM mineralization. Recently, we have also implicated PHOSPHO1 function in the biogenesis of MVs. However, how MVs are formed is still unclear and little is known about how apatite crystals formed within MVs propagate onto the collagenous matrix. Understanding MV biogenesis and function is critical to being able to develop rational approaches for the prevention of ectopic calcification.

Disclosure
I have received grants, consolation fees and speaker engagement fees from Alexion Pharmaceuticals. I have received a grant and consultation fees from AM Pharma.

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Biographical details
José Luis Millán
Professor José Luis Millán received his early training in clinical chemistry and biochemistry at the University of Buenos Aires, Argentina, and his PhD in Physiological Chemistry at the University of Umeå, Sweden, in 1983. Professor Millán is currently based at the Children’s Health Research Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, USA. He studies the mechanisms of initiation of skeletal and dental mineralization, the pathophysiology of hypophosphatasia and other soft-bones conditions, as well as dystrophic calcification, with a particular focus on medial vascular calcification.
The mechanobiology of the growing skeleton

Bettina Willie
Montreal, Québec, Canada

Bone is a tissue that continually adapts to changing external loading conditions (so-called modeling) and has the capacity for self-repair and renewal (remodeling). These processes construct and reconstruct the skeleton by the removal and formation of bone packets that mediate the size, architecture, mass, and consequently the bone’s strength, allowing bones to perform their mechanical functions successfully over long periods of time. Both adaptation and self-repair are believed to be mediated by osteocytes, which act as a mechano-strain sensor embedded within the bone tissue. Although bone is able to accommodate changes in loading circumstances during growth, the adaptive capacity seems to diminish with age, contributing to compromised material and structural properties. Either the skeleton’s ability to form new bone declines with increasing age or the appropriate stimulus required to form new bone in an aged skeleton is not perceived. The underlying mechanism(s) responsible for this alteration are largely unknown, although recently developed imaging methods are providing new insights. Until recently, bone formation and resorption were primarily measured using biochemical markers of bone turnover or histomorphometry. However, advances in computed tomography allow for following structural changes in cortical and trabecular bone of living animals and human patients in four dimensions, 3D space and over time. My research group and others have developed 3D time lapse tomography-based methods that allow the monitoring of bone formation and resorption as well as tracking surface modeling and remodeling processes in vivo in mice by using registered longitudinal tomography data. With these new methods, detailed information on biological processes can be provided, in addition to or instead of standard histomorphometry. The lecture reviews current knowledge about skeletal mechanobiology in animal disease models during bone growth and aging, and discusses how novel tomography-based imaging methods are providing insights.

Disclosure
Amgen provided me with Sclerostin neutralizing antibody. Novartis provided me with SOST KO mice.

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Biographical details
Bettina Willie
Bettina Willie is an Associate Professor in the Department of Pediatric Surgery at McGill University and an investigator at Shriners Hospitals for Children - Canada. She is an Associate Member of the Departments of Biomedical Engineering and Surgery. She earned a doctoral degree in Bioengineering from the University of Utah. She performed postdoctoral training at the University of Ulm, Hospital for Special Surgery, and led a research group at the Charité-Universitätsmedizin Berlin. Her research focuses on the importance of the mechanical environment in bone for adaptation, regeneration, and aging. Her work involves in vitro, in vivo, and in silico studies to understand the mechanism(s) responsible for alterations in the response of the skeleton to mechanical strain. These studies center on unravelling the important cellular and mechanical factors regulating mechanoreception in bone cells to improve targeted therapies for treating and preventing bone loss and delayed bone healing.
A role for leptin as a myokine mediating muscle-bone interactions

Mark W Hamrick
Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, USA

Muscle mass and strength are known to enhance pediatric gains in bone mineral and bone cross-sectional area, providing a rationale for targeting muscle early in life as a means of improving bone health. We have recently found that the cytokine-like hormone leptin, a well-established adipokine, is abundant in skeletal muscle. Leptin levels normalized for total protein are actually higher in mouse skeletal muscle than in mouse adipose tissue, and studies in human subjects have demonstrated that muscle actively secretes leptin. The long form of the leptin receptor is abundant in skeletal muscle, and treatment of isolated primary myoblasts with leptin increases the expression of myogenic genes. Leptin treatment in vivo also increases the expression of myogenic microRNAs in skeletal muscle. Finally, recent data suggest that leptin stimulates production of follistatin, a potent antagonist of the atrophy-related factor myostatin. Dietary amino acids such as leucine are thought to induce leptin secretion in adipocytes by activating mTor, and we have shown that the dietary amino acid tryptophan can activate the mTor pathway in skeletal muscle and increase protein levels of muscle-derived leptin and follistatin. These studies point to a key role for leptin in mediating the impact of dietary amino acids on muscle and bone accrual in both children and adults.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.IS07

Biographical details

Dr Hamrick
Dr Hamrick is Regent’s Professor in the Department of Cellular Biology & Anatomy at the Medical College of Georgia, Augusta University (formerly Georgia Health Sciences University), Augusta, Georgia, USA. He received his PhD in Cellular & Integrative Biology from Northwestern University and completed postdoctoral studies in Anatomy at Duke University. He is currently Associate Editor at the Journal of Musculoskeletal & Neuronal Interactions and Section Editor for Muscle and Bone at Current Osteoporosis Reports. His research focuses on the role of soft tissues, particularly muscle and fat, in bone mineral accrual during growth and bone loss with aging. His research on muscle-bone interactions has received funding support from the US National Institutes of Health, the Department of Defense, and the National Science Foundation.
Next generation sequencing and genome editing, game changers in the field of skeletal research

Uwe Kornak
Berlin, Germany

The massive amount of genetic information made available by next generation sequencing (NGS) has already changed our clinical and scientific approach to skeletal disorders. As one consequence the gap between rare monogenic disorders and common complex disorders is becoming more permeable. On the one hand this is due to the finding that rare variants with larger effect sizes also have significance for common disorders. On the other hand, broader testing strategies reveal a considerable portion of rare disorders among the label of common disorders. As another consequence, analysis of a growing portion of the non-coding genome is becoming normality. The interpretation of non-coding variants is still a major challenge, but important lessons have been learned from the investigations of structural variants. These novel insights would not have been possible without an improved understanding of the 3D structure of the genome, which again was deciphered by NGS technology. At the same time, genome editing has greatly facilitated the generation of models for a better understanding of genetic variants identified by NGS. However, there are still major bottlenecks preventing a sufficient throughput of variant testing. These different points will be highlighted using different skeletal malformations, skeletal dysplasias, and disorders of bone homeostasis as examples.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.IS08

Biographical details

Uwe Kornak
Uwe Kornak, MD PhD, is leader of a research group at the Institute of Medical Genetics and Human Genetics, and the Berlin-Brandenburg Center for Regenerative Therapies, Charité-Universitätsmedizin Berlin and the Max Planck Institute for Molecular Genetics, Berlin, Germany. Through his work as a human geneticist he has a broad experience with clinical and molecular genetic diagnostics of rare human disorders with a special focus on neuromuscular and skeletal phenotypes. He has established several gene panels for diagnostics of metabolic and skeletal disorders and helped to develop bioinformatics tools for data evaluation. As a basic researcher, he is most interested in understanding the cellular pathophysiology of hereditary disorders of the skeleton and of connective tissues. An important focus has always been the regulation of trafficking and ion homeostasis of intracellular compartments with a focus on Golgi-related processes including glycosylation. During his PhD project as a biochemist at the Center for Molecular Neurobiology Hamburg Uwe Kornak became involved in the generation and interdisciplinary analysis of mouse models for human disorders. Up to now he not only identified several genes associated with human disorders, but also analysed the effect of these gene defects in in vitro and in vivo models using transgenic mice and zebrafish. Uwe Kornak has been reviewer for different human genetics journals and in 2007 received the Ian T Boyle award of the European Calcified Tissue Society and in 2011 the Ulmer Dermatologiepreis. He is member of three consortia on rare diseases (DIMEOs, SYBIL, and EURO-CDG).
Skeletal dysplasia

Melita Irving
Consultant and Honorary Reader in Clinical Genetics, Evelina London Children’s Hospital, Guy’s and St Thomas’ NHS Foundation Trust and The Division of Medical and Molecular Genetics, King’s College London, London, UK

Skeletal dysplasia is a heterogeneous group of more than 450 disorders characterised by abnormalities of cartilage and bone growth, resulting in abnormal modelling of the skeleton and disproportion of the long bones, spine, and head. It affects an estimated 1 in every 4000–5000 live births. Pathogenic variants in genes encoding proteins key in skeletal growth and development underlie skeletal dysplasia. These genes express proteins involved in cartilage extracellular matrix, signalling pathways and transcription and growth factors. Disruption of these finely tuned processes result in fundamental dysfunction of the bones and cartilage. In addition, epigenetic factors are known to be associated with skeletal dysplasia, as exemplified by paternal uniparental disomy of chromosome 14 and related mechanisms. In this session, an overview of the different genetic and epigenetic factors causing skeletal dysplasia will be discussed, using illustrative examples to explain the wide spectrum of disorders. In addition, an overview of how new technological advancements are revolutionising the management of skeletal dysplasia and the prospects for new therapies will be provided. Emphasis remains on the multidisciplinary approach to treating skeletal dysplasia and the Evelina London Children’s Hospital experience will be shared.

Disclosure
The authors declared no competing interests.

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Biographical details

Dr Melita Irving
Dr Melita Irving is a consultant in clinical genetics and joint head of service at Guy’s and St Thomas’ Hospital NHS Foundation Trust with the Evelina London Children’s Hospital. She is a trained general geneticist and sub-specialises in skeletal dysplasia, particularly achondroplasia and other genetic dwarfing conditions. She has developed clinical whole exome sequencing for skeletal dysplasia conditions. Melita completed her research higher degree in clinical and molecular studies in skeletal dysplasia and is chief investigator for a number of clinical trials and projects in achondroplasia. In 2011, she received the Maroteaux Award for advancing knowledge of skeletal dysplasia conditions. She is a keen teacher and trainer in skeletal dysplasia and clinical genetics, and is part of the team recruiting families with rare diseases to the 100 000 Genomes Project through the Genomic Medicine Centre South London, which she helped to establish. In addition, she is co-lead for the Genomics England Clinical Interpretation Partnership (GECIP) for skeletal dysplasia, recruiting patients and undertaking research across a number of conditions, including undiagnosed skeletal dysplasia.
Arterial calcification syndromes: causes and treatments

Frank Rutsch
Department of General Pediatrics, Münster University Children’s Hospital, Münster, Germany

Arterial calcification is now considered as an actively regulated process resembling osteogenesis orchestrated by a number of systemic or constitutively expressed mediators. Genetic studies of rare inherited syndromes have identified key regulators of arterial calcification. Based on the pathogenic principles causing the diseases these can be classified into three groups:

i) Disorders of an increased extracellular inorganic phosphate/inorganic pyrophosphate ratio a) Generalized Arterial Calcification of Infancy (GACI) caused by mutations in ENPP1 and ABCC6, b) Pseudoxanthoma Elasticum (PXE) caused by mutations in ABCC6 and ENPP1, c) Arterial Calcification and Distal joint Calcification (ACDC), caused by mutations in NT5E, d) Progeria, caused by mutations in LMNA, e) Idiopathic Basal Ganglia Calcification (IBGC), caused by mutations in SLC20A2, XPR1, PDGFRB and PDGFB, and f) Hyperphosphatemic Familiar Tumoral Calcinosiis (HFTC), caused by mutations in KL, GALNT3 and FGF23.

ii) Interferonopathies (Singleton-Merten syndrome), caused by mutations in IFIH1 and DDX58.

iii) Deficiency of Matrix-Gla protein (Keutel syndrome), caused by mutations in MGP. Although some of the identified causative mechanisms are not easy to target, it has become clear that a disturbed extracellular phosphate/pyrophosphate ratio is a major force triggering arterial and cardiac valve calcification. Further studies will focus on this target to effectively prevent and treat the underlying disease phenotypes.

Disclosure
Receipt of research support from Alexion Pharmaceuticals. Receipt of research support and consultation fees from BioMarin.

DOI: 10.1530/boneabs.6.IS10

Biographical details

Frank Rutsch
Frank Rutsch is a consultant and Associate Professor in Pediatrics at Münster University Children’s Hospital, Münster, Germany. He graduated from Münster University Medical School in 1992 and took part in the Pediatric residency program in Dresden University and Dortmund Municipal Hospital, Germany. After spending a postdoctoral research fellowship at the Department of Rheumatology/Immunology, UCSD, San Diego, USA, he became the leader of an independent research group at Münster University Children’s Hospital in 2004. His main research interests are focused on the discovery of the underlying genetic defects and translational aspects in rare Pediatric metabolic and autoimmune disorders. In this respect, with the help of several consortia, his group discovered the genetic cause of generalized arterial calcification of infancy, Crisponi syndrome, certain defects of intracellular cobalamin metabolism, subtypes of Aicardi-Goutières syndrome and Singleton-Merten syndrome. His current projects include experimental studies in animal models of some of these rare disorders.
Hans van Leeuwen

Biographical details

Hans van Leeuwen studied biology in Amsterdam, and did his PhD study on the mechanism of action of PTH in Leiden, The Netherlands. Currently he is professor at the Erasmus University Medical Center in Rotterdam, The Netherlands, leading the research program on Calcium and Bone Metabolism. Main research focus is on regenerative medicine with emphasis on control of mesenchymal stem cell and osteoblast differentiation, on the impact of bone metabolism on hematopoietic stem cell control and tumor cell metastasis, and on aging and calcium and bone homeostasis.

- Professor of Calcium and Bone Metabolism.
- Director of Research and Education of Erasmus University Medical Center.
- Board member of the Advances in Mineral Metabolism from 2011 to 2014.
- Secretary on the Executive Board of the European Calcified Tissue Society from 2000 to 2009.
- Served on the board of scientific, academic committees and grant-awarding bodies.
- Served on over 35 organizing and program committees of national and international scientific meetings within as well as outside the bone field.
- Initiated the genetics of osteoporosis research program at the Erasmus University Medical Center.
- Published over 210 peer reviewed papers and 25 book chapters.
- Founder of the biotech companies Therosteon and Arcarios BV.
- Initiated a GRID computing program in collaboration with the RABO bank.
- Obtained about 40 Dutch, EU and industrial research grants.

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The ERNS as a tool for the European research on rare diseases

Luca Sangiorgi, on behalf of BOND ERN

Bologna, Italy

Between 5000 and 8000 rare diseases affect the daily lives of around 30 million people in the EU. Many of those affected by a rare or complex conditions frequently undergo to late diagnosis and inadequate care and do not have access to high quality treatment. European reference networks (ERNs) for rare diseases should serve as research and knowledge centres, updating and contributing to the latest scientific findings, treating patients from other Member States and ensuring the availability of subsequent treatment facilities where necessary. The networks will also foster collaborative research and facilitate translation of research into care driving innovation for new research development and therapeutical approaches to address true patients unmet needs. To this aim, ERNs are working in close collaboration with patient organisations. The backbone instruments that support research and leverage networking synergy are interoperability and harmonization. This term means in general ‘to operate together to achieve a common goal’. It complies standardization, integration, cooperation and synergy. All these elements have a key role in speed up research activities intra-ERN and inter-ERNs. One goal of ERNs is the improvement of the overall quality and value of research at European level, in a cluster of orphan diseases with similar needs, achieved by complementing, supporting and providing added value to the existing studies and projects and exchanging expertise among partners. A better and more innovative research needs bio-specimens exchange, data integration, sharing of protocols, individuation of SOPs and guidelines to obtain harmonized biobanking procedures, interoperable registries and databases, unique epidemiological definitions, unanimous lab protocols and guidelines allowing researcher to optimize diseases studies and to innovate research approach. Same importance has the pooling of expertise and knowledge for training and education of health professionals and patients. As a matter of fact, the contribution to continuous education, training, development and maintenance of competence of all stakeholders (both researchers and patients) is of undeniable relevance. This exchange of resources can be covered more effectively thanks to ERNs contribution and their tight collaboration with patients’ associations.

Disclosure

The authors declared no competing interests.

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Biographical details

M Kassim Javaid

After completing medical training at Charing Cross and Westminster Medical School, I specialized in adult rheumatology at the Wessex Deanery. During that time, I completed a PhD examining the maternal determinants of intra-uterine bone growth as part of an ARC Clinical Fellowship at the University of Southampton followed by a travelling fellowship and worked with the OA group in UCSF to study the role of vitamin D and bone in lower limb OA. Since my return to the UK, I have been appointed as Honorary Consultant Rheumatologist and am the Associate Professor in Metabolic Bone disease at the University of Oxford. My research interests include the role of epidemiology of musculoskeletal diseases focusing in secondary fracture prevention and rare bone diseases (www.rudystudy.org).
Osteoporosis in boys with Duchenne muscular dystrophy: morbidity, mechanisms and the path forward

Leanne M Ward

Ottawa, Canada

Osteoporosis in Duchenne Muscular Dystrophy (DMD) is arguably one of the most severe bone fragility conditions among children with chronic illnesses. This is hardly surprising, given the deleterious effects of the myopathy and glucocorticoid (GC) therapy on bone strength. The severity of the osteoporotic phenotype is highlighted by observations that 60% of boys will sustain long bone fractures during childhood and a third will present with back pain due to vertebral fractures (VF). Since VF prevalence studies in other GC-treated pediatric cohorts underscore that VF are frequently asymptomatic, studies to date have likely grossly under-estimated the total VF burden in DMD. Long bone fractures can lead to premature permanent loss of ambulation, and death due to fat embolism syndrome following long bone injury has also been described. Long bone fractures can occur prior to the onset of GC therapy, while VF manifest on average 1-2 years (and as early as 6 months) following GC start. VF are detected in their earliest stages when spine health is routinely monitored through periodic lateral spine imaging starting no later than the time of GC initiation. Left untreated, boys with DMD are at risk for “the vertebral fracture cascade” (more painful, numerous and severe collapse following an initial VF event); prevention of the cascade is one of the main goals of intervention, particularly important in DMD since vertebral body reshaping following VF has never been reported in this setting. Treatment to date has largely been restricted to bisphosphonates given their long-standing track record in pediatric osteoporotic conditions. With such an approach, back pain and further vertebral collapse are mitigated and overall the benefits of late-stage osteoporosis intervention are out-weighed by early fracture detection and timely initiation of osteoporosis therapy. Trans-iliac bone histomorphometric and density distribution studies show classic signs of a low-turnover osteoporosis prior to bisphosphonate therapy (reduced trabecular bone volume, thin cortices, low bone formation rates and an increased number of highly mineralized areas), followed by further reductions in bone turnover and a drop in mineralization heterogeneity after anti-resorptive intervention. Reductions in bone turnover both pre- and post-bisphosphonate therapy point to the pressing need for anabolic and growth-promoting therapies; intervention trials which aim to prevent first fractures are also needed.

Disclosure

Consulting honoraria and active participation in a clinical trial for Novartis. Consulting honoraria and active participation in a clinical trial for Amgen.

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Biographical details

Leanne Ward

Dr Leanne Ward is an Associate Professor of Pediatrics at the University of Ottawa where she has held a Research Chair in Pediatric Bone Health since 2010. She is the Medical Director of the Pediatric Bone Health Clinical and Research Programs at the Children’s Hospital of Eastern Ontario. Dr Ward’s research program is dedicated to the study of bone development and the treatment of pediatric bone disorders, with particular emphasis on bone health in children with chronic illnesses. She has served as an advisor to numerous national and international organizations on various aspects of skeletal health in children, including the Centres for Disease Control Clinical Care Guidelines for Duchenne Muscular Dystrophy. Dr Ward has received a number of awards for her work in pediatric bone health, including a Canadian Child Health Clinician Scientist Career Development Award, a Canadian Institutes for Health Research New Investigator Award and a Canadian Child Health Clinician Scientist Career Enhancement Award.
Bone, body composition and metabolic abnormalities after allogeneic hematopoietic stem cell transplantation during childhood

Sogol Mostoufi-Moab

The Children’s Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA

Allogeneic hematopoietic stem cell transplant (AlloHSCT) is an established treatment for benign and malignant hematologic disorders. Five-year cure rates for childhood alloHSCT currently exceed 60%. Unfortunately, chemotherapy, total body irradiation (TBI), glucocorticoid therapy, immune dysregulation, graft versus host disease, and treatment-related endocrine disorders result in significant late effects, including poor bone health and metabolic derangements. Consequently, medical attention has shifted to prevention and treatment of long-term alloHSCT-related morbidities. AlloHSCT survivors exhibit bone deficits and excess adiposity, related to altered mesenchymal stem cell differentiation into osteoblasts and adipocytes. The location of fat deposition is implicated in adverse cardiovascular and bone outcomes. We recently reported DXA measures of total body fat and lean mass in 55 long-term survivors of childhood alloHSCT. Although body mass index Z-scores did not differ between alloHSCT and a large group of reference participants, alloHSCT recipients demonstrated significant sarcopenic obesity. Furthermore, AlloHSCT survivors had substantial deficits in trabecular volumetric bone mineral density and cortical geometry by peripheral quantitative CT compared with reference participants. On tibia micro-MRI, survivors had lower bone volume fraction and abnormal bone microarchitecture with a greater number of vertebral deformities ($P < 0.01$). These abnormalities were more pronounced in survivors with a history of TBI and growth hormone deficiency. DXA whole body (WB) and leg-lean mass (LM) Z-scores were also significantly lower in alloHSCT compared to reference participants ($P < 0.001$ for both), and the magnitude of LM deficits was more pronounced for leg-LM, compared with WB-LM Z-scores. AlloHSCT survivors had significantly greater WB-fat mass (FM) Z-scores ($P < 0.001$). DXA visceral adipose tissue, subcutaneous adipose tissue, and marrow adipose tissue (measured by MR spectroscopy) were significantly higher in alloHSCT. Muscle density was significantly lower, indicative of greater fat infiltration of muscle ($P < 0.05$ for all). None of these group differences were attenuated after adjustments for greater WB-FM in alloHSCT. Importantly, alloHSCT demonstrated significant insulin resistance independent of physical activity. In conclusion, the markedly increased marrow adiposity, abnormal bone microarchitecture, and abnormal fat distribution highlight risks of long-term treatment-related morbidity and mortality in alloHSCT recipients. These findings underline the need for lifelong specialized healthcare to institute appropriate and timely intervention strategies.

Disclosure

The authors declared no competing interests.

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**Biographical details**

**Sogol Mostoufi-Moab**

I am an Assistant Professor of Pediatrics at the Children’s Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, and board certified in pediatric oncology and endocrinology. I have advanced epidemiology training and my research program is focused on bone, body composition, and metabolic abnormalities in survivors of childhood malignancies. Clinically, I practice as a pediatric oncologist with a focus in thyroid cancer as well as endocrine late effects after childhood cancer therapy. My NIH-funded research program is focused on examining the mechanisms of skeletal deficits and body composition abnormalities after childhood hematopoietic stem cell transplantation (HSCT), with an emphasis on the fat-bone axis and its contribution to the bone and metabolic complications following childhood HSCT. My ongoing research goal is identification of targeted interventions to form the basis of future studies to conduct randomized clinical trials. When not at work, I enjoy Pilates, Yoga, cooking, gardening, and theatre.
Bone in chronic kidney diseases: a systemic problem

Craig B Langman

The Isaac A Abt MD Professor of Kidney Diseases, and Head, Kidney Diseases, Feinberg School of Medicine, Northwestern University, and the Ann and Robert H Lurie Children’s Hospital of Chicago, Chicago, USA

Chronic kidney disease (CKD) is defined according to the presence of kidney damage and level of kidney function – irrespective of the type of kidney disease (diagnosis). Among individuals with chronic kidney disease, the stages are defined based on the level of glomerular filtration rate. From infancy through young adulthood, the major causes of CKD arise from congenital abnormalities of the kidneys and urinary tract (CAKUT), acquired or congenital forms of nphrotic syndrome, genetic forms of renal tubular dysfunction, and a host of other diseases affecting the glomerulus and/or the tubule-interstitium of the kidney, often with a genetic component. Changes in normal bone function occur early in the course of CKD and worsen as CKD progresses into the need for renal replacement therapies of dialysis or kidney transplantation. The entity of bone disease associated with CKD is not termed CKD-Mineral Bone Disturbance (CKD-MBD). The clinical manifestations of CKD-MBD in bone include frank rickets, deformities, fractures, and linear growth failure. The mechanisms for these manifestations relate to the biochemical findings of CKD, including metabolic acidosis, changes in blood calcium and phosphorus, and multiple hormonal disturbances, including those in parathyroid hormone, fibroblast growth factor 23, sclerostin and wnt-signalling, among others. As important as the changes in bone cell function are, is the understanding that the disturbance in mineralization in bone is transferred to the cardiovascular system, leading to pathologic vascular calcifications throughout the body. Recent and novel mechanisms for this will be discussed, and the relevance to other bone diseases in which vascular calcification occur will be elucidated. Treatment of CKD-MBD is fraught with absence of evidence for optimal protocols, no clinical trials in childhood, and few approved therapeutic agents. Thus, the clinician is often faced with uncertain nodal choices for each and every patient, leading to uncertain outcomes and ways to document success. Trial networks must be established to best understand meaningful outcomes, especially in the cardiovascular system.

Disclosure


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Biographical details

Craig B Langman

Professor Craig B Langman, is the Isaac A Abt, MD, Professor of Kidney Diseases at the Feinberg School of Medicine, Northwestern University, Head of Kidney Diseases at the Ann and Robert H Lurie Children’s Hospital of Chicago. His research focuses on the basic and clinical expression of inherited or acquired disorders of calcium, phosphorus vitamin D, and FGF23 metabolism, inherited genetic diseases (cystinosis, oxalosis, kidney stones, atypical HUS, hypophosphatasia), and the rehabilitation of patients around the world with chronic kidney disease. Professor Langman has published more than 235 articles, reviews and chapters in his discipline.
Body composition and physical activity

Bonny Specker
South Dakota State University, Brookings, USA

Periods of growth are thought to be the best time to improve bone health through increased loading due to the high rates of bone modeling and remodeling. Although numerous observational studies find higher aBMD and greater bone size in physically active children than sedentary children, this may be a result of confounding and publication bias. Randomized trials are the gold standard in determining causality. A meta-analysis of 22 pediatric exercise trials was conducted using Metafor in R. The majority of trials measured bone by DXA; few trials provided information on lean mass changes with exercise. There was a greater overall percent increase in femoral neck (FN), hip, and spine (LS) BMC and FN and LS aBMD among prepubertal children randomized to exercise compared to controls, with a mean percent difference ranging from 0.6% for FN aBMD to 3.7% for LS BMC. BMC results were not significant among postpubertal children and too few trials in postpubertal children reported aBMD. Including calcium intake and length of intervention in the statistical analysis reduced heterogeneity among studies in LS aBMD results, while all BMC analyses had significant heterogeneity. Results related to periosteal circumference and cortical thickness were inconsistent, perhaps due to the variety of measurement methods used. There was evidence that other factors may modify the bone response to exercise, including baseline BMI, baseline activity levels and calcium intake. In summary, only prepubertal children randomized to exercise had greater increases in BMC and aBMD than control children, but few studies have been done in postpubertal children. Other factors may modify the bone response to exercise.

Disclosure
The authors declared no competing interests.

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Biographical details

Bonny Specker
Dr Bonny Specker is currently Director and Chair of the E.A. Martin Program in Human Nutrition at South Dakota State University. Prior to moving to SDSU in 1997, she was at the University of Cincinnati and Cincinnati Children’s Hospital Medical Center where she received her PhD in Epidemiology and spent 15 years in research and teaching as Professor of Pediatrics. She has published extensively in the area of bone, calcium and vitamin D metabolism. She was the Principal Investigator of the South Dakota Rural Bone Health study, which was designed to determine how lifestyle (diet and activity) and genetics influence bone density and later bone loss, and the SDSU Study Center of the National Children’s Study. Her and her group at SDSU have been working with the South Dakota Department of Health and the Northern Plains Tribal Epidemiology Center on maternal child health issues.
Factors influencing peak bone mass

Nicholas C Harvey\textsuperscript{1,2}

\textsuperscript{1}MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK; \textsuperscript{2}NIHR Southampton Nutrition Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK

Peak bone mass is a major determinant of osteoporosis risk and subsequent fragility fractures in older age. There is a wide range of factors influencing peak bone mass, ranging from those acting very early in life, for example in utero and periconception, to those acting through childhood and adolescence into young adulthood. In this presentation I will give an overview of some overarching themes and principles of relevance to peak bone mass, using specific clinical scenarios to illustrate key points. Bone mass increases through growth in childhood to a peak in young adulthood, with the age of peak bone mass varying by individual bone site. Influences on peak bone mass are many and varied, and include common factors which have a small individual contribution, and act at the level of the population, for example nutrition, physical activity, smoking and alcohol intake. The biggest individual effects come from illnesses; almost any severe childhood illness may have an adverse influence on growth. Longer term effects of chronic or repeated illness may result in linear growth failure and/or deleterious effects on bone accrual. One of the key principles in the assessment of bone mass in childhood disease is to differentiate between linear growth failure and a specific adverse effect on bone. The clinical scenarios have been chosen to illustrate several important mechanistic points, of relevance to clinical assessment and treatment, and will include those of chronic inflammation associated with diseases such as Crohn’s disease and inflammatory arthritis, malabsorption through inflammatory bowel disease and coeliac disease, associations with physical activity in terms of trauma and effects on bone, and hormonal issues such as amenorrhea and weight loss. Acute lymphoblastic leukaemia serves as an example of a disease and its treatment both having negative effects on bone quality. Finally I will consider the increasing evidence for the role of environmental factors in utero, such as maternal gestational 25(OH)D concentration, on long term bone development and potential underlying mechanisms. The clinical implications of the issues discussed will be considered, together with important principles in the assessment of bone mass in children.

Disclosure

N Harvey has received consultancy, lecture fees and honoraria from Alliance for Better Bone Health, AMGEN, MSD, Eli Lilly, Servier, UCB, Shire, Consilient Healthcare and Internis Pharma.

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Biographical details

Nicholas Harvey

Nicholas Harvey was appointed to a personal chair at the University of Southampton in 2015, and leads, with Professor Cooper and Professor Dennison, an MRC programme focused on the lifecourse epidemiology of bone and joint disease, as part of the MRC Lifecourse Epidemiology Unit. He is working to i) translate epidemiological observations linking early life influences on later bone health into potential novel public health strategies (e.g. gestational vitamin D supplementation) aimed at optimising childhood bone mineral accrual and reducing risk of later fracture; and ii) elucidate underlying mechanisms. He has won several Awards at national and international meetings, is an investigator/author on >£50 m grant funding, has published over 130 peer-reviewed papers, and is a member of the National Osteoporosis Society (UK) Scientific Programme Committee, UK Biobank Imaging Working Group, International Osteoporosis Foundation Committee of Scientific Advisers, Bone Research Society (UK) Committee, Arthritis Research UK PRC.

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Phosphate and FGF23 signaling

Justine Bacchetta

Bron, France

The vision of calcium/phosphate metabolism has been completely modified during the last decade with the description of the FGF23/Klotho regulation axis. Renal regulation of phosphate handling in the proximal tubule is a complex and highly-regulated process. At least three transport proteins are responsible for renal phosphate reabsorption: NAPI-IIa (SLC34A1), NAPI-IIc (SLC34A3) and PIT-2 (SLC20A2). These transporters are highly regulated by various cellular mechanisms and factors including acid–base status, electrolyte balance and hormones such as dopamine, glucocorticoids, IGF1, calcitriol, parathyroid hormone, FGF23 and Klotho. Hypophosphatemic rickets are secondary to increased FGF23 levels, due to various mutations directly in the FGF23 gene or in its regulators (PHEX, DMP1, Klotho, …). Mutations in the NAPI-IIc or NAPI-IIb transporters as well as mutations in the sodium-hydrogen exchanger regulatory factor 1 (NHERF1) can induce hypophosphatemia and/or bone demineralization and/or hypercalciuria. The objectives of this talk are therefore: 1/ to detail renal phosphate handling in physiology, 2/ to develop genetic diseases associated with renal phosphate handling such as hypophosphatemic rickets but also genetic nephrolithiasis, and 3/ to discuss their impact on bone status.

Disclosure

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Biographical details

Justine Bacchetta

Justine Bacchetta (MD 2009, PhD 2011) is an Associate Professor of Pediatrics in Lyon, France. She is specialized in pediatric nephrology and pediatric diseases of calcium and phosphate metabolism. After a research fellowship at UCLA (Los Angeles, USA), she has four main research topics of interest: bone and mineral disorders associated to chronic kidney disease (CKD-MBD), bone disease in pediatric chronic diseases, bone disease in rare inherited renal diseases and bone physiology during growth. She is a council member of the European Society for Paediatric Nephrology (ESPN), and board-member of the CKD-MBD working group of the ESPN. She has published more than 100 publications in peer-reviewed journals. She has given 49 invited lectures in international and national conferences, and she received in 2016 the Renee Habib award from the International Pediatric Nephrology Association (IPNA).
Signalling pathways and their significance for bone health and disease. PTH/cAMP/PKA

Anya Rothenbuhler

Pediatric Endocrinology Department, Centre for Rare Diseases of the Calcium and Phosphorus Metabolism, Bicêtre Hospital, Université Paris Sud, Le Kremlin-Bicêtre, France

GNAS-Gsalpha based disorders lead to heterogeneous diseases associated with abnormal bone development via two distinct mechanisms. At the level of the growth plate in bones, the PTHrP/PTH1R/Gsalpha/cAMP/PKA/PDE signalling pathway regulates endochondral ossification. PTHrP binds to the PTH receptor (PTH1R) which then couples with the stimulatory G protein (Gsalpha) leading to cAMP formation. cAMP binds to the regulatory 1A subunits (R1A) of the PKA. Upon binding the catalytic subunits dissociate from the R1A and phosphorylate numerous target proteins including CREB, which activates transcription of cAMP responsive genes. Phosphodiesterases (PDE’s) regulate intracellular cAMP levels. Down-regulation of this signalling pathway due to mutations or methylation changes in genes coding for proteins spanning throughout the pathway from PTH1R (Blomstrand dysplasia) to PDE’s (acrodysostosis) lead to end-organ resistance to the action of PTHrP, abnormal endochondral ossification thus resulting in various degrees of chondrodysplasia. Other then chondrodysplasia, another hallmark of GNAS based disorders are de novo formations of extra skeletal qualitatively normal bone in skin and subcutaneous fat due to abnormal differentiation of mesenchymal stem cells (MSC’s). Based on studies from transgenic mice, Gsalpha seems to be a key regulator of osteoblast differentiation by maintaining the balance between two key signalling pathways: Wnt-beta catenin and Hedgehog. Gsalpha’s role may be to prevent bone formation in tissues where bone should not form. Historically most GNAS based diseases were classified under the term pseudohypoparathyroidism (PHP). Recently, a novel classification including a larger span of diseases, based on their common mechanism, was proposed using the term inactivating PTH/PTHrP signalling disorders (iPPSD).

Disclosure

I have received honoraria and travel grant from Kyowa Kirin and Ultragenyx.

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Biographical details

Anya Rothenbuhler

Anya Rothenbuhler became MD at the Faculty of Medicine Lille 2 in France in 2003 with a primary specialization in Pediatrics. She then became assistant Professor in the Pediatric Endocrinology Department in Cochin-Saint Vincent de Paul Hospital, Université Paris Descartes and trained to become a pediatric endocrinologist with a special interest in rare diseases of calcium and phosphorus metabolism. Dr Rothenbuhler is now a full time senior clinician in the Department of Pediatric Endocrinology at Bicêtre University Hospital in France working for the national reference center for rare disorders of the mineral metabolism. She has an over 10-year clinical experience in treating children from birth throughout adolescents with rare mineral disorders.
IS20

Canonical Wnt signaling in bone health and disease

Wim Van Hul

Department of Medical Genetics, University of Antwerp, Antwerpen, Belgium

About two decades ago, evidence was generated that canonical Wnt signaling plays an important role in bone accrual mainly based on the identification of mutations in genes from the Wnt pathway resulting in extremely low or high bone mass. Since then, it became clear that genetic variation in a lot of genes from this pathway have an influence on bone mass both in a number of skeletal dysplasias as well as in the general population. This is the case for a number of wnt ligands (wnt1, wnt3, wnt4, wnt5B, wnt16,…), the co-receptor LRP5 and some intracellular partners of this pathway (Axin1 and CTNNB1). Because of its important role in several biological processes, there is a need for strong regulation of the Wnt signaling pathway. This is being done at different levels. Extracellularly, a number of inhibitors are identified. The sFRP’s bind directly to wnt ligands and genetic variance in the sFRP4 gene influences bone mass. The DKK gene family includes 4 inhibitors of the pathway with especially DKK1 being involved in bone homeostasis. Sclerostin encoded by the SOST gene is a bone specific inhibitor secreted from osteocytes. Absence of functional sclerostin results in Van Buchem disease and sclerosteosis, both characterized by extremely high bone mass. Its crucial role in regulating bone homeostasis was corroborated by the evidence that specific missense mutations in the co-receptor LRP5 which disrupt the binding between sclerostin and LRP5 also result in high bone mass. Along the same lines, some missense mutations in LRP4, that seems to act as an anchor for sclerostin in the bone, result in disruption of the binding with sclerostin and subsequently high bone mass. Finally, the R-spondins are transmembraneous activators of canonical Wnt signaling and especially RSPO3 seems to affect bone mass. In conclusion, a lot of evidence has been generated, initially in genetic studies and subsequently corroborated by in vitro functional studies and animal models, to illustrate the essential role of canonical Wnt signaling in bone formation and homeostasis. Therefore, this pathway has been considered an interesting target to identify novel treatments for osteoporosis with very promising results.

Disclosure

The authors declared no competing interests.

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Biographical details

Wim Van Hul

Wim Van Hul is full professor of Molecular biology and genetics at the University of Antwerp, Belgium. He obtained a bachelor degree in Chemistry from the University of Louvain (Belgium) and a master degree in biochemistry. He obtained his PhD on molecular genetics in 1993 from the University of Antwerp. He started his own research group aiming at the identification and characterization of genes underlying skeletal disorders and obesity. His team was successful in identifying and characterizing several disease causing genes including the SOST gene encoding the sclerostin protein. He authored and co-authored more than 200 publications and is on the editorial board of several journals. He is currently chair of the educational committee of biomedical sciences at the University of Antwerp, Belgium.
BOOSTB4: Boost Brittle Bones Before Birth
A clinical trial on pre- and/or postnatal stem cell transplantation for treatment of osteogenesis imperfecta

Cecilia Götherström on the behalf of the BOOSTB4 consortium (BOOSTB4.EU)
Division of Obstetrics and Gynaecology, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

Osteogenesis imperfecta (OI), or brittle bone disease, is a heterogeneous inherited condition and severe forms present already in utero. Persons with severe OI is affected throughout their lifetime with repeated, multiple fractures, short stature and orthopaedic problems, considerable pain and handicap. There is no curative or sufficiently effective symptomatic treatment for OI. Preliminary clinical experience indicates that transplantation of fetal liver derived mesenchymal stem cells (MSC) before and after birth may ameliorate symptoms. The main objective of the international Boost Brittle Bones Before Birth (BOOSTB4) phase I/II multicentre trial is to evaluate the safety and efficacy of pre- and/or postnatal MSC transplantation in severe vital forms of OI (type III and severe type IV). The study will include three groups:
   i) Prenatal and postnatal transplantations in circa 15 patients, inclusion during pregnancy
   ii) Postnatal transplantation in circa 15 patients, inclusion before one year of age
   iii) Historical and prospective controls, at least 30 cases

Over 12 months, the patients will receive four postnatal infusions of same-donor MSC at 4-month intervals. The primary outcome is safety for the fetus, child and pregnant woman. Secondary outcomes relate to efficacy, including fracture frequency, time to fracture, number if fractures at birth, growth, bone mineral density, biochemical bone turnover and clinical OI status. Rapid exome sequencing using a panel targeted for skeletal disorders for definitive molecular diagnosis of OI will be developed. Experience, impact and perception of the therapy will be evaluated in both treatment groups. Non-invasive prenatal diagnosis of OI based on analysis of cell free DNA will be developed. We have established a European network centred around four clinical hubs in Stockholm, Cologne, London and Utrecht/Leiden. Ethical and regulatory applications are underway to conduct this clinical trial. The BOOSTB4 consortium welcomes clinical cases for diagnosis of OI using rapid exome sequencing and for inclusion in the clinical trial. Contact Cecilia Götherström for more information: Cecilia.Gotherstrom@ki.se and BOOSTB4.EU

Disclosure
The authors declared no competing interests.
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Biographical details
Cecilia Götherström
Cecilia Götherström is Associate Professor in Stem Cell Research at Karolinska Institutet and her research is in the field of perinatal regenerative medicine. She was one of the first in the world to isolate and characterize human fetal mesenchymal stem cells. Dr Götherström has developed fetal mesenchymal stem cells for prenatal and postnatal transplantation purposes and since then the cells has indeed been used clinically to treat fetuses and children suffering from severe osteogenesis imperfecta with promising results. Dr Götherström is leading an international multicentre trial to evaluate the clinical effect of mesenchymal stem cell transplantation in the treatment of severe osteogenesis imperfecta.
Role of microRNAs in the development of osteosarcoma

Eric Hesse
Heisenberg Group for Molecular Skeletal Biology, Department of Trauma, Hand and Reconstructive Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Osteosarcoma is the most prevalent primary bone tumor that occurs mainly during adolescence. Osteosarcoma is an aggressive mesenchymal tumor that often arises due to mutations in the tumor suppressor gene p53. Treatment of osteosarcoma patients includes chemotherapy, radiation therapy and surgical approaches. Nevertheless, at advanced stages the survival rate is rather low. Thus, a better understanding of the underlying biology of osteosarcoma might help to develop novel therapeutic approaches to improve the current treatment options. Runx2 is a transcription factor that promotes differentiation and restricts proliferation of osteoblasts under physiological conditions. In osteosarcoma, expression of Runx2 is often increased and its function is deregulated. We defined pathological roles of Runx2 in the etiology of osteosarcoma and mechanisms by which Runx2 expression is increased. Runx2 is often highly expressed in human osteosarcoma biopsies and cell lines. Small interference RNA (siRNA)-mediated depletion of Runx2 inhibits growth of U2OS osteosarcoma cells. Runx2 levels are inversely linked to loss of p53, which predisposes to osteosarcoma, in distinct osteosarcoma cell lines and osteoblasts. Runx2 protein levels decrease upon stabilization of p53 with the MDM2 inhibitor Nutlin-3. Elevated Runx2 protein expression is post-transcriptionally regulated and directly linked to diminished expression of several validated Runx2 targeting microRNAs (miRNAs) in human osteosarcoma cells compared to mesenchymal progenitor cells. The p53-dependent miR-34c is the most significantly down-regulated Runx2 targeting miRNA in osteosarcoma. Exogenous supplementation of miR-34c markedly decreases Runx2 protein levels, while 3’UTR reporter assays establishes Runx2 as a direct target of miR-34c in osteosarcoma cells. Importantly, Nutlin-3 mediated stabilization of p53 increases the expression of miR-34c and decreases the abundance of Runx2. Thus, a novel Runx2-p53-miR34 network controls cell growth of osseous cells and is compromised in osteosarcoma. This regulatory network could provide the opportunity for novel therapeutic interventions, i.e. the exogenous reconstitution of osteosarcoma with miR-34c. MicroRNAs are already in the development as potential drugs and could also be useful for future cancer therapies.

Disclosure
Received consultation and speaker fee from Lilly and Amgen.

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Biographical details

**Eric Hesse**

Eric Hesse studied Medicine at Hannover Medical School in Germany where he became MD in 2003. He was trained in Orthopedic Surgery and graduated as PhD in 2007 in Genetics & Cell Biology in Hannover, Germany. In 2005, he moved as a Postdoctoral Fellow funded by the German Research Foundation to the laboratory of Dr Roland Baron at Yale University School of Medicine. The laboratory moved to Harvard University Schools of Medicine and Dental Medicine in 2008, where he continued his work as a Postdoc and later as Junior Faculty until 2011. During this time, he worked on clinical and basic science projects focusing on osteoblast biology and bone homeostasis, leading to publications in top tier journals including JCB, JBMR, Dev Cell, Bone, and the NEJM. He received numerous awards and fellowships, including the ASBMR Young Investigator-, John Haddad- and Harold Frost Award, the ECTS New Investigator Award, the Harvard Deans Fellowship, and the Gideon & Seygi Rodan IBMS Fellowship. By the end of 2011, he moved to the University Medical Center Hamburg-Eppendorf in Germany, where he established an independent international research group as full, endowed, tenure-track Heisenberg-Professor. His research continues to focus on translational aspects of osteoblast function and bone remodeling as well as on cancer-induced bone diseases and is funded by the German Research Foundation, the German Federal Ministry of Education and Research, the European Union, the Helmholtz Association, and several Foundations. In addition, he was Co-Chair of the IBMS Young Investigator Committee and serves as a member of the ASBMR Professional Practice Committee, the ECTS Training Committee, the IBMS Awards Committee, the IBMS Publication Committee, the ORS Sun Valley Workshop Advisory Board, and as Director of Research of the Molecular Skeletal Biology Laboratory and of the Department of Trauma, Hand, and Reconstructive Surgery, in which he is practicing as Orthopedic Surgeon. Furthermore, he is Adjunct Professor in the Department of Anatomy and Cell Biology at Indiana University School of Medicine in the USA and serves as spokesperson of the BMBF/ANR bi-national Consortium ‘Integrative Biology of Osteoanabolic Networks in the Epigenome (iBONE)’.
Oral Communications
OC1
NBAS variants causing a novel form of inherited bone fragility
Meena Balasubramanian1,2, Jane Hurs1, Catherine DeVile1, Nick Bishop1,2, Paul Arundel1,2, Amaka Olihia1,2, Rebecca Pollitt1,2, David Hughes1,2, Dasa Longman1, Javier Caceres1 & Tim Skerry1
1University of Sheffield, Sheffield, UK; 2Sheffield Children’s NHS Foundation Trust, Sheffield, UK; 3Great Ormond Street Hospital, London, UK; 4Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK; 5MRC Human Genetics Unit, Edinburgh, UK.

Background
Osteogenesis imperfecta (OI), the commonest inherited bone fragility disorder, affects 1/15,000 live births resulting in frequent fractures and reduced mobility, with significant impact on quality of life. Early diagnosis is important, as therapeutic advances can lead to improved clinical outcomes.

Methodology and results
Triplo exome sequencing in patients with OI identified, in two patients, compound heterozygous mutations in NBAS (Neuroblastoma amplified sequence). Patient 1: c.5741G>T p.(Arg1914His); c.3010C>T p.(Glu678*) in a 10-year old boy with significant short stature, bone fragility requiring bisphosphonate treatment, developmental delay and immunodeficiency. A transiliac bone biopsy, following recurrent low-trauma fractures, demonstrated osteoporosis with high bone turnover with marked sub-periosteal bone resorption, different to classical OI. Patient 2: c.5741G>T p.(Arg1914His); c.2032C>T p.(Glu678*) in a 5-year old boy with bone fragility, developmental delay and immunodeficiency. Studies in human patient fibroblasts (hpf) showed reduced collagen expression, compared to control cells. RNAseq studies, in bone cells showed NBAS expression in osteoblasts and osteocytes of rodents and primates; Western blot analysis showed reduced level of NBAS protein in hpf, compared to control cells, implying that NBAS mutations compromise the stability of NBAS protein. CRISPR-Cas9 technology has been used to generate stable knockout NBAS cell lines in human SAOS2 osteoblast cells.

Discussion
These findings provide proof-of-concept that NBAS mutations have mechanistic effects in bone, and NBAS mutations are a novel cause of bone fragility, distinguishable from ‘Classical’ OI. Since NBAS has been proposed to function in the non-sense mediated decay (NMD) pathway and is also part of the Golgi-ER transport, the effect on bone fragility may be attributable to either pathway in isolation or to both. In our clinical practice, patients with NBAS variants have responded positively to bisphosphonate treatment, with marked improvement in their bone health and quality of life.

Conclusions
Here we report on NBAS mutations as a novel cause of bone fragility. Further studies are ongoing to elucidate the precise mechanism of action of NBAS and its role in bone fragility. We have since identified more patients with NBAS variants and will explore the phenotypic variability with special attention to their effect on bone health.

Disclosure
The authors declared no competing interests.

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OC2
Methylation patterns at the novel DMR of GNAS (GNAS-AS2) in pseudohypoparathyroidism IB (PHP1B or iPPD3) subtypes
Patrick Hanna1, Anne Rochus2, Harald Jueppner1, Deborah Mackay4, Bruno Francou1, Jerôme Bouligand1, Anne Mantel3, Elii Anagnostou2, Elips Vlachopapadopoulou2, Dominique Gaillard1, Brigitte Delemer9 & Agnès Linglart1,8
1INSERM U1169, Bicêtre Paris Sud, France; 2Department of Pediatrics, University Hospitals, Leuven, Belgium; 3MGH and Harvard Medical School, Boston, MA, USA; 4Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, UK; 5Laboratory of Molecular Genetics, APHP Bicêtre, Bicêtre Paris Sud, France; 6Division of Human Reproduction, Athens University Medical School, Athens, Greece; 7Genétique et Biologie de la Reproduction, Centre Hospitaller Universitaire, Reims, France; 8APHP, CMR Calcium-Phosphore, Bicêtre Paris Sud, France; 9Endocrinologie, CHU, Reims, France.

PHP1B & iPPD3 per the new proposed classification- is a rare disorder characterized in most patients by proximal tubular resistance to PTH resulting in hypercalcaemia, hyperphosphataemia and elevated PTH. Loss-of-methylation (LOM) at the Differentially Methylated Region (DMR) at GNAS exon A/B occurs in all PHP1B patients, but methylation changes at other DMRs within GNAS occur in some familial and most sporadic PHP1B cases. All patients with autosomal dominant PHP1B (AD-PHP1B) due to a maternal deletion that comprises the STX16 region (delSTX16 +) present with LOM restricted to the GNAS-A/B DMR, while sporadic cases (sporPHP1B) present with broad GNAS methylation defects, including LOM at a novel, recently identified DMR within the GNAS locus referred to as antisense DMR2 (GNAS-AS2).

Objectives and patients
Characterize the methylation pattern at the GNAS-AS2 DMR in AD-PHP1B delSTX16 + (n=9) and delSTX16 − (n=5) patients; furthermore, sporPHP1B (n=10) and healthy controls (n=10) were investigated. STX16 and GNAS deletions were excluded in the delSTX16 − patients by MLPA, genomic multiplex and quantitative PCR of the GNAS and STX16 regions.

Results:
1- The methylation index at the GNAS-AS2 DMR was significantly higher in delSTX16 − patients (32 ± 14%) than in controls (24 ± 6%), delSTX16 + (5 ± 2%) and sporPHP1B patients (3 ± 1%) (P<0.0001).
2- Bisulfité-treated DNA of PHP1B patients with delSTX16 − was PCR amplified across the GNAS-AS2 DMR and products were cloned into pcDNA3.1. First, we identified 2 CG-rich subdomains (SD1 and SD2) within the GNAS-AS2 DMR that are separated by 184 bp. Second, in delSTX16 − patients we observed a unique pattern of methylation including an gain of methylation at SD1 and a methylation pattern at SD2 similar to that of controls, whereas both delSTX16 + and sporPHP1B patients displayed full LOM at SD1 and SD2.

Conclusion
We have further refined the GNAS-AS2 DMR and identified a subgroup of PHP1B patients with a specific pattern of methylation at the GNAS-AS2 DMR.

Disclosure
The authors declared no competing interests.

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OC3
Principal component-derived bone density phenotypes and genetic regulation of the pediatric skeleton
Jonathan Mitchell1,2, Alessandra Chesi1, Shana McCormack1,2, Diana Cousminer3,4, Heidi Kalkwarf5, Joan Lapre6, Vicente Gilsanz5, Sharon Oberfield6, John Shepherd7, Andrea Kelly7,11, Babette Zemel1,8 & Grannt Struan1,2
1Children’s Hospital of Philadelphia, Philadelphia, PA, USA; 2University of Philadelphia, Philadelphia, PA, USA; 3Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA; 4Creighton University, Omaha, NE, USA; 5Children’s Hospital Los Angeles, Los Angels, CA, USA; 6Columbia University Medical Center, New York, NY, USA; 7University of California San Francisco, San Francisco, CA, USA.

Objectives
To define if genetic variants associated with principal component-derived areal bone mineral density (abMD) loading scores.

Methods
Our sample comprised 1,293 children of European ancestry enrolled in the longitudinal Bone Mineral Density in Childhood Study (52% female). The participants completed up to 7 annual study visits. From dual energy X-ray absorptiometry scans, sex and age-specific abMD Z-scores were calculated for total hip, femoral neck, spine and distal radius. Principal component analysis, applied to the four Z-scores, generated new integrated abMD phenotypes. Linear mixed effects models, adjusted for age, Tanner, BMI, diet, calcium and physical activity, were used to test associations between a genetic score (percentage abMD-lowering alleles carried at 63 GWAS-implicated loci) and the loading scores. We also performed a GWAS, using the baseline data, to identify loci associated with the loading scores.

Results
Four principal components (PC1-PC4) were identified that explained 68.1, 18.6, 10.5, and 2.8% of the variance, respectively. A higher PC1 loading score indicated higher bone Z-scores across all four sites. The genetic score was associated with lower PC1 loading score (beta = 0.05, P = 3.9 x 10^-10); from the GWAS, rs114260199 (LMO2/CAPRIN1, P = 5.9 x 10^-8) and rs75321045 (ZMAT4, P = 2.3 x 10^-10), females) were associated with PC1 loading score. A higher PC2 loading score indicated higher distal radius Z-score only. The genetic score was not associated with PC2; from the GWAS rs67991850 (CPED1, P = 2.5 x 10^-11) was associated with PC2 loading score. A higher PC3 loading score indicated higher spine Z-score only. The genetic score was not associated with PC3; from the GWAS rs88689746 (RAB11FIPS, P = 4.8 x 10^-5, females) was associated with PC3 loading score. A higher PC4 loading score indicated lower total hip Z-score, but higher femoral neck Z-score. No genetic associations were observed for PC4.

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Conclusion
We identified four integrated pediatric aBMD phenotypes, including non-site-specific (PC1), distal radius-predictive (PC2) and spine-specific phenotypes (PC3). An established genetic bone fragility score associated with the non-site-specific phenotype, but not the site-specific phenotypes. Novel variants near LMO2/CA-PRR1, ZMAT4, and RAB11FIP5 associated with non-site specific or spine specific phenotypes. These results highlight the utility of an integrated skeletal phenotyping approach, which may help identify additional genetic loci associated with skeletal development.

Disclosure
The authors declared no competing interests.

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OC5
Eight-year longitudinal analysis of physical activity and bone strength during adolescence: The Iowa Bone Development Study
Kathleen Janz, Elena Letuchy & Steven Levy
University of Iowa, Iowa City, IA, USA.

Objectives
Conventional wisdom suggests that bone is most responsive to physical activity during the growing years, especially the period just before puberty. Few studies have addressed the entire period of adolescence and even fewer have done so using bone imaging techniques to capture structural outcomes which contribute to bone strength. Using a well-defined cohort (The Iowa Bone Development Study, IBDS), this report examined the magnitude and consistency of the association between physical activity and structural bone strength measures from adolescence to young adulthood.

Methods
IBDS members with at least three bone scanning visits between age 11 and 19 year were studied (n = 551, 280 females, 1844 total records). DXA scans of hip, used with the hip structural analysis program, and pQCT scans of tibia at 4% (trabecular) and 38% (cortical) sites were obtained. Outcomes included femoral neck (FN) section modulus (Z), FN cross-sectional area (CSA), tibia bone strength index (BSI), cortical thickness (CTb), and tibia torsion strength (pSST). Physical activity was assessed using the self-report PAQ-A and Actigraph accelerometry (hip worn) analyzed with the Evesnon equation. Age at peak height velocity (PHV age) was estimated from the Mirwald equation. Sex-specific bone trajectories were developed as two-level growth models with up to five repeated measurements. Models included height, weight, and PAQ-A as time-varying covariates. Models included cubic polynomial for time (biological age as years from peak height velocity age) with random effects for intercept and time at the individual level to describe growth over time. All models were repeated using Actigraph measured minutes per day of vigorous physical activity (VPA). Fewer records were available for the Actigraph measured minutes per day of vigorous physical activity for males.

Results
PAQ-A was positively associated (P < 0.05) with all bone strength measures for males and females with the exception of pSST for females. VPA was positively associated (P < 0.05) with BSI, Z, and CSA in males and BSI, pSST, Z, and CSA in females.

Conclusion
Bone remains responsive to the mechanical loading effects of physical activity throughout adolescence and into young adulthood. Greater attention should be placed on promoting bone-strengthening physical activity after the pre-pubertal years when adult exercise patterns are more likely to be formed.

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The authors declared no competing interests.

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OC6
Lean mass accretion increases during summer and positively associates with vitamin D status in healthy children 2-8 years
Neil Brett, Catherine Vanstone & Hope Weiler
McGill University, Sainte-Anne-de-Bellevue, QC, Canada.

The relationship between vitamin D status and lean mass accretion in young children is not well understood.

Objective
To explore vitamin D status in relation to lean mass outcomes over 12 mo in children 2-8 years.

Methods
This was a secondary analysis of trial data (clinicaltrials.gov: NCT02097160, NCT023587892) in Montreal, Canada. Children consumed their normal vitamin D intake for 6 mo (Apr-Oct 2014, n = 39) and 12 mo (Apr 2014–Apr 2015). Vitamin D status (serum 25(OH)D: Liaison, Diasorin) was measured at all visits (Apr, Oct 2014, Jan, Apr 2015). Standardized anthropometry, skin colour (forehead, forearm and lower leg; spectrophotometer: CM-700d/600d, Konica Minolta), demographics, activity and diet were assessed. Whole body lean and fat mass were measured at baseline, 6 and 12 mo using DXA (Hologic Discovery, APEX v13.3). Spearman correlations, linear regression and a mixed model ANOVA were used.

Results
In Apr 2014, children were 5.1 ± 1.9 years, 54% (21/39) male, with BMI Z-score of 0.72 ± 0.06. Vitamin D intake (222 ± 9 IU/d) did not change across the 12 mo and was not related to 25(OH)D. Serum 25(OH)D increased (P = 0.01) from 0 to 6
mo (Apr: 62.0±14.1 nmol/l; Oct: 73.5±13.4 nmol/l). Summer change of skin colour did not correlate with Δ 25(OH)D even though there was significant tanning of skin over summer (individual typological angle 0-6 mo: Δ: -120±5%). Using linear regression, the summer Δ in 25(OH)D was 3.3 nmol/l less for every 10 nmol/l increment in April 25(OH)D (r²=0.60, P<0.01). The summer Δ in lean mass positively correlated with Apr 25(OH)D (r=0.37, P=0.02) and was greater in summer than winter (summer: 8.3±3.7, winter: 5.7±3.5, P=0.04). In the subgroup, 25(OH)D decreased (P=0.01) from 6 to 12 mo (Oct: 71.6±15.1 nmol/l; Apr: 61.3±16.3 nmol/l). The 12 mo Δ in lean mass was higher by 1.5% for every 10 nmol/l increment in Oct 25(OH)D (r²=0.66).

Conclusion

These results suggest 25(OH)D concentration may be an important factor for lean mass accrual in young children. (Clinical trial funding: Dairy Farmers of Canada, Canada Research Chairs and Canada Foundation for Innovation).

Disclosure: The authors declared no competing interests.

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OC7

The effect of antenatal iron supplementation on fibroblast growth factor-23 concentration in mothers and infants: a randomised controlled trial in rural Kenya

Vickie Breathwaite1, Ayse Demir2, Martin Mwangi1,4, Pauline Andang’o4, Andrew Prentice3,4, Ann Prentice1,5 & Hans Verhoef6,7

1MRC Elsie Widdowson Laboratories, Cambridge, UK; 2Meander Medical Centre, Laboratory for Clinical Chemistry, Amersfoort, The Netherlands; 3Division of Human Nutrition, Wageningen University, The Netherlands; 4School of Public Health and Community Development, Maseno University, Kenya; 5MRC Unit The Gambia, Banjul, Gambia; 6MRC International Nutrition Group, London, UK; 7Cell Biology and Immunology Group, Wageningen University, The Netherlands.

Objectives

Murine studies have shown that iron deficiency during pregnancy can cause abnormal phosphate and bone metabolism in offspring by elevating concentrations of fibroblast growth factor-23 (FGF23). FGF23 exists in plasma as an intact phosphate- and vitamin D-regulating hormone and its C-terminal fragment, a cleavage product that possibly antagonises the intact hormone. These findings are pertinent to low-income countries, where the prevalence of iron deficiency in pregnant women exceeds 50% and rickets is the most common non-communicable disorder of children. We aimed to determine the effect of antenatal oral iron supplementation on maternal and infant FGF23 and bone mineral metabolites.

Methods

Pregnant women in rural Kenya (n=470) were randomised to daily supplementation with iron (60 mg, as ferrous fumarate) or placebo from 13–23 weeks gestation until 1 month post-partum. We collected EDTA blood samples at baseline and at 6 months gestation, 35 days post-partum, 1 month and 12 months post-partum. Serum FGF23 was measured using an enzyme-linked immunosorbent assay (ELISA) kit (C-terminal fragment, R&D Systems, Inc). Infant FGF23 was measured in the infant serum at 3 months post-partum.

Results

Iron supplementation improved maternal iron status (as seen by effects on intact form (I-FGF23), or both intact FGF23 together with its C-terminal fragment (C-FGF23). Iron supplementation led to reduced C-FGF23 concentrations in mothers (medians: 71.6 nmol/l vs 93 nmol/l, Z=4.91 RU/ml vs 570 RU/ml, Z=4.91). The 12 mo Δ in I-FGF23 was higher by 7.3% for every 10 nmol/l increment in Oct 25(OH)D (r²=0.66).

Conclusion

These results suggest 25(OH)D supplementation led to reduced C-FGF23 concentrations in mothers (medians: 71.6 nmol/l vs 93 nmol/l, Z=4.91 RU/ml vs 570 RU/ml, Z=4.91). The 12 mo Δ in I-FGF23 was higher by 7.3% for every 10 nmol/l increment in Oct 25(OH)D (r²=0.66). iron supplementation improved maternal iron status (as seen by effects on intact form (I-FGF23), or both intact FGF23 together with its C-terminal fragment (C-FGF23).

Disclosure: The authors declared no competing interests.

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OC9
Calcium carbonate supplementation of pregnant rural Gambian mothers alters offspring IGF-1 at age 7.5 years in a sex-dependent manner
Ann Prentice1,2, Saillja Nighdira & Sophie Moore3,4
1MRC Elsie Widdowson Laboratory, Cambridge, UK; 2MRC Unit The Gambia, Kenema, Gambia; 3Division of Women’s Health, King’s College London, London, UK.

Objective
We have observed sex-specific effects of pregnancy calcium carbonate supplementation in 8-12 year old Gambian children, indicating slower growth after infancy in girls born to calcium-supplemented mothers but faster in boys.1,2 IGF-1 is a key driver of growth and is responsive to calcium supplementation.3 The aim of this study was to determine whether the pregnancy supplement had resulted in sex-specific effects on mid-childhood IGF1 of the offspring.

Methods
Archived data from a study of the offspring of Gambian mothers who participated in ISRCTN96502494 were used. These women were randomised to 1500 mg Ca/d as calcium carbonate (Ca) or placebo (P) from 20 weeks pregnancy to delivery. Of the 546 children born, 389 were followed-up and 290 had plasma IGF-1 and IGF-BP3 assayed by Immulite 1000 (n’group: female(F)-Ca = 77, F-P = 73, Male(M)-Ca =64, M-P=76). The IGF-1 distribution was normalised using square root transformation. Maternal supplement effects were considered using regression with sexes separated and then together to test for a sex*supplement interaction (P-values given). IGF-1 summaries are presented as geometric mean (±1gSE, +1gSE); group differences as sympercents±SE (difference/mean).

Results
The children were 7.5 (±1.2) years, there were no significant sex or supplement group differences in age, weight or height. Mean IGF-1 concentrations (ng/ml) were F-Ca =99.5 (±8.3,5), F-P=118.9 (±6.4,8), M-Ca=78.1 (±4.3,5), M-P=67.7 (±3.4,6). Girls had higher IGF-1 than boys in both supplement groups (P<0.001). IGF-1 was lower in F-Ca than F-P (=12.7±%, P=0.01) but higher in M-Ca than M-P (+14±%, P=0.05); sex*supplement P=0.001. IGF-BP3 differences were in similar direction but effect sizes were smaller. Although attenuated after IGF-BP3 adjustment (F-Ca v F-P=−15±%, P=0.02; M-Ca v M-P=+8±7%, P=0.02), sex*supplement remained significant (P=0.008).

Conclusion
Calcium carbonate supplementation of pregnant rural Gambian mothers resulted in lower IGF-1 in girls and higher IGF-1 in boys during mid-childhood before effects on growth were apparent. These results are consistent with the observed effects of the maternal supplement on the growth trajectories of the offspring. Funded by European Union Sixth Framework (FOOD-CT-2005-007036), MRC programmes U105960371, U123261351, MC-A760-5QX00 and DfID under the MRC/DFID Concordat.

Disclosure
The authors declared no competing interests.

References

OC11
The effect of whole body vibration training on bone and muscle function in children with osteogenesis imperfecta and limited mobility: a randomized controlled pilot trial
Wolfgang Högener1,2, Nick Bishop3, Paul Arundel3, Janis Scott1, Zulf Mughal1, Raja Padiella4, Peter Nightingale5, Nick Shaw2 & Nicola Crabtree6
1Department of Endocrinology and Diabetes, Birmingham Children’s Hospital, Birmingham, UK; 2Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK; 3Academic Unit of Child Health, Sheffield Children’s Hospital, Sheffield, UK; 4Department of Endocrinology, Royal Manchester Children’s Hospital, Manchester, UK; 5Wellcome Trust Clinical Research Facility, University Hospitals Birmingham, Birmingham, UK.

Objectives
Osteogenesis imperfecta (OI) is a bone fragility disorder associated with reduced muscle size, dynamic muscle function and mobility. This paired randomised controlled pilot study assessed the effect of whole body vibration (WBV) training on bone density and geometry, muscle size and function, and balance in children with OI.

Methods
Twenty-four children (5-16 years) with OI types 1,4 and limited mobility (defined as a Childhood Health Assessment Questionnaire (CHAQ) score ≥0.13), were recruited in gender- and pubertal stage-matched pairs. Incident fractures in both legs and a WBV arm) led to exclusion of two prepubertal male pairs. Matched pairs were randomised to either 5 months of WBV training (3×3 min twice daily) using a rotational WBV device, or regular care. Bone and muscle variables measured by dual-energy X-ray absorptiometry (lumbar spine, hip, total body) and peripheral quantitative computed tomography (distal and proximal tibia). Mobility assessed by six-minute walk tests and CHAQ, and dynamic muscle function by mechanography using single two-leg jumping, multiple one-leg hopping, chair- and heel-rising tests, and balance tests.

Results
At baseline, all participants had reduced six-minute walking distances (median Z-scores = −2.34 (−6.51 to −0.58)) and dynamic muscle function (P<0.001). BMI Z-score was associated with higher CHAQ scores (rho 0.552; P=0.005), reduced walking distance and two-leg jumping outcomes (rho −0.405 to −0.533, P<0.05). The WBV and control groups did not differ in the 5-month changes in bone density or geometry. Total lean mass increased more in the WBV group (+1.191 g (+224 to +1744)) compared to controls (+0.635 g (−951 to +1066)), P<0.01, without improving mobility, muscle function or balance.

Conclusion
This first randomised controlled trial in OI children demonstrated that WBV training increased lean mass without changes in dynamic muscle function or bone mass. This suggests reduced biomechanical responsiveness of the muscle-bone

OC10
Inadequate vitamin D status adversely affects trabecular bone mineral density in 14-18 year old adolescents
Taryn Smith1, Laura Tripkovic1, Camilla Damsgaard2, Christian Mølgaard2, Aine Hennessey3, Kirsten Dowling4, Kevin Cashman1, Mairead Kiely1, Susan Lantham-New1 & Kathryn Hart1
1University of Surrey, Guildford, UK; 2University of Copenhagen, Copenhagen, Denmark; 3University College Cork, Cork, Ireland.

We have previously shown a high prevalence of vitamin D inadequacy (serum 25-hydroxyvitamin D [S25(OH)D] <50 nmol/l) in adolescents (14-18 years) in the UK (51%7). It is well recognised that vitamin D deficiency (S25(OH)D < 25 nmol/l) increases the risk of rickets and impaired growth in adolescents, however the optimal vitamin D status for bone health is debated. The aim of this study was to investigate the effects of vitamin D status on bone health parameters in white male and female adolescents aged 14-18 years. A total of 110 adolescents (mean age 15.9 ± 1.4 years; 43% male) were recruited onto a vitamin D dose-response randomised controlled trial. At baseline, anthropometric and dietary information was collected, S25(OH)D was measured and bone geometry of the non-dominant radius was assessed by peripheral quantitative computed tomography (pQCT). Mean S25(OH)D concentration was 49.1 ± 12.3 nmol/l. When S25(OH)D concentrations were stratified by tertile, adolescents in the lowest tertile (≤44.7 nmol/l) had lower trabecular volumetric bone mineral density (vBMD) than those in the highest tertile (≥52.4 nmol/l) (P=0.012). These differences persisted after controlling for sex, age, Tanner stage, height, weight and calcium intakes (ANCOVA P=0.003). Trabecular vBMD z-score, calculated using published reference data for UK white children and adolescents5,6, was lower in the lowest vs the highest tertile of S25(OH)D (0.30 ± 1.22 and 0.90 ± 0.88 respectively; P=0.050). There were no differences in other pQCT measured bone parameters across the S25(OH)D tertiles. In conclusion, while debate regarding the optimal vitamin D status for bone health continues, this study has shown that adolescents with higher S25(OH)D concentrations (>53 nmol/l) had greater trabecular vBMD than those with lower concentrations. However it is not known whether this association arises due to lower bone remodelling and this requires further investigation. If maintaining a circulating 25(OH)D concentration above 50 nmol/l is confirmed to be beneficial for bone accretion in adolescents, we have shown that dietary vitamin D intakes of 30 μg/day would be required to achieve this7. This study was funded by the European Community’s Seventh Framework Programme, Grant Agreement 613977 for the ODIN Project.

Disclosure
The authors declared no competing interests.

Reference

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unit in Ol children and discourages the use of rotational WBV therapy as a tool to increase bone formation in Ol. The association of overweight with impaired mobility highlights the need for active weight management in children with Ol.

Disclosure
The authors declared no competing interests.

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**OC12**

**Fracture incident rate and growth in a nationwide cohort of boys with Duchenne Muscular Dystrophy**

S Joseph1,2, K Bushby1, M Guglieri3, I Horrocks1, V Straub1, SF Ahmed1, SC Wong1 & Northstar Clinical Network4
1Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK; 2Paediatric Neurosciences Research Group, Department of Paediatric Neurology, Royal Hospital for Children, Glasgow, UK; 3Institute of Human Genetics, University of Newcastle upon Tyne, Newcastle, UK; 4Northstar Clinical Network, UK.

**Background**
Fracture incidence rate and growth according to different glucocorticoid (GC) regimen in Duchenne Muscular Dystrophy (DMD) is currently unknown.

**Objective**
To determine the extent of skeletal morbidity and the influence of GC regimen on fracture incidence rate and growth in a contemporary cohort of DMD in the UK.

**Method**
Clinical details of 832 boys with DMD in the North Star database (2006–2015) from 23 centres were analysed. Fracture incidence rate per 10,000 person-years were determined for the group and according to GC regimen. Adjusted models using linear regression was used to evaluate factors associated with change in height standard deviation score (SDS).

**Results**
A total of 62 vertebral fracture (VF) episodes were observed in 52/832 (6%) and 118 non-VF episodes were observed in 112/832 (13.5%) boys. Median age at first VF and non-VF were 12 years (95% CI 10.5, 13.5) and 10.9 years (95% CI 10.3, 11.9), respectively. Kaplan-Meier analysis showed that 50% probability of first fracture was observed after 7.4 years (95% CI 6.3, 8.4) of GC therapy. Among the correlates of first incident fracture, ambulant status was associated with statistically significant increase in first fracture risk (Hazard ratio 2.5; 95% CI 1.1: 5.6; P = 0.03). Over the follow-up period, fracture incidence rate was 682/10,000 (95% CI 580,780) person-years. Fracture incidence rate was 254/10,000 (95% CI 29,887) person-years in GC naive boys. The highest fracture incident rate was observed in those treated with daily Deflazacort 1367/10,000 (95% CI 796, 2188) person-years. Using adjusted multiple regression models (age, height SDS baseline, duration of follow-up), change in height was positive for Enterococccus faecium (RA was positively associated with TB-BMD (FDR Z 278; 13%) was 0.25 S.D. higher (P = 0.03), though this effect was no longer significant after adjustment for BMI (P = 0.2).

**Conclusion**
Human gut microbiota influences bone mineral density in children. Enterococcus faecium, an active component of the Lactiferm probiotic has a positive effect on BMD, however, not fully independent of BMI. Our results are in line with animal models showing that incorporation of Enterococcus faecium in the diet increases the absorption of magnesium and phosphorus exerting positive effects in bone growth and immunological resistance. Replication of these findings will allow establishing probiotics (Enterococcus faecium) in the diet as beneficial to bone health of children.

Disclosure
The authors declared no competing interests.

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**OC13**

**Enterococcus faecium abundance in gut microbiome is associated with higher bone mineral density in school age children**


**Aim**
Human gut microbiota is an important determinant of health and disease. Discoveries from recent microbiome studies have been postulated as actionable targets to treat malnutrition, diabetes, obesity among other conditions. The role of the gut microbiome on the development of the human musculoskeletal system is yet to be established. The aim of our study was to investigate the association between bacterial operational taxonomic units (OTUs) of the gut in relation to bone mineral density levels in healthy children.

**Methods**
This study is embedded in the Generation R Study, a prospective multiethnic birth cohort in Rotterdam, The Netherlands. We included 2,173 children with a mean age of 9.8 (S.D. = 1.0 years) with total body DXA measurements (GE-Lunar iDXA) and 16S rRNA v3-v4 microbiome profiles determined from stool samples. We assessed the association of total body (less head) BMD (TB-BMD) with OTU relative abundance (RA) using the MASSLIN approach, followed by mean comparison of TB-BMD levels between bacterial profile carriers and linear testing of OTU/RA in the children where the OTU was present. All analyses were adjusted for age, sex, BMI, genomic principal components and technical covariates. Multiple testing was accounted for using FDR <0.05.

**Results**
Enterococcus faecium RA was positively associated with TB-BMD (FDR = 0.008), but not after correction for BMI (FDR = 0.051). TB-BMD of children positive for Enterococcus faecium (n = 278; 13%) was 0.23 S.D. higher (P = 0.001) than that of children in which this bacteria was absent, even after adjustment for BMI (P = 0.001). In children positive for Enterococcus faecium TB-BMD was 0.65 S.D. higher per RA unit increase (P = 0.03), though this effect was no longer significant after adjustment for BMI (P = 0.2).

**Conclusion**
Gut microbiota affects bone mineral density in children. Enterococcus faecium, an active component of the Lactiferm probiotic has a positive effect on BMD, however, not fully independent of BMI. Our results are in line with animal models showing that incorporation of Enterococcus faecium in the diet increases the absorption of magnesium and phosphorus exerting positive effects in bone growth and immunological resistance. Replication of these findings will allow establishing probiotics (Enterococcus faecium) in the diet as beneficial to bone health of children.

Disclosure
The authors declared no competing interests.

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**OC14**

**Body composition and associated biomarkers as determinants of bone mineral density in children 6–8 years of age – The Physical Activity and Nutrition in Children (PANIC) study**

Sonja Soininen1,2, Virpi Sidoroff1, Virpi Lindi1, Anitta Mahonen1, Liisa Kröger2, Heikki Kröger2,3, Jarno Jääskeläinen2, Mustafa Atalay1, David Laaksonen1,3, Toni Laitinen1,4 & Timo A. Lakka1,4
1University of Eastern Finland, School of Medicine, Kuopio, Finland; 2Social and Health Centre, City of Varkaus, Varkaus, Finland; 3Department of Pediatrics, North-Karelia Central Hospital, Joensuu, Finland; 4Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland; 5Department of Orthopedics and Traumatology, Kuopio University Hospital, Kuopio, Finland; 6Kuopio Musculoskeletal Research Unit (KMRU), University of Eastern Finland, Kuopio, Finland; 7Department of Internal Medicine, Kuopio University Hospital, Kuopio, Finland; 8Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland.

**Background and objectives**
Lean mass (LM) has been positively associated with bone mineral density (BMD), but the impact of increased adiposity on bone especially in children is controversial. Several biomarkers, secreeted by adipose tissue, skeletal muscle, or bone, may have important roles in the bone health. Our aim was to study the association of body composition, adipokines, myokines, inflammation-related cytokines, growth factors, and serum 25-hydroxyvitamin D (S-25(OH)D) with BMD in children.

**Methods**
A population sample of 472 prepubertal Finnish children (245 boys) aged 6–8 years was studied. BMD and body composition were determined using whole-body dual-energy x-ray absorptiometry and the biomarkers were analysed from fasting blood samples. The associations of LM, percent of body fat (%BF), and the biomarkers with BMD of the total body without head were analysed and the differences in means of BMD, adjusted for height and age, in gender-specific tertiles of LM and %BF were compared.

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Results
In linear regression models adjusted for gender, age, and height, higher LM (standardized regression coefficient $\beta=0.708$, $P<0.001$), %BF ($\beta=0.361$, $P<0.001$) insulin ($\beta=0.102$, $P=0.010$), homoeostasis model assessment for insulin resistance (HOMA-IR; $\beta=0.087$, $P=0.028$), leptin ($\beta=0.275$, $P<0.001$), and irisin ($\beta=0.079$, $P=0.048$), high-sensitive CRP (hs-CRP; $\beta=0.088$, $P=0.023$), S-25(OH)D ($\beta=0.086$, $P=0.036$), DHEAS ($\beta=0.100$, $P=0.012$), and lower leptin receptor levels ($\beta=-0.260$, $P<0.001$) were associated with higher BMD. Insulin, HOMA-IR, leptin, hs-CRP and DHEAS were not associated with BMD after adjustment for %BF, and HOMA-IR, S-25(OH)D, and DHEAS were not associated with BMD after adjustment for LM. Leptin receptor and irisin were associated with BMD independent of adjustments. Children who were in the lowest tertile of both LM and PRBF had the lowest BMD (mean: 0.695 g/cm$^2$; 95% confidence interval: 0.685–0.704). Children who were in the highest tertile of both LM and %BF had the highest BMD (0.765; 0.755–0.774).

Conclusions
Both LM and %BF had positive associations with BMD in a population sample of mainly normal-weight prepubertal Finnish children. Irisin had a positive and significant association with BMD independent of LM and %BF. The role of these biomarkers as possible mediating factors between body composition and BMD need further research.

Disclosure
The authors declared no competing interests.

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OC15
Fractures in school age children in relation to sex, ethnic background and bone mineral density: the generation R Study
Olja Grgic$^{1,2}$, Carolina Medina-Gomez$^{1,2}$, Katerina Trajanoska$^{1,2}$, Enisa Shevrjoja$^{1,2}$, Fjorda Koromani$^{1,2}$, Andre Uitterlinden$^{1,2}$, Vincent Jaddoe$^{1}$, Eppo Wolvius$^{1,3}$ & Fernando Rivadeneira$^{1,2}$

$^1$The Generation R Study, Erasmus MC, Rotterdam, The Netherlands; $^2$Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; $^3$Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus MC, Rotterdam, The Netherlands.

Objectives
Previous studies indicate that about half of boys and one fourth of girls suffer a fracture before the age of 16 years. Similarly, children of European descent are at a higher risk of fracture at a mean age of six (OR 1.19–2.30; $P<0.003$). One S.D. decrease in TB-BMD was associated with 26% ($Z_{10}=-4.26$, 95%CI 0.102, $P=0.048$), high-sensitive CRP (hs-CRP, $Z_{10}=1.04$, 95%CI 0.84–1.28; $P=0.048$), DHEAS ($Z_{10}=0.102$, $P=0.275$), Irvine ($Z_{10}=-0.088$, $P=0.023$), and lower leptin receptor levels ($Z_{10}=-0.260$, $P<0.001$) were associated with higher BMD. Insulin, HOMA-IR, leptin, hs-CRP and DHEAS were not associated with BMD after adjustment for %BF, and HOMA-IR, S-25(OH)D, and DHEAS were not associated with BMD after adjustment for LM. Leptin receptor and irisin were associated with BMD independent of adjustments. Children who were in the lowest tertile of both LM and PRBF had the lowest BMD (mean: 0.695 g/cm$^2$; 95% confidence interval: 0.685–0.704). Children who were in the highest tertile of both LM and %BF had the highest BMD (0.765; 0.755–0.774).

Conclusions
Both LM and %BF had positive associations with BMD in a population sample of mainly normal-weight prepubertal Finnish children. Irisin had a positive and significant association with BMD independent of LM and %BF. The role of these biomarkers as possible mediating factors between body composition and BMD need further research.

Disclosure
The authors declared no competing interests.

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OC16
Pediatric hypophosphatasia – a retrospective single-center chart review of 50 children
Marius Vogt$^1$, Hermann Josef Girschick$^2$, Annette Holt-Wieden$^3$, Lothar Seefried$^1$, Franz Jakob$^1$ & Christine Hofmann$^1$

$^1$Pediatric Rheumatology and Osteology, University Children’s Hospital Wuerzburg, Wuerzburg, Germany; $^2$Children’s Hospital, Vivantes Hospital im Friedrichshain, Berlin, Germany; $^3$Orthopedic Department, Orthopedic Center of Musculoskeletal Research, University of Wuerzburg, Wuerzburg, Germany.

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

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

Results
The cohort comprises 4 (8%) perinatal, 17 (34%) infantile, 28 (56%) childhood and 1 (2%) Odonto-HPP. 2 patients were deceased at the time of the data collection. Diagnosis was based on available characteristic clinical symptoms (72%), available low AP activity (88%), accumulating substrates (56%) and X-ray findings (36%). Genetic analysis was performed in 47 patients (33 compound heterozygous) allowing investigations on genotype-phenotype correlations. Median age at first clinical symptoms was 3 months (min 0, max 107) and median time to diagnosis was 13 months (min 0, max 103) based on anamnestic data. Common symptoms included: delay of motor development (in 38 cases, 76%), bone pathologies (36, 72%), failure to thrive (31, 62%), premature loss of teeth (31, 62%) and musculoskeletal pain (24, 48%). Fourteen patients started medical treatment with asfotase alfa.

Conclusion
Findings reported support our clinical impression of a chronic multi-systemic disease with often delayed diagnosis. Our natural history information provides detailed insights into the prevalence of different symptoms which can help to improve and to shorten diagnostics and thereby lead to an optimised medical care.

Disclosure
The authors declared no competing interests.

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OC17
Vertebral Fractures in children with chronic inflammatory and/or disabling conditions: the SNAP study
Nicola Crabtree$^{1,2}$, Wolfgang Hogler$^{1,2}$, Dee Chapman$^3$, Jacky Walford$^3$ & Nicholas Shaw$^{1,2}$

$^1$Birmingham Women’s and Children’s NHS Foundation Trust, Birmingham, UK; $^2$Institute of Metabolism & Systems Research, Birmingham, UK; $^3$University Hospital Birmingham NHS Foundation Trust, Birmingham, UK.

Objectives
The SNAP study is a prospective fracture study of children with chronic inflammatory and/or disabling conditions. The overall study aim is to assess causal links between body-size adjusted bone density and low trauma fracture. Methods
330 children aged 5–18 years were recruited from seven disease groups namely; acute lymphoblastic leukaemia (ALL), rheumatological disease, inflammatory bowel disease, cystic fibrosis, coeliac disease, Duchenne muscular dystrophy (DMD) and cerebral palsy. At baseline, bone density by DXA (lumbar spine (LS) and total body less head (TLBH BMD)), forearm pQCT (trabecular density at the 4% site (Trab vBMD)), and ratio of bone/muscle area at the 66% site (Radius BA/MA), hand radiographs (Bone health index (BHI), BoneXpert), lateral spinal radiographs and medical history were assessed. A threshold of

Disclosure
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Z-score $< -2.0$ was set to dichotomise the bone density Z-scores and used in conjunction with a binary prediction model to assess diagnostic accuracy.

**Results**

Spinal radiographs identified 71 children (21.5%) with vertebral fractures, with highest incidence for children with ALL (26/51) and DMD (14/42) ($P < 0.001$). Steroid exposure, back pain and immobility were reported in 50, 37 and 14% of patients, respectively. Bone density Z-scores were significantly lower in the fracture group for LS BMAD, Trab vBMD, Radius BA/MA, and BHI. The variables most predictive of vertebral fracture were Trab vBMD, BHI and BA/MA ($P < 0.05$), with corticosteroid exposure and back pain also significant (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio (96%CI)</th>
<th>Sensitivity (96%CI)</th>
<th>Specificity (96%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trab vBMD</td>
<td>3.4 (1.6–7.0)</td>
<td>0.29 (0.17–0.41)</td>
<td>0.89 (0.85–0.93)</td>
</tr>
<tr>
<td>Hand BMH</td>
<td>2.5 (1.4–4.6)</td>
<td>0.45 (0.36–0.57)</td>
<td>0.77 (0.71–0.82)</td>
</tr>
<tr>
<td>Radius BA/MA</td>
<td>2.3 (1.0–5.3)</td>
<td>0.20 (0.09–0.32)</td>
<td>0.60 (0.56–0.64)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>1.7 (1.0–2.9)</td>
<td>0.61 (0.49–0.72)</td>
<td>0.53 (0.47–0.60)</td>
</tr>
<tr>
<td>Back Pain</td>
<td>1.7 (1.0–2.8)</td>
<td>0.46 (0.35–0.58)</td>
<td>0.68 (0.60–0.77)</td>
</tr>
<tr>
<td>TBH BMAD</td>
<td>1.4 (0.8–2.6)</td>
<td>0.27 (0.17–0.38)</td>
<td>0.75 (0.69–0.84)</td>
</tr>
<tr>
<td>LS BMAD</td>
<td>1.3 (0.5–3.2)</td>
<td>0.10 (0.03–0.17)</td>
<td>0.80 (0.74–0.85)</td>
</tr>
<tr>
<td>Immobility</td>
<td>1.0 (0.5–2.1)</td>
<td>0.14 (0.06–0.22)</td>
<td>0.86 (0.81–0.90)</td>
</tr>
</tbody>
</table>

**Conclusion**

Disease itself, back pain and corticosteroid exposure are significantly associated with risk of vertebral fracture. However, the variables most predictive of vertebral fracture were low trabecular density measured by pQCT and BHI by BoneXpert. Evidence of the predictive power of these measurements will only be confirmed with future follow-up of this group. Funded by NHR Clinical Development Fellowship (HCS/P10/009).

**Disclosure**

The authors declared no competing interests.

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**OC18**

**Stimulation of angiogenesis and osteogenesis by enhanced preosteoclast platedet growth factor type BB attenuates glucocorticoid-induced osteoporosis in growing mice**

Ping Yang1,2, Yan Wang1,2, Zhuying Xia1,3, Xucao1 & Janet Crane1

1Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2Shandong University, Jinan, China; 3Second Xiangya Hospital, Central South University, Changsha, Hunan, China.

Survival of chronic diseases in childhood is often achieved utilizing glucocorticoids. However, the survival comes at a cost to the growing skeleton, resulting in impairment in the acquisition of peak bone mass and is the major etiology of secondary osteoporosis in children. We recently found that preosteoclasts secrete increased trap positive cells in both the primary and secondary spongiosa with increased bone volume seen in the Csk$^-/-$ mice relative to wild type littermates at 2, 4, and 8 weeks of age. Osteoprogenitors were similarly increased at all time points, with a more significant difference noted in the secondary spongiosa compared to primary spongiosa. We then established a glucocorticoid-induced mouse model in young Csk$^-/-$ mice and wild type littermates with prednisolone at 10 mg/m2 per day beginning at 2 weeks of life and continued for 4 weeks. Overall, the osteoporotic phenotype as assessed by microcomputed tomography noted in wild type mice treated with prednisolone relative to vehicle treatment was prevented in Csk$^-/-$ mice treated with prednisolone relative to vehicle. Trap staining and co-staining with PDGF-BB demonstrated that osteoclast numbers decreased in response to prednisolone, whereas loss of ctsk in Csk$^-/-$ mice was largely by increasing Trap-positive mononuclear cells in the bone marrow. Serum concentrations of PDGF-BB showed a similar pattern. The decreased angiogenesis and osteogenesis, as assessed by H-type vessels and osteocalcin staining, observed in wild type mice treated with prednisolone were attenuated in Csk$^-/-$ mice treated with prednisolone. These data suggest that inhibition of osteoclast resorption that preserves osteoclast coupling factors may be a potential preventive treatment strategy against glucocorticoid-induced osteoporosis in the growing skeleton.

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**Funding**

Pediatric Endocrine Society Clinical Scholar Award and US National Institute of Health 1K08AR064833.

**Disclosure**

The authors declared no competing interests.

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**OC19**

Mesenchymal stromal stem cells in pediatric orthopedic oncology, focus on osteosarcoma

Valérie Trichet1, Louis-Romée Le Nail1,2, Romain Guillo1, Pierre Layrolle1 & Françoise Redini1

1INSERM Université de Nantes, Nantes, France; 2Centre Hospitalier Régional Universitaire de Tours, Service de Chirurgie Orthopédique et Traumatologique, Tours, France.

**Introduction**

Conventional therapy of osteosarcoma, a primary malignant bone tumor, includes surgical excision with wide resection, which leads to physical and aesthetic defects. Allografts for reconstruction of bone and joints and adipose tissue autologous grafts for soft tissue defects can be supplemented with mesenchymal stromal/stem cells (MSCs). Additionally MSCs may be used in tumor-targeted cell therapy. However MSCs may have adverse effect on osteosarcoma development, being stromal component of the tumor microenvironment.

**Methods**

MSC-like cells were derived from the bone marrow of healthy or osteosarcoma patients and from osteosarcoma biopsies (OS-derived stromal cells). After characterization (ability to form clones, surface markers and differentiation potential), MSC-like cells were used to produce conditioned media that were tested on osteosarcoma cells for osteoclastigenesis-condition or in non-adherent spheres, favouring propagation or quiescent stage, respectively. Proliferation and cell cycle were analyzed. MSC-like cells were co-injected with osteosarcoma (OS) cells in nude mice. Additionally MSCs have been modified to express tumour-necrosis-factor related apoptosis inducing ligand (TRAIL). Results: MSCs secreted factors activated osteosarcoma cell cycle from G1 to mitosis phases, but did not promote the transition from quiescent G0 to G1 phases. OS-derived stromal cells showed similar immature markers than bone marrow MSCs, but a higher osteogenic differentiation potential, a higher clone-forming potential and a different oxidative metabolism. They had a normal genotype that distinguished them from tumor cells. These stromal cells alone did not induce tumor in immunodeficient mice (SCID). However, when co-injected with OS cells in nude mice, they increased the local tumor development and for one patient, they increased the metastatic progression to lungs. When TRAIL expressing MSCs were co-injected with OS cells in nude mice, the tumor development was similar to that observed with OS cells alone, but it was not inhibited by TRAIL expression.

**Conclusion**

MSC-like cells may be safe in reconstructive surgery following tumor treatment as they did not change the quiescent state of dormant osteosarcoma cells. In contrast, they accelerated cell cycle of proliferating osteosarcoma cells in vitro and in vivo and they did not appear as good candidates for osteosarcoma cell therapy.

**Disclosure**

The authors declared no competing interests.

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**OC20**

Autoimmune hyperphosphatemic tumoral calcinosis

Mary Scott Ramnitz1, Peter Burhobo1, Daniela Egli-Spichtig2, Farzana Perwad1, Christoph Romeo3, Shojo Ichiikawa4, Emily Farrow4, Michael Econs5, Lori Guthrie1, Rachel I. Gafni1 & Michael T. Collins1

1National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA; 2University of California San Francisco, San Francisco, CA, USA; 3Icahn School of Medicine at Mount Sinai, New York, NY, USA; 4Indiana University School of Medicine, Indianapolis, IN, USA; 5Children’s Mercy Hospital, Kansas City, MO, USA.

**Background**

Hyperphosphatemic familial tumoral calcinosis (HPT),hyperostrosis-hyperphosphatemia syndrome (HPS) is an autosomal recessive disorder due to deficiency
activating mutations in Fibrous dysplasia is a rare bone and endocrine disorder resulting from somatic mutation of \( \mathbf{FGF23} \), \( \mathbf{GALNT3} \), or \( \mathbf{KLOTHO} \) have been identified as causative for HFTC/HHS. Here we present the first case of autoimmune hyperphosphataemic tumoral calcinosis.

Case
A 6-year old boy presented with right hip pain and a firm lesion on the lateral aspect of the hip, biopsy-confirmed as tumoral calcinosis. Biochemical evaluation showed hyperphosphataemia (7.2 mg/dl; nl 3–5.7), increased TRP (98%), and inappropriately normal 1.25D (68 pg/ml; nl 24–96). Intact and C-terminal FGF23 were elevated at 9600 pg/ml (nl 22–63) and 28,500 RU/ml (nl <230), respectively, findings suggestive of FGF23 resistance due to a \( \mathbf{KLOTHO} \) or possibly an \( \mathbf{FGFR1} \) mutation. However, no causative mutation was identified in \( \mathbf{GALNT3}, \mathbf{FGF23}, \mathbf{KLOTHO} \), or \( \mathbf{FGFR1} \). Exome sequencing did not reveal any variants that could explain the phenotype. The subject was prescribed a low phosphate diet, sevelamer and acetazolamide, with subsequent decrease in blood phosphorus and tumor size. Eight months later, he presented with a 2-week history of polyuria and polydipsia. Blood glucose was 433 mg/dl, insulin 4 mcU/ml, and hemoglobin A1c 10.7%, with positive urine antigen two antibodies. Given this new diagnosis of type 1 diabetes, investigation was undertaken to evaluate for possible autoimmune causes of his tumoral calcinosis. Luciferase immunoprecipitation systems (LIPS) were used to evaluate autoantibodies against FGF23, FGF1, KLOTHO and several other autoantigen targets. LIPS revealed significantly elevated autoantibodies against FGF23 in the patient that were over 50-fold higher than healthy controls and other subjects with HFTC/HHS. In contrast, there were no detectable autoantibodies against FGF1, or KLOTHO. FGF23 functional assays showed anti-FGF23 autoantibodies in the patient’s plasma blocked FGF23 downstream signaling in a dose-dependent manner.

Conclusion
This is the first reported case of autoimmune hyperphosphataemic tumoral calcinosis with autoantibodies against FGF23. Identification of this novel pathophysiology suggests that immunomodulatory therapy may be an effective treatment.

Disclosure
The authors declared no competing interests.

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**OC22**

**Type I collagen C-propeptide cleavage deficiency increases bone mineralization and alters bone cell differentiation**

Aileen Barnes, Joseph Perosky, Stéphane Bouc, M. Helen Rajpar, Basma Khoury, Mary Ann Weis, Klaus Klaushofer, Paul Roschger, David Eyré, Nadja Fratzl-Zelniker, Kenneth Kozloff & Joan Marini

1NICHD/NIH, Bethesda, MD, USA; 2University of Michigan, Ann Arbor, MI, USA; 3Ludwig Boltzmann Institute of Osteology, Vienna, Austria; 4University of Washington, Seattle, WA, USA.

High Bone Mass (HBM) osteogenesis imperfecta (OI) is caused by dominant mutations in the C-propeptide cleavage site of COL1A1 or COL1A2, characterized by bone hypermineralization. To elucidate the role of C-propeptide processing in bone mineralization and development, we generated heterogeneous HBM mice with both residues (Ala-Asp) of the COL1A1 cleavage site substituted (Thr-Asn) to prevent processing by BMP1. Two, 6- and 12-month WT and HBM bones were examined for histology, mineralization, mechanics, and cell differentiation. HBM mice are smaller than WT in weight and length throughout life. Their femoral extracts contain pC-collagen and increased monomeric COL1A1 C-propeptide, resulting in a ‘barbed-wire’ appearance to bone collagen fibrils, thin cortices and decreased BTV/TV. By 6 months, HBM mice hind limb joints fuse with severe osteoarthrits. HBM femora have decreased stiffness, yield and fractures and increased implant with age. These femora are also extremely brittle; post-yield displacement is ~15% of WT (P<0.001). HBM femora have decreased collagen content (59% of WT) with an increase in mature (HP) and total (HP+LP) crosslinks. Femoral aBMD is decreased at 2-months but is near normal (95%) at 1 year. On µ-CT, HBM cortical and trabecular TMD are normalized at 1 year. Using quantitative backscattered electron imaging (qBEI) to assay mineral content, HBM cortical bone had increased CaMean, CaPeak and CaHigh at 2- and 6- and 12-months compared to WT. HBM CaPeak increased significantly between 6- and 12-months, although WT levels peaked at 6 months. There is a complementary decrease in HBM percent body fat at 6- and 12-months. Bone cell differentiation was also affected in HBM. Femoral Bglap transcripts are decreased, as was osteoblast collagen secretion. HBM femora had fewer osteocyte lacunae (P<0.01) but increased lacunar area at 2-, 6- and 12-months (P<0.001). HBM serum TRAP and PINP were significantly increased, consistent with increased femoral transcripts of Ctsk, Asp5 and the Rankl/Opn ratio. Murine HBM bone mineralization is increased throughout life and increases with age, even after WT mineralization has peaked, raising concerns for long-term patient status. Alterations in multiple bone cell populations support a putative C-propeptide trimer signalling function, influencing bone matrix and mineralization.

Disclosure: The authors declared no competing interests.

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Objective

Children with hypophosphatasia (HPP) treated with asfotase alfa in a Phase 2 study (NCT00952438) and its open-label extension (NCT01203826) experienced significant improvements in skeletal mineralization and physical function that were sustained through 5 years of treatment (1). Herein, we report data from these studies with a maximum of 7 years of treatment.

Methods

Children with HPP aged 6–12 years at baseline received asfotase alfa (3 mg/kg per wk subcutaneously, later increased to 6 mg/kg per wk). Radiographs of the hand/wrist and knees were assessed using the Radiographic Global Impression of Change (RGI-C) scale and Rickets Severity Scale (RSS). Additional outcomes included growth, functional ability/disability (6-Minute Walk Test (6MWT); Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2)), Strength and Agility Composite Standard Score; Child Health Assessment Questionnaire Disability Index (CHAQ-DI), and safety.

Results

All 12 children who entered the extension phase received asfotase alfa for 7 years. Final RGI-C and RSS scores measured improved HPP-related skeletal manifestations that were sustained through end of study (Table 1). Improved growth (height/weight Z-scores) and function (6MWT, BOT-2 Strength and Agility, CHAQ-DI) were also sustained through end of study (Table 1). Mild-to-moderate injection site reactions were reported in all patients (e.g., erythema, macule, lipohypertrophy); 1 event of injection site atrophy was assessed as severe. No serious adverse events, including deaths, were reported.

Conclusion

Improvements in HPP-related skeletal manifestations, growth, and functional ability with asfotase alfa treatment in children with HPP were sustained for up to 7 years. Treatment was generally well tolerated.

Disclosure

This study was sponsored by Alexion Pharmaceuticals, Inc. MPW and WHM are clinical trial investigators and have received honoraria, travel support, and research grant support from Alexion Pharmaceuticals, Inc. CRG is a clinical trial investigator and has received honoraria, travel support, and research grant support from Alexion Pharmaceuticals, Inc., for consulting and participating on advisory boards. SM and AED are employees of and may own stock/options in Alexion Pharmaceuticals, Inc., which sponsored the study.

Reference


Table 1

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Reference range for healthy peers</th>
<th>Baseline (n=13a)</th>
<th>Last observation (n=13a)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGI-C</td>
<td>NAa</td>
<td>2.8 (2.0, 3.0)</td>
<td>6.7 (5.0, 8.1)</td>
<td>0.0005</td>
</tr>
<tr>
<td>RSS</td>
<td>0–no rickets</td>
<td>3.0 (3.0, 4.0)</td>
<td>3.1 (2.0, 4.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>Height Z-score</td>
<td>2b–2c–2d</td>
<td>−1.26 (−6.0, 0)</td>
<td>−0.69 (−5.4, 0.4)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Weight Z-score</td>
<td>2b–2c–2d</td>
<td>−1.21 (−8.2, 2.3)</td>
<td>−0.15 (−5.4, 2.7)</td>
<td>0.0004</td>
</tr>
<tr>
<td>FHPIC</td>
<td>% predicted</td>
<td>61% (20%, 85%)</td>
<td>61% (20%, 85%)</td>
<td>0.0005</td>
</tr>
<tr>
<td>BOT-2, Strength and Agility Composite Standard Score</td>
<td>40–60</td>
<td>28 (20, 57)</td>
<td>51 (24, 62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHAQ-DI</td>
<td>0=no disability</td>
<td>1.2 (0.2, 2.5)</td>
<td>0.0 (0.5)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

aOne patient withdrew after 1 month for elective surgery. bBaseline value not applicable (NA) because RGI-C descreases from baseline; RGI-C range: 3 (worst) to 0 (complete/mild disability). cWilcoxon signed-rank test assessing if median is 0. dWilcoxon signed-rank test assessing if median change from baseline is 0. eWilcoxon signed-rank test assessing if median change from baseline is 0. fWilcoxon signed-rank test assessing if median change from baseline is 0. gCHAQ-DI range: 0 to 3, with higher scores indicating greater disability. nD—not determined.

OC25

Biochemical and physical function outcomes after 5 years of treatment with asfotase alfa in adolescents and adults with hypophosphatasia: phase 2 study results

Priya S. Kishnani1, Cheryl Rockman-Greenberg2,3, Andrew E. Denker4, Scott Moseleya & Michael P. Whytene

1Duke University Medical Center, Durham, NC, USA; 2Rady Faculty of Health Sciences, Max Rady College of Medicine, The University of Manitoba, Winnipeg, Manitoba, Canada; 3Children’s Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada; 4Alexion Pharmaceuticals, Inc., New Haven, CT, USA; 5Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children-St. Louis, St. Louis, MO, USA.

Objectives

To evaluate safety and efficacy after 5 years of treatment with asfotase alfa in adolescents and adults with hypophosphatasia (HPP) in a Phase 2, open-label, randomized, dose-ranging study (NCT01163149).

Methods

Treatment with subcutaneous asfotase alfa 0.3 or 0.5 mg/kg per d was compared with no treatment (control) for 6 months in patients aged 13–66 years. After 6 months, all patients (treatment and control groups) received active treatment at 0.5 mg/kg per d; dose was increased after 6–12 months to 1 mg/kg 2–6 times/wk (lowest approved dose). The primary safety outcome was tolerability. Coprimary efficacy outcomes included median change at 6 months in plasma inorganic pyrophosphate (Pi) and pyridoxal-5'-phosphate (PLP) levels. Other metrics included 6-Minute Walk Test (6MWT) and Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2). Data from treatment groups were pooled and reported below as median (min, max).

Results

The study randomized 19 patients (6 aged 13–18 years; 13 aged ≥18 years; 15/19 (79%) completed 5 years of treatment. One patient withdrew due to injection-site hypersensitivity and anaphylactoid reaction (1 episode each). No deaths occurred. The most common treatment-emergent adverse events were...
Objectives
In XLH, FGF23-mediated hypophosphatemia leads to defective bone mineralization and rickets. Investigational product KRN23 binds FGF23 and inhibits its activity. The objective of this Phase 2 study was to evaluate the safety and efficacy of KRN23 in 52 children with XLH (ages 5–12 years, n=52).

Methods
Patients were randomized to receive KRN23 biweekly (Q2W) or monthly (Q4W) by SC injection. KRN23 dose was titrated (maximum 2 mg/kg) to achieve age-appropriate serum phosphate (Pi) concentrations which were measured Q2W. Efficacy endpoints included change in rickets severity (Thacher Rickets Severity Score [RSS]) and Radiographic Global Impression of Change (RGI-C); −3=severe worsening; 0=no change; +3=complete healing), pharmacodynamic parameters, and growth. The primary analysis was at Week (Wk) 40; extended analysis was at Wk64.

Results
Rickets was evident at baseline (mean RSS 1.8) despite ~7 years of prior oral phosphate/active vitamin D therapy. Serum Pi increased in all patients to near normal levels (mean increase from baseline to Wk38 was 0.33 mmol/l; P<0.001) and was more stable in Q2W group. No hyperphosphataemia occurred. At Wk40, mean RSS improved by 61% for the Q2W group, 37% for Q4W, and 50% overall (P<0.001 all groups). In subjects with higher-severity rickets (baseline RSS ≥1.5; N=34), mean RSS improved by 71% for Q2W, 48% for Q4W, and 61% overall (P<0.0001 all groups). At Wk40, mean RGI-C scores of +1.72 for Q2W, +1.41 for Q4W, and +1.56 overall (P<0.0001 all groups) also indicated improvement. In the subset with baseline RSS ≥1.5, substantial healing of rickets was observed with a mean RGI-C+ 2.04 for Q2W; +1.78 for Q4W, and +1.91 overall (P<0.0001 all groups). Efficacy was sustained at Wk64. Most treatment-related adverse events (AEs) were mild. Transient injection site reactions (33%) were most frequent. One child experienced a serious AE, was hospitalized for fever/muscle pain that resolved, and continues in the trial. No clinically meaningful changes occurred in serum or urine calcium, serum iPTH, or renal ultrasounds.

Summary
KRN23 improved serum Pi and rickets in children with XLH, and was generally safe and well tolerated.

Disclosure
Hogler: travel and consulting fees from Ultragenyx; Portale: travel and advisory panel for Ultragenyx; Carpenter: grant support and travel from Ultragenyx; Imel, Boot, Linglart, van’t Hoff: travel and consulting fees from Ultragenyx; Padidela: consulting.

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Poster Presentations
Bone mass tracks into teenage years

Maria Ratne1, Malene Heideman2, Anlers Schou3, Jens Ole Laursen2,
Niels Wedderkopp3, Steffen Husby4 & Christian Mølgaard1
1Hans Christian Andersen Children’s Hospital, Odense University Hospital,
Odense, Denmark; 2Emergency Department, Hospital of Southern Jutland,
Aabenraa, Denmark; 3Research of Childhood Health, Faculty of Health
Sciences, University of Southern Denmark, Odense, Denmark.

Objectives
Bone mass development in childhood and adolescence is crucial for peak bone
mass (PBM) and low PBM may lead to osteoporosis later in life. The stability of
bone mineral status through childhood and adolescence is known as tracking. The
objective of this study is to determine the degree of tracking according to bone
mass from pre-puberty into puberty in healthy Danish children.

Methods
190 healthy Danish children (97 boys) with mean age 9.25 years (range 8.0–11.1
years) at baseline (2008) were followed for 7 years. Whole body DXA-scan
and anthropometric measurements were performed three times in 2008, 2010 and
2015 respectively. Children were aged 14–17 years at the last follow-up and all
were prepubertal at the start of the study. Intermittent longitudinal DXA values
were used for the analyses, and the study is based on the method described
previously.

Results
Performance in vertical jump (except for femoral neck aBMD and narrow neck
width) and standing long jump (except for narrow neck width) was positively
associated with all bone outcomes in models 1 and 2 (P < 0.006). In model 3, most
previous associations disappeared except those between standing long jump and
total hip and trochanter aBMD (P = 0.004 and 0.003, respectively), which were
slightly attenuated.

Conclusion
VPA did not explain muscular fitness associations on bone outcomes and both
muscular fitness and the skeletal benefits of sport participation appear to be a
function of lean mass in young males.

Funding Sources
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Union Seventh Framework Programme (FP7/2007-2013) under grant agreement
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Abstract withdrawn.

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Disclosure
The authors declared no competing interests.
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P006
Bone density and body composition in post-pubertal adolescents treated with GnRH analogues in a gender identity development service
Xanthippe Eseteopoulo1, Nadia Amin & Talat Mushtaq
Department of Paediatric Endocrinology, Leeds Teaching Hospitals NHS Trust, Leeds, West Yorkshire, UK.

Objectives
Gender Identity Disorder (GID) occurs when a person’s gender identity differs from their biological sex, causing distress. GID presenting in childhood can dissipate at puberty. If it persists, they may progress to physical interventions. This involves the use of a GnRH analogue (GnRHa) for one year following sex hormones.

Methods
As part of the clinical assessments, adolescents had body composition measurements and annual bone density scans. Two related studies were undertaken; 1) Cross sectional study to compare the body composition data between bone densitometry (iDXA) vs Tanita measurements (85 children) and 2) Longitudinal changes in iDXA bone density and body composition in 32 adolescents who had been on GnRHa for a year.

Results
The cross-sectional pre-GnRHa study included 85 patients (30 male, 55 female) with a mean age of 16.2 years (range 14.4–17.9). The mean height, weight and BMI SDS were 0.0, 0.9 and 0.43. Investigations were performed at 0.2 (1.0), TBLH (0.1) and 0.1 years. The iDXA recorded 5.4% more body fat (mean 4.6 kg, s.d. 3.2) and 8.2% less lean mass (mean 7 kg, s.d. 3.5) than the Tanita scales. Following sub-analysis, the iDXA recorded 3.3 kg more body fat in females and 6.9 kg in males, with 5.5 kg and 9.6 kg less lean mass.

Conclusions
The present results suggest that, after a weight loss, geometric indices of bone strength at weight bearing sites in young males are lower than expected to allow positive adaptation of bone parameters. Moreover, reduced load appeared to cause higher fragility at the NN than at the shaft. Conflict of interest
Nothing to declare.
Disclosure
The authors declared no competing interests.
DOI: 10.1530/boneabs.6.P007

P008
Cardiorespiratory fitness, bone mineral density and hip geometry in young males: the PRO-BONE study
Esther Ubago-Guisado1, Dimitris Vlachopoulos2, Augusto César de Moraes1, 3, 4, Ana Torres-Costos3, Kelly Wilkinson2, Brad Metcalf2, 6, Daniel Courteix1,3

Objective
The main aim was to evaluate associations between cardiorespiratory fitness and bone outcomes, including hip geometry estimates in young males.

Methods
One hundred twenty one males (13.1 ± 0.1 years) were included: 41 swimmers, 37 footballers, 29 cyclists and 14 non-athletes. Lean mass, areal bone mineral density (aBMD) and hip structural estimates were measured using dual-energy X-ray absorptiometry. Relationships of physical fitness test (20 m shuttle run test) with bone outcomes, including hip geometry estimates in young males were analysed using three regression models: Model 1, adjusted for age and stature; Model 2, model 1 + height; and model 3, model 2 + lean mass. Bonferroni correction was applied and only values of P < 0.006 were considered statistically significant.

Results
In 20 m shuttle run test (except for narrow neck width) was positively associated with all bone outcomes in models 1 and 2 (P < 0.006). Interestingly, all significant associations but the one at lumbar spine and femoral neck aBMD (P = 0.097 and 0.008, respectively) remained significant after adding lean mass as a covariate (model 3).

Conclusion
Most of the associations found between cardiorespiratory fitness and bone outcomes in young males do not seem to be a function of neither VPA nor lean mass.

Funding sources
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1Department of Pediatrics, Klatovy Hospital, Klatovy, Czech Republic; 2Department of Clinical Biochemistry, Klatovy Hospital, Klatovy, Czech Republic; 3Bone Disease Center, Klatovy Hospital, Klatovy, Czech Republic.

Discussion

Neonatal hypocalcemia is defined as S-Ca <2 mmol/l in fullterm newborns and <1.75 mmol/l in preterm newborns. Neonatal hypocalcemia is either early onset (<3 days of age) or late onset (>3 days of age). Newborns with hypocalcemia are often asymptomatic, but may present with hypotonia, apnea, poor feeding, jitteriness, seizures, cardiac failure. Signs of hypocalcemia rarely occur unless S-Ca drops below 1.75 mmol/l. Case presentation and clinical management

We present three boys (two with gestational age 39 weeks, one 36 weeks; none of them with either asphyxia or sepsis) with mild hypotonia, where S-Ca in the range of 1.67–1.9 mmol/l was detected within the first three days of life, together with hyperphosphatemia (S-P 2.5–2.6 mmol/l), normonagnesemia (S-Mg 0.77–0.88 mmol/l), normal alkaline phosphatase activity (S-ALP 2.8–4.5 kKat/l) and high S-PTH (40–51 pg/ml; normal 5–28). In spite of i.v. calcium supplementation and increase in S-Ca within 2–6 days, the elevated S-PTH persisted until day 6, together with normal or low-to-normal S-Ca, high or normal-to-high S-P and no increases in S-ALP. The mothers’ S-Ca, P, Mg, ALP, PTH levels were within normal reference ranges. 

Discussion

Neonatal hypocalcemia can be a result of hypoparathyroidism (transient or primary), increased serum calcitonin, sepsis, asphyxia, hepatopathy, hypomagnesemia, high phosphate load and, rarely, transient neonatal hypoparathyroidism (transient resistance to biological actions of parathyroid hormone – PTH; 38 children reported so far). With regard to Table 1, the diagnosis of transient pseudohypoparathyroidism (due to immaturity of PTH-receptors) is suggestive and highly probable in these three neonates.
Table 1 Differential diagnosis of neonatal hypocalcemia.

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Disclosure
The authors declared no competing interests.
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P012
Determinants of bone outcomes in adolescent athletes at baseline: The PRO-BONE study

Luis Gracia-Marco1,3, Dimitris Vlachopoulos1, Esther Ubago-Guisado2, Alan R Barker1, Ioannis G Fatouros1, Alexandra Avloniti1, Karen K Knap1, Luis A Moreno1 & Craig A Williams1
1University of Exeter, Exeter, UK; 2University of Castilla-La Mancha, Toledo, Spain; 3University of Granada, Granada, Spain; 4University of Zaragoza, Zaragoza, Spain; 5University of Thessaly, Trikala, Greece; 6Democritus University of Thrace, Komotini, Greece.

Objectives
The determinants of areal bone mineral density (aBMD) and hip geometry estimates in adolescent athletes are poorly understood. This study aimed to identify the determinants of aBMD and hip geometry estimates in adolescent male athletes.

Methods
One hundred twenty one males (13.1 ± 0.1 years) were measured: 41 swimmers, 37 footballers, 29 cyclists and 14 controls not engaged in these sports more than 3 hours per week in the last three years. Bone mineral content (BMC) was measured using dual-energy x-ray absorptiometry (DXA) at the femoral neck and lumbar spine. Bone geometry estimates at the femoral neck were measured using hip structural analysis (HSA) and bone microarchitecture of the lumbar spine using trabecular bone score (TBS). Bone formation was measured using procollagen type 1 amino terminal propeptide (P1NP), bone resorption using isomer of the Carboxy-terminal telopeptide of type 1 collagen (CTX-1) and blood markers of total calcium serum and 25 hydroxyvitamin D [25(OH)D]. Moderate to vigorous physical activity (MVPA) was measured for 7 days using accelerometer. Bone acquisition was compared after controlling for age, height, lean mass, MVPA and baseline bone outcomes.

Results
Footballers had significantly (P<0.05) higher BMC acquisition at the lumbar spine and femoral neck compared to cyclists and at the lumbar spine compared to swimmers. Footballers had significantly (P<0.05) higher acquisition in all HSA outcomes at the femoral neck compared to cyclists, and significantly (P<0.01) higher acquisition in TBS score at the lumbar spine compared to cyclists and swimmers. There were no significant differences in bone acquisition between swimmers and cyclists. At T1 footballers had significantly higher P1NP compared to swimmers and cyclists, and 25(OH)D was significantly higher in footballers and cyclists compared to controls and swimmers.

Conclusions
This longitudinal study demonstrates for first time superior changes in bone mass, geometry and metabolism in adolescent male footballers compared to swimmers and cyclists. Therefore, this study aimed to investigate the longitudinal differences in bone acquisition and bone metabolism between adolescent males participating in osteogenic (football) and non-osteogenic (swimming, cycling) sports compared to a control group over 1 year.

Methods
A total of 116 adolescent males aged 12–14 years at baseline (T0) were measured and followed up for 1 year (T1): 37 swimmers, 37 footballers, 29 cyclists and 14 controls not engaged in these sports more than 3 hours per week in the last three years. Bone mineral content (BMC) was measured using dual-energy x-ray absorptiometry (DXA) at the femoral neck and lumbar spine. Bone geometry estimates at the femoral neck were measured using hip structural analysis (HSA) and bone microarchitecture of the lumbar spine using trabecular bone score (TBS). Bone formation was measured using procollagen type 1 amino terminal propeptide (P1NP), bone resorption using isomer of the Carboxy-terminal telopeptide of type 1 collagen (CTX-1) and blood markers of total calcium serum and 25 hydroxyvitamin D [25(OH)D]. Moderate to vigorous physical activity (MVPA) was measured for 7 days using accelerometer. Bone acquisition was compared after controlling for age, height, lean mass, MVPA and baseline bone outcomes.

Results
Footballers had significantly (P<0.05) higher BMC acquisition at the lumbar spine and femoral neck compared to cyclists and at the lumbar spine compared to swimmers. Footballers had significantly (P<0.05) higher acquisition in all HSA outcomes at the femoral neck compared to cyclists, and significantly (P<0.01) higher acquisition in TBS score at the lumbar spine compared to cyclists and swimmers. There were no significant differences in bone acquisition between swimmers and cyclists. At T1 footballers had significantly higher P1NP compared to swimmers and cyclists, and 25(OH)D was significantly higher in footballers and cyclists compared to controls and swimmers.

Conclusions
This longitudinal study demonstrates for first time superior changes in bone mass, geometry and metabolism in adolescent male footballers compared to swimmers and cyclists.

Funding sources
The research leading to these results has received funding from the European Union Seventh Framework Programme ([FP7/2007-2013] under grant agreement n°. PCIG13-GA-2013-618496.

Disclosure
The authors declared no competing interests.
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P014

Longitudinal changes in bone mineral content and bone stiffness in adolescent male athletes: The PRO-BONE study

Dimitris Vlachopoulos1,3, Alan R Barker2, Craig A Williams4, Esther Ubogo-Guasado2, Robinson Ramirez-Velez5, Antonio Garcia-Hermoso6, Jose Castro Pinero7, Francisco B Ortega8, Jonatan R Ruiz9, Luis A Moreno1, & Luis Gracia-Marco1,3

1University of Exeter, Exeter, UK; 2University of Castilla-La Mancha, Toledo, Spain; 3Universidad del Rosario, Bogota, Colombia; 4University of Santiago, Santiago, Chile; 5University of Cadiz, Cadiz, Spain; 6University of Granada, Granada, Spain; 7University of Zaragoza, Zaragoza, Spain.

Objectives

Bone development can be enhanced by exercise during growth, but not all types of exercise may be beneficial. Weight bearing and non-weight bearing sports have different effects on bone outcomes during adolescence. However, there is no longitudinal evidence comparing the effects of popular sports in the UK on bone acquisition in adolescent males.

Methods

In the present study 116 adolescent males (13.1±0.1 years: 37 footballers, 37 swimmers, 28 cyclists engaged in these sports more than 3 hours per week in the last three or more years and 14 controls not engaged in these sports more than 3 hours per week in the last or more three years) were measured at baseline and after 1 year of sports specific training. Bone mineral content (BMC) was measured by dual-energy X-ray absorptiometry (DXA) and bone stiffness by quantitative ultrasound (QUS). Moderate to vigorous physical activity (MVPA) was measured for 7 days using accelerometers. BMC and bone stiffness acquisition after 1 year were compared after adjusting for age, height, lean mass, MVPA and baseline bone status.

Results

Longitudinal participation in football was associated with significantly higher adjusted BMC acquisition at the total body, total hip, shaft, Ward’s, legs and bone stiffness acquisition compared to cyclists. Also, footballers had significantly higher adjusted BMC acquisition at total body, shaft and legs compared to swimmers. There was no difference between swimmers and cyclists for any bone outcomes. Longitudinal participation in swimming and cycling had no difference in acquisition across the bone outcomes, and both groups had non-significantly lower acquisition in bone outcomes at most sites of the skeleton compared to controls.

Conclusions

This novel longitudinal evidence shows that one year of football participation was associated with significantly greater improvements in BMC and bone stiffness compared to cycling and swimming in adolescent males. Furthermore, participation in swimming and cycling might induce lower bone acquisition compared to controls suggesting that weight-bearing exercises may be needed to improve bone development in adolescent males engaged in these non-osteo-genic sports.

Funding sources

The research leading to these results has received funding from the European Union Seventh Framework Programme ([FP7/2007-2013] under grant agreement n°. PCIG13-GA-2013-618496.

Disclosure

The authors declared no competing interests.

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P016

Necrotizing enterocolitis during the neonatal period is related to lower bone mass at 5 years of age, compared to matched controls

Amanda Magnusson, Diana Swolin-Eide & Anders Elvin

Institution of Clinical Sciences, Department of Pediatrics, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Objectives

Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease, mainly affecting preterm infants. NEC-survivors may have short or dysfunctional bowel with malnutrition as a result. Osteopenia of prematurity is well described among preterm infants. To the best of our knowledge there are no studies that follow preterm NEC-survivors to 5 years of age, regarding growth and bone mass. The aim was to study whether children diagnosed with NEC during their neonatal period are shorter and have lower bone mineral content (BMC) and bone mineral density (BMD) at 5 years of age, compared to matched no-NEC-controls.

Methods

In this pilot longitudinal follow-up study a total of 36 children at age 5 years were included. The 18 NEC-patients had been medically or surgically treated for NEC during their neonatal period at Queen Silvia Children’s Hospital, Gothenburg, Sweden. To every case a no-NEC-control matched for gestational age and gender, was included. Height and weight were measured at follow-up. Bone mass was measured by Dual-energy X-ray absorptiometry (DXA). Information from hospital charts regarding the neonatal period, and surgical data, was collected. Data were evaluated using Mann-Whitney U test. Statistical significance was assumed at P<0.05.

Results

The NEC-children were significantly shorter (P<0.05), had lower weight (P<0.001) and lower BMI (P<0.05) at 5 years of age compared to no-NEC-controls. Total body less head (TBBLH) BMC were significantly lower among NEC-children (P<0.001). Regarding TBBLH BMD, no significant differences were found. TBBLH BMD Z-scores ranged from -2.5 to +0.4 s.d. for cases and from -1.5 to +2.2 s.d. for controls. (P=0.052). The NEC-children had significantly lower lumbar spine (L1-L4) BMC (P<0.05), BMD (P<0.05) and BMC Z-score (P<0.05).

Conclusion

Children with NEC during their neonatal period were shorter, had lower body weight, a decreased TBBLH BMC, and a lower lumbar spine BMD than no-NEC-controls at 5 years of age. NEC-survivors are at increased risk of osteopenia and growth disturbance during childhood, however there may be confounding factors related to other morbidities during the neonatal period and further longitudinal studies are warranted.

Disclosure

The authors declared no competing interests.

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P015

Structural geometry of bones is prominently associated with risk of fracture in children

Carolina Medina-Gomez1, Olja Grigie1, Enisa Shevroja1,3, Katerina Trajanoska1, Andre G Uitterlinden1, Vincent W Jaddoe1, Tom J Beck2 & Fernando Rivadeneira3

1Erasmus MC, Rotterdam, The Netherlands; 2Beck Radiological Innovations, Inc., Baltimore, Maryland, USA; 3Lausanne University Hospital, Lausanne, Switzerland.

Background

Low total body BMD (TB-BMD) is an established risk factor for fractures in healthy children. However, bone strength depends not only on bone mass and density, but also on the structural geometry of bones. Hip structural analysis (HSA) is a technique applied on hip DXA scans to calculate several bone geometry parameters. The aim of our study was to evaluate other bone geometrical parameters that can constitute determinants of fracture risk. Specifically, we examined the association between femoral structural parameters including the geometry-derived femoral stress index (FSI) and risk of fracture in children.

Methods

We studied 1,851 children from the Generation R study, with whole body and hip scans measured using the same densitometer (GE-Lunar iDXA) at a mean age of 6.2 years. Hip DXA scans underwent HSA with derivation of FSI. This stress index considers both bending and axial forces acting on the femoral neck and is adjusted for lean mass fraction. Fractures at any skeletal site were assessed using questionnaire reports obtained before a mean age of 9.8 years. Risk (odds) of fracture was estimated from logistic regression models adjusted for sex, age, weight and ethnicity.

Results

Fractures were observed in 251 children (13.7%). A significant increase in the odds of fracture was observed for every standard deviation (SD) decrease in TBBLH-BMD (OR=1.28 95% CI 1.05–1.56; P=0.01). Similarly, an increase in the odds of fracture was observed for every reduction in one SD of femoral neck BMD (OR=1.23 95% CI 1.06–1.43; P=0.005) and narrow neck BMD (OR=1.26 95% CI 1.08–1.46; P=0.005). The FSI showed the strongest association with fracture, where every increment of one SD in the FSI resulted in 28% increased odds of fracture (OR=1.28 95% CI 1.13–1.45; P=0.0001). After inclusion of both the FSI and each of the BMD variables in the multiple regression model, only the stress variable remained significantly associated with risk of fracture.

Conclusions

Femoral and total body BMD parameters are associated with fracture in children. The stress index which considers in addition to quantity, the distribution of bone in the region, constitutes a biomechanical assessment which captures fracture propensity of children.

Disclosure

TJB is founder of Beck Radiological Innovations, Inc. All other authors state that they have no conflicts of interest.

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Bone mineral density in children and adolescents with neurofibromatosis type I: mineralization during growth and pubertal development

Giulia Rodari1, Giulietta Scuvera2, Fabio M Olivieri1, Francesca Menni3, Veronica Salletti1, Silvia Esposito3, Erislotta Prokta1, Silvia Bergamaschi1, Cristina Eiller Vainicher1, Maura Arosio4, Susanna Esposito5 & Claudia Giavoli1
1Endocrinology and Metabolic Diseases Unit, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy; 2Pediatric Highly Intensive Care Unit, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy; 3Bone Metabolic Unit, Division of Nuclear Medicine, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milan, Italy; 4Developmental Neurology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; 5Pediatric Clinic, Università degli Studi di Perugia, Perugia, Italy.

Objectives
The present study aims at evaluating bone mineral density (BMD) in a population of children with Neurofibromatosis type I (NF1), with particular focus on changes occurring during growth and pubertal development, trying to understand the magnitude and timing of onset of BMD impairment in this multisystemic and progressive disease, the latter poorly defined so far.

Methods
Bone metabolic markers (total calcium, phosphorus, bone alkaline phosphatase, parathyroid hormone, 25OH vitamin D, urinary calcium/creatinine ratio) and bone mineral status (by dual energy X-ray absorptiometry scans of the total body and lumbar spine with morphometric analysis) were assessed in fifty children (33 males; mean age ± s.d., 11.6±4 years). Bone mineral apparent density (BMD) and trabecular bone score of the lumbar spine were also obtained.

Results
In our cohort areal BMD (aBMD) Z-score was below the mean in 88% of patients at lumbar spine (LS, 70% after correction for bone size) and in 86% considering total body (TB) scans. However, aBMD Z-score was <−2 in 14% (12% after correction for bone size) and 12% patients at LS and TB, respectively. Though BMD at all sites was higher in older and pubertal patients, LS aBMD Z-score (r = −0.54, P < 0.0001), LS BMAD Z-score (r = −0.53, P < 0.0001) and TB Z-score (r = −0.39, P = 0.005) showed a negative correlation with growth and pubertal development (P = 0.0077, P = 0.02, P = 0.01, respectively), as suggesting that patients failed to gain as much as expected for age. Hypovitaminosis D was highly prevalent, as 98% patients had 25OHID concentrations below 30 ng/mL (75 nmol/L) and 18% less than 12 ng/mL (30 nmol/L). No statistically significant correlation between biochemical and densitometric data was found.

Conclusion
Bone mineral density impairment seems to become more evident with growth and pubertal development in NF1 patients, thus identifying childhood as the best aim for the molecular characterization of tcf12 expression, we could detect tcf12 expressing cells in cranial sutures, skull bones, and neural tissues of zebrafish. With the establishment of tcf12 and twist1 loss-of-function fish we clarify that even heterozygous mutations in tcf12 can lead to local fusions of the cranial sutures in zebrafish, whereas individuals with mutations in twist1 do not exhibit suture fusions.

Disclosure
The authors declared no competing interests.

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Zebrafish as model organism for craniosynostosis
Rabea Bluemel, Eva Klopopck & Daniel Liedtke
Institut for Human Genetics, Würzburg, Germany.

Objectives
Craniosynostosis is a skeletal birth defect, resulting in premature fusion of cranial sutures. The patency of the sutures is essential to enable the growth of the skull in compliance to the developing brain. Mutations in TWIST1 and TCF12 have been identified in patients with Saethre-Chotzen syndrome, which is typically associated with coronal synostosis. Studies in mouse models suggest that the quantity of TCF12-TWIST1 heterodimers is one critical factor for the patency of coronal sutures. We present zebrafish (Danio rerio) as supportive disease model to further visualize the specific function of TCF12 and TWIST1 during suture development and emergence of craniosynostosis.

Methods
To characterize the dynamic expression patterns of tcf12 during development, we generated transgenic zebrafish, in which the green fluorescent protein (GFP) is expressed under the control of the tcf12 zebrafish promoter. Using customized bone imaging techniques we perform in vivo imaging of developing bones and are able to correlate tcf12 expression to skull development. By use of CRISPR/Cas9-mediated genome editing we additionally established a number of loss-of-function mutations in conserved regions of tcf12 and twist1.

Results
The GFP-transgenic zebrafish reveal a broad range of expression patterns of tcf12 in developing embryos with high levels of expression in tissues like the developing heart and in neurons of the epiphysis, the otic vesicle, and the spinal cord. Most importantly tcf12 expressing cells are localized at the edges of the skull bones during development and could also be identified inside all cranial sutures in adult zebrafish. Our loss-of-function experiments further reveal that heterozygous mutations in tcf12 can lead to local fusions of the cranial sutures in zebrafish, whereas individuals with mutations in twist1 do not exhibit suture fusions.

Conclusion
We present zebrafish as a valuable disease model to gain deeper insight into the function and interaction of craniosynostosis genes. By generating transgenic individuals for tcf12 expression, we could detect tcf12 expressing cells in cranial sutures, skull bones, and neural tissues of zebrafish. With the establishment of tcf12 and twist1 loss-of-function fish we clarify that even heterozygous mutations can have minor effects on the maintenance of suture patency. Further experiments aim for the molecular characterization of tcf12 expressing cells detected in the sutures and for the generation of double knockout mutants.

Disclosure
The authors declared no competing interests.

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Age at onset of walking affects bone mineral content in early childhood
Scara Onkama, Heli Viljakainen, Elisa Holmlund-Suila, Jenni Rosendahl, Outi Mäkitie
Children’s Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.

Objectives
The aim of this study was to evaluate the association between the age at onset of walking and bone mineral content (BMC) in healthy 2-year-old children participating in the Vitamin D in Infants Trial (VIDI).

Methods
Altogether 253 children (120 girls, 133 boys) with a daily vitamin D3 intake of 10 µg and a sufficient serum 25-hydroxyvitamin D (25-OHD) concentration (>50 nmol/L) were included in the analysis. BMC was measured at 20% of the distal length of the left tibia with a peripheral quantitative computed tomography (pQCT) (Stratec Medizintechnik). Scans were analysed using the loop function with a threshold of 180 and 400 mg/cm2 for total and cortical bone, respectively. During the study visit at 2 years, data on onset of walking was collected retrospectively with a questionnaire. Anthropometric measurements and a blood sample for 25-OHD concentration were obtained.

Results
Serum 25-OHD ranged between 50.1 and 153.5 nmol/L. The age at onset of walking did not differ between sexes (P = 0.844) but boys were heavier than girls at 2 years (mean 12.9 vs. 12.0 kg, P < 0.001). BMC was higher in boys than girls (mean 56.5 vs. 52.2 mg/mm, P < 0.001). Weight associated with BMC in boys (r = 0.475, P < 0.001) and girls (r = 0.649, P < 0.001). Serum 25-OHD and BMC

Abstract withdrawn.

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did not associate either in boys or girls ($P=0.543$ and $P=0.704$). In a linear model, adjusted with weight and 25-OHD, the age at onset of walking associated with BMC in both sexes ($P=0.002$ and $P=0.046$) (Table 1). Conclusion These findings indicate that the onset of walking and weight are more important determinants of bone mineral content than serum 25-OHD in vitamin D sufficient healthy toddlers.

Disclosure The authors declared no competing interests.

Table 1 Linear regression analysis of determinants of bone mineral content at 2 years.

<table>
<thead>
<tr>
<th></th>
<th>BOYS</th>
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<th>GIRLS</th>
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<td>P</td>
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<tr>
<td>Serum 25-OHD</td>
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<td>-0.038</td>
<td>0.540</td>
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**P021**
Sexual dimorphism in bone size, density, micro-architecture and strength is site-specific and manifested in favour of boys already in childhood

Saija Kontulainen, Kelsey Bjorkman, Chantal Kawalilak, Whitney Duff, Hassanali Vatanparast & J.D. Johnston

University of Saskatchewan, Saskatoon, Canada.

Sex-differences in bone strength manifest at late puberty likely due to sex-specific hormonal stimulus to bone development. Comparisons of bone structural properties between sexes in years preceding the pubertal growth are lacking. Our objective was to assess sex-differences in bone size, strength, density and micro-architecture in childhood. We scanned distal and shaft sites of the radius and tibia from 85 girls and 75 boys (mean age 10.8, s.d. 1.8 years) using peripheral quantitative computed tomography (pQCT) and high resolution pQCT.

We defined biological age by calculating age from the estimated age at peak height velocity (aPHV). We included participants with a biological age of 0 to 4 years from aPHV and excluded post-menarcheal girls. We measured and compared (t-tests) anthropometrics, physical activity levels and dietary intakes of protein, calcium and vitamin D between sexes to identify covariates (for bone outcomes) that differed between sexes. We used MANCOVA (covariates: biological age, height and weight) to compare total, cortical and trabecular bone areas and densities, trabecular micro-architecture (thickness and number) and bone volume fraction, and estimated bone strength against compression at distal sites between girls and boys. We also compared total and cortical areas, cortical density and bone strength against torsion at the shaft sites of the radius and tibia. At the distal sites, girls had 10–19% smaller total, cortical and trabecular area, 7–10% lower total and trabecular density, 4% fewer trabeculae and 20% lower estimated bone strength against compression. At the shaft sites, girls had 20% smaller total area and 12% lower bone strength against torsion at the radius. We did not observe any sex-differences at the tibia shaft. Results suggest that boys have favourable bone size, density, micro-architecture and strength particularly at the distal radius and shaft radius already in childhood. In contrast, tibia bone properties at the shaft did not differ between sexes. These findings suggest that in addition to systemic factors (e.g. hormones) local factors (e.g., mechanical loading) may contribute to sex-specific bone development in childhood. A better understanding of sex-specific development in bone structure, micro-architecture and strength, along with information of modifiable factors, will guide investigations and strategies aiming to optimize primary prevention of osteoporosis and bone fragility, particularly in females.

Disclosure The authors declared no competing interests.

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**P022**
Bone densitometric parameters and body composition in preterm and term infants at the age of forty weeks of gestational age

Natascia Di Jorgi1 & Paola Diana2

1University of Genova, Genova, Italy; 2Endocrine Unit, Department of Pediatrics, Giannina Gaslini Institute, Genova, Italy.

Background Limited data are available on body composition of preterm compared to born at term infants. The aim of our study was to compare bone mass, fat mass-FM and free fat mass-FFM in preterm and term infants at 40 weeks of gestational age (GA).

Methods Thirty-four preterm infants (14M, 20F) born at 31.0±2.2 weeks of GA (range 26–36+3 weeks) and n=8 term neonates (4M, 4F) born at 39±1.0 weeks of GA underwent a total body DXA scans less head (TBLH, LUNAR Prodigy, Infant software) at 40.9±1.8 weeks of GA. Bone mineral content (BMC, g), bone mineral density (BMD, g/cm²), FM (g) and FFM (gr) were obtained at the TBLH and at the trunk (based on an automatic designed ROI).

Results Preterm were lighter than born at term infants (1597.2±35.3 kg vs 3465.2±260.1 kg, $P<0.0001$) and shorter (42.4±2.51 vs 50.7±1.7 cm, $P=0.0002$) at the time of birth, but comparable for weight and length at the time of evaluation; they displayed more FM (19.4±2.1% vs 16.7±2.6%, $P=0.19$) and similar FFM. Moreover, preterm infants had a lower BMC (2.070±0.048 vs 2.755±0.011 g/cm², $P=0.0004$), BMC (25.6±2.3 vs 48.2±6.8 g, $P<0.0001$) and area (126.6±25.5 vs 175.0±20.1 cm², $P<0.0001$) at the TBLH and lower BMD (0.195±0.046 vs 0.252±0.020, $P=0.0016$), BMC (13.5±4.1 vs 26.1±4.4, $P<0.0001$) and area (71.1±18.4 vs 103.4±12.9, $P<0.0001$) at the spine compared to born at term neonates. All bone parameters were related to birth weight ($r$’s range 0.13–0.52, all $P<0.05$) and GA ($r$’s range 0.17–0.37, all $P<0.05$) in preterm infants. However, multiple regression analysis showed that in preterm FFM was a positive ($r$ 0.4736e−5, $P=0.0305$) and FFM an negative predictor ($r$=−0.003, $P=0.0078$) of TBLH BMD (adj $r^2=0.466$, $P<0.0001$), after adjustment for GA and birth weight.

Conclusions Our preliminary data demonstrate that preterm infants exhibit early recovery in weight and length, but deficient gain in bone mass at the TB and the spine compared to born at term infants; interestingly, already at the age of ‘correspondent 40 weeks of GA’ FFM seems to be more important than birth weight or prematurity for bone mass development, while fat mass might have a negative impact.

Disclosure The authors declared no competing interests.

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**P023**
Tracking differences in morphology and regulation between the spine and long bones in a pig model

Adalbert Raaymann1, Alireza Javannardari1, Monika Egberger2 & Gabriele Hauesler1

1Division of Pediatric Pulmonology, Allergology and Endocrinology, Department of Pediatrics & Adolescent Medicine, Medical University of Vienna, Vienna, Austria; 2Institute of Anatomy, Histology and Embryology, University of Veterinary Medicine Vienna, Vienna, Austria.

Objectives The skeleton is not a single functional unit but consists of different, well-organized and modularized compartments with specific functions, developmental aspects and regulations. Differences in the regulation of spinal and long bone elongation are mirrored clinically by the age course in body proportions. Whereas growth plates (GPs) in long bones can easily be discriminated, vertebral GPs are part of the growing GPs of long bones. Further investigation is needed to decipher the molecular mechanism will ultimately improve assessment of clinically essential characteristics of spinal growth, such as vertebral elongation potential and growth plate fusion.

Methods 2- and 6-week-old piglet GP (GPs) of three vertebral segments (cervical, thoracic, lumbar) and eight long bones (proximal and distal radius, humerus, tibia, femur) were analyzed morphometrically. Further, estrogen receptor (ER), proliferation marker and growth factor expression was examined by immunohistochemistry.

Results Individual vertebral GPs were smaller in width and contained fewer chondrocytes than long bone GPs, although their proliferation activity was similar. Whereas the expression pattern of growth hormone-associated factors such as Insulin-like Growth Factor (IGF-1) and IGF1-receptor was similar, ERβ and IGF-2 were distinctly expressed in the vertebral samples.

Conclusions Vertebral GPs display differential growth, with measurements similar to the slowest growing GPs of long bones. Further investigation is needed to decipher the molecular basis of differential growth of the spine and long bones. Knowledge on the distinct mechanisms will ultimately improve assessment of clinically essential characteristics of spinal growth, such as vertebral elongation potential and growth plate fusion.

Disclosure The authors declared no competing interests.

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Spondyloepiphyseal dysplasia: A rare cause of short stature

Eilpis Vlachopapadopoulou1,2, Eirini Dikaiakeou1,2, Ioannis Panagiotopoulos1,2, Christos Zambaki2,2, Elii Anagnostou1,2, Vassilios Papadaki1,2, & Stefanos Michalacos1,2

1Children’s Hospital P.A. Kyriakoy, Athens, Greece; 2Agia Sofia Children’s Hospital, Athens, Greece.

Background

Short stature is a very common reason for referral to a pediatric endocrinologist. Endocrine and non endocrine causes are involved. Short stature can be classified as symmetrical or non-symmetrical. Numerous monogenic causes of growth disorders have been identified.

Presenting problem

To present two brothers with familial spondyloepiphyseal dysplasia presenting with short stature and very low growth velocity starting at the age of 8 years. The older brother was the product of an uneventful pregnancy with a birth weight of 2950 gms. Father had short stature with a height of 161 cms and mother was of normal stature with a height of 163 cms. He grew along the 25th ile until the age of 8 years. Since then, growth rate decelerated to less than 3 cm/yr and height was at the 3rd percentile at the age of 11 years.

Clinical management

He was tested for growth hormone deficiency and peak growth hormone was 6 ng/ml. Growth hormone therapy with no good response. Reexamining the diagnosis and appreciating the low U/L ratio the possibility of spondyloepiphyseal dysplasia was raised and proven by the spine x-ray. Growth hormone therapy was discontinued and final height was 150 cms. The younger brother who was overweight, he also suffered low growth rate at the age of 10 years. His near final height is 150 cms. He did not undergo any growth hormone stimulation test and a spine x-ray revealed spondyloepiphyseal dysplasia. Molecular analysis is pending.

Conclusion

Spondyloepiphyseal dysplasia should be considered as a rare cause of short stature. Of importance is that growth rate deceleration as well as skeletal asymmetry with low U/L segment occurs at the peripubertal age and thus it is not easy to diagnose in early childhood.

Disclosure

The authors declared no competing interests.

P025

The impact of Haemophilia A on bone health

Artemis Doulgeraki1, P. Kafaki2, H. Pergantou2, H. Athanasopoulos1 & H. Platonou2

1Department of Bone and Mineral Metabolism, Institute of Child Health, Athens, Greece; 2Haemophilia Center/Haemostasis Unit, Aghia Sophia Children’s Hospital, Athens, Greece.

Objectives

Haemophilia A (FVIII deficiency) is an X-linked disorder of haemostasis with bleeding tendency, mainly in joints and muscles. Recurrent haemarthroses, subclinical immobilization and avoidance of contact sports, may affect these patients’ skeletal health.

Methods

Evaluation of bone health was performed in 51 children with Haemophilia A (severe: 41, all on prophylaxis treatment), mean age: 11.7±3.6 years. Dual-energy X-Ray absorptiometry (DXA) of total body less head (TB) and lumbar spine (LS) was performed. Also, laboratory markers for bone formation (boneALP, osteocalcin, type I procollagen carboxy-terminal propeptide (PICP)) and bone resorption (urinary deoxypyridinoline/creatinine (uDPD/urCr), urinary calcium excretion (uCa/uCr), tartrate-resistant acid phosphatase (boneTRAP5b)) as well as vitamin D (25-OH-D) and PTH were measured.

Results

Mean LS bone mineral density (BMD) Z-score was −0.51 ± 0.98 (10% with low Z-score ≤−2, 20% with low-normal Z-score, i.e. between −1 and −2). Mean TB BMD Z-score was 0.18 ± 0.85 (9.1% with low-normal Z-score). Compared to the laboratory reference values, osteocalcin was significantly lower (19.06 ± 5.8 ng/ml, p<0.05), whereas the other bone formation markers were normal. Moreover, osteocalcin was positively but weakly correlated with LS and TB BMD Z-scores (r=0.337 and r=0.313 respectively, p<0.05). Increased uDPD/urCr (mean value 32.35 ± 14.6 mmol/mmol) was found in 77.6% of the patients and it was negatively and strongly correlated to both LS and TB BMD Z-scores (r =−0.677, −0.569 respectively, p<0.01). Patients with increased uDPD/urCr had lower LS BMD Z-scores (−0.82 ± 0.85 vs −0.20 ± 0.97, p<0.05) and TB BMD Z-scores (mean −0.08 ± 0.69 vs −0.37 ± 0.91, p<0.05).

Conclusions

In our study, lumbar BMD was more severely affected than TB BMD and bone metabolism was also disturbed, with more significant changes in bone resorption. This is important for prompt detection of patients at risk, as metabolic bone markers tend to change earlier than BMD. Finally, one in three patients had vitamin D deficiency. Taken together, these results emphasize the negative impact of haemophilia A on bone health and underline the need for close surveillance of this population.

Disclosure

The authors declared no competing interests.

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P026

Own research experience of bone tissue metabolism in patients with the Ehlers-Danlos syndrome

Yuriy Demyan1, Iurii Guk1, Oleksandr Magomedov1, Andrii Zyva1, Andrii Cheverda2, Tamara Kincha Polishchuk 1 & Natalia Balacka2

1Institute of Orthopedics and Traumatology, Kiev, Ukraine; 2Institute of Gerontology, Kiev, Ukraine.

Purpose

To explore the features of bone metabolism and create a system of medical correction of violations in patients with Ehlers-Danlos syndrome.

Materials and methods

Based on the analysis of the survey results of 12 patients with different types of EDS aged 3 to 10 years (males - 8 patients, female - 4 patients) who were treated in Institute of Orthopedics and Traumatology, National Academy of Medical Sciences, Kiev, Ukraine from 2005 to 2015 years. Bone metabolism was studied by examining markers of bone turnover as recommended by the International Organization of osteoporosis (International Osteoporosis Foundation) by ELISA on the analyzer “ELECSYS” firm ROCHE (Roche Diagnostics, Germany) using test systems Cobas in terms of biochemical laboratory control "ITO NAMS". Among the markers of bone formation were: propeptydy procollagen of type I (boneALP, osteocalcin, type I procollagen carboxy-terminal propeptide (PICP)) and calcium excretion (uCa/uCr), tartrate-resistant acid phosphatase (boneTRAP5b).

Results and discussion

Changes in bone markers and vitamin 25 (OH) D in most patients show the violation in bone metabolism in patients with EDS, including the synthesis and degradation of type I collagen, imbalance between the processes of bone formation and osteo resorption; differing vectors changes in bone formation, increased bone resorption in most patients, acceleration remodeling at both types of EDS; reducing the concentration of vitamin 25 (OH) D, which negatively affects the formation and mineralization of bone. So, all of the above written points to the need and feasibility of developing a system of drug correction of change of bone metabolism.

Conclusion

Indicators of bone markers and vitamin 25 (OH) D show the violation in bone metabolism in patients with EDS, synthesis and degradation of collagen type I, imbalance between the processes of bone formation and bone resorption.

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P026
Sex and iron modify fibroblast growth factor 23 concentrations in 1-year-old children
Elisa Holmlund-Suila 1,2, Maria Enlund-Cerullo 1,2, Saara Valkama 1,2, Helena Hauata-alus 1, Jenni Rosendahl 1,2, Otto Helve 3, Heli Viljakainen 1,2, Sture Andersson 1,3 & Outi Mäkitie 1,3
1University of Helsinki, Helsinki, Finland; 2University of South Florida, Tampa, Florida, USA; 3Osteogenesis Imperfecta Foundation, Gaithersburg, Maryland, USA

Objectives
The regulation of fibroblast growth factor 23 (FGF23) metabolism during infancy is inadequately characterized. We previously observed a distinct sex difference in intact FGF23 at 3 months of age. In this study we aimed to further examine the role of sex and iron status in FGF23 metabolism in 1-year-old children.

Methods
This was a cross-sectional study including 731 1-year-old Caucasian children participating the Vitamin D intervention in infants (VIDI) trial in Finland. In this double-blind trial, healthy term infants are randomized to receive 10 or 30 µg vitamin D3 daily from 2 weeks to 2 years. We analyzed intact and C-terminal FGF23, 25OHD, PTH, calcium, phosphate and markers of iron status at 1 year.

Results
Intact FGF23 was higher in girls than in boys (median 44.3 vs 41.0 pg/ml, \( P<0.001 \)) and C-terminal FGF23 did not differ between sexes (median 2.9 vs 2.8, \( P=0.403 \)). These findings persisted after adjusting with growth parameters. Boys were bigger and had lower ferritin concentrations (median 18 vs 26 µg/l) than girls (\( P<0.001 \) for both). Iron status was positively associated with intact FGF23 and inversely with C-terminal FGF23 (\( P<0.001 \) for both). Iron was the strongest modifier of intact FGF23 concentration when season, sex, 25OHD, ionized calcium and ferritin were also included in the model: higher iron associated with higher intact FGF23 (\( P<0.001 \)). In both boys and girls, iron (\( P<0.001 \) and \( P=0.001 \)) and season (\( P<0.001 \) and \( P=0.031 \)) remained significant modifiers. Furthermore, in girls, 25OHD (\( P<0.001 \) and ionized calcium (\( P=0.003 \)) were positively, and ferritin (\( P=0.043 \)) inversely associated with intact FGF23.

Conclusion
Intact FGF23 was higher in girls than in boys, likely due to differences in growth and the demand for phosphate during infancy. Higher iron associated with higher intact FGF23 and lower C-terminal FGF23. Other modifiers of intact FGF23 included season, 25OHD, ionized calcium and ferritin.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P027

Outcomes of zoledronic acid use in paediatric conditions
Angelina Lim 1,2, Peter Simm 1,3, Simon James 4 & Margaret Zacharin 1,3
1 Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 2Monash University, Melbourne, Victoria, Australia; 3Royal Childrens Hospital, Melbourne, Victoria, Australia; 4Deakin University, Melbourne, Victoria, Australia

Objectives
Intravenous bisphosphonates have been used in children for various primary and secondary bone fragility disorders for three decades but beyond osteogenesis imperfecta, there is very limited information published in relation to outcomes. We report the experience at the Royal Children’s Hospital (RCH), Melbourne using Zoledronic acid (ZA), describing outcomes based on the underlying condition for which treatment was given, with the aim of informing future protocols and guidelines for bisphosphonate use.

Methods
A retrospective review of all RCH patients administered at least one dose of intravenous ZA from 2002–2015 was undertaken. All outcome data was collected from existing hospital medical records.

Results
In the 13 years of ZA use, 325 children had at least one dose of ZA; 12 of these were excluded from study inclusion due to either missing documentation regarding use of ZA or indications of extreme prematurity, renal osteodystrophy or intra-arterial calcification. Children included in this review were not taking any other bisphosphonates. Of the 313 patients, Table 1 describes preliminary data regarding use of ZA or indications of extreme prematurity, renal osteodystrophy or intra-arterial calcification. Children included in this review were not taking any other bisphosphonates. Of the 313 patients, Table 1 describes preliminary data outcomes grouped for each condition; more analysis is currently being done on changes per year and after first year of treatment. Mild to moderate first dose acute phase reaction occurred in most with symptomatic acute day 2 hypocalcaemia in several who had steroid induced osteoporosis.

Conclusion
Zoledronic acid demonstrated a good efficacy profile, with improved bone density for osteoporotic conditions, significant pain relief in all treated bone abnormality indications, and stabilization of lesion size with reduced incidence of bone collapse in AVN.
Valproic acid induces Fanconi syndrome and reversible hypophosphataemic rickets via upregulation of fibroblast growth factor 23

Valproic acid (VPA) is a commonly used antiepileptic drug in the management of childhood epilepsy. Renal dysfunction presenting as Fanconi syndrome (FS) is a rare side effect of VPA use. This can lead to renal tubular phosphate loss, resulting in hypophosphataemic rickets, low bone mass and fractures. We report 6 children with VPA induced FS from three tertiary paediatric metabolic bone centres across England.

Presenting problem
P1: Global developmental delay of unknown cause, cortical blindness and sensory neural hearing loss

Clinical case presentation
Vrinda Saraff1, Raja Padidela2, Talat Mushtaq3, Sophia Sakka1, Rubina Mandlik1, Veena Ekbote1, Shital Bhote2, Neha Jadhav1, Jwala Pawar1, Shiriram Narwade1, Vaman Khadilkar1, Raja Padidela2, Zulf Mughal2 & Anuradha Khadilkar1

1Birmingham Children’s Hospital, Birmingham, UK; 2Royal Manchester Children’s Hospital, Manchester, UK; 3University of Birmingham, Birmingham, UK.

Background
Valproic acid (VPA) is a commonly used antiepileptic drug in the management of childhood epilepsy. Renal dysfunction presenting as Fanconi syndrome (FS) is a rare side effect of VPA use. This can lead to renal tubular phosphate loss, resulting in hypophosphataemic rickets, low bone mass and fractures. We report 6 children with VPA induced FS from three tertiary paediatric metabolic bone centres across England.

Presenting problem
P1: Global developmental delay of unknown cause, cortical blindness and sensory neural hearing loss

Objective
Our earlier study using dual energy x-ray absorptiometry had shown that longer duration of type 1 diabetes (T1DM) in children was associated with small and slender bones. The objective of this study was to assess bone geometry in children and adolescents with T1DM using a pQCT.

Methods
We studied 69 children (8.3 to 18.7 years of age, 29 boys) with T1DM. Anthropometry and biochemical assessments (glycosylated Hb (HbA1c), Vitamin D and PTH) were performed. pQCT (STRATEC XCT-2000) of the radius of non-dominant hand at 4% and 66% was performed, and P4, however P2 and 5 received intravenous Zoledronic acid infusions due to further long bone fractures. In one patient (P5), post VPA weaning, Fibroblast growth factor 23 (FGF23) normalised to 63 RU/ml (0–99).

Clinical management
Proximal tubular dysfunction and serum phosphate normalised within 6–12 months of stopping VPA. Marked radiological improvement was noted in P1, P3 and P4, however P2 and 5 received intravenous Zoledronic acid infusions due to further long bone fractures. In one patient (P5), post VPA weaning, Fibroblast growth factor 23 (FGF23) normalised to 63 RU/ml (0–99).

Discussion
The exact mechanism of VPA induced FS is not clearly understood. Elevated FGF23 levels in three patients suggest VPA mediated dysregulation, leading to worsening renal phosphate loss and resulting in osteopenia (osteomalaclia). In addition, immobility in this severely disabled subgroup of children on VPA, contributes to poor bone health.

Disclosure
The authors declared no competing interests

DOI: 10.1530/boneabs.6.P030

P032
Bone health status in Indian children with type 1 diabetes as assessed by peripheral quantitative computer tomography (pQCT)

Rubina Mandlik1, Veena Ekbote1, Shital Bhote2, Neha Jadhav1, Jwala Pawar1, Shiriram Narwade1, Vaman Khadilkar1, Raja Padidela2, Zulf Mughal2 & Anuradha Khadilkar1

1Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India; 2Royal Manchester Children’s Hospital, Manchester, UK.

Objective
Our earlier study using dual energy x-ray absorptiometry had shown that longer duration of type 1 diabetes (T1DM) in children was associated with small and slender bones. The objective of this study was to assess bone geometry in children and adolescents with T1DM using a pQCT.

Methods
We studied 69 children (8.3 to 18.7 years of age, 29 boys) with T1DM. Anthropometry and biochemical assessments (glycosylated Hb (HbA1c), Vitamin D and PTH) were performed. pQCT (STRATEC XCT-2000) of the radius of non-dominant hand at 4% and 66% was performed, t-scores were computed from data provided by Stratech. Children were classified in tertiles of disease duration (<2.2 years, 2.3 to 4.5 years and > 4.5 years).

Results
The mean height (HAZ), weight and BMI for age Z-scores of children were −0.77 ± 1.5, −0.69 ± 1.1, −0.39 ± 0.8 respectively (p > 0.1, between genders). The mean HbA1c was 10.0 ± 2.1. Eighty-three% of children were vitamin D deficient (serum 25 OHD <50 nmol/l) with mean 25 OHD concentrations of 35.8±20.7 nmol/l. The mean PTH concentration was 7.2 ± 4.3 (35% above 7.6 nmol/l). The HAZ was significantly lower in children with disease duration of >4.5 years. The mean trabecular density, total density at 4%, cortical density...
and strength strain index (SSID) at 66% for age Z-score were $-1 \pm 1.0$ ($15\% < -2$), $-0.7 \pm 1.0$ ($7\% < -2$), $-0.1 \pm 1.3$ ($10\% < -2$) and $-1.3 \pm 0.71$ ($20\% < -2$) respectively, and than zero ($P<0.0001$) except for cortical density ($P=0.079$). When these measurements were assessed across the disease duration, it was found that the SSIPol3 for age Z-score was significantly lower ($P<0.05$) in children with disease duration of $>4.5$ years ($-1.57 \pm 0.73$) than $2.3-4.5$ years ($-1.07 \pm 0.72$) indicating that there may be an increased risk of fracture as the disease duration increases.

Conclusion

Indian children with poorly controlled T1DM had hypovitaminosis D and poor bone health as judged by low trabecular density and SSI. Disease duration is likely to increase the risk of poor bone health because of short stature and lower SSI. Disease duration increases.

A significant decrease in BMD is observed in children with severe cerebral palsy as judged by low trabecular density and SSI. Disease duration is likely to increase the risk of poor bone health because of short stature and lower SSI. Disease duration increases.

The authors declared no competing interests.

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P033

Nutritional status influence upon bone mineral density of children with cerebral palsy after reconstructive hip joint surgery

Svetlana Kharchenko, Anna Popотовец, Lyudmila Grigoriechева, Michael Lobanov & Vadim Kozhevnikov

FSBI “Federal center of traumatology, orthopedics and endoprosdosis replacement” Ministry of Health of Russian Federation, Barnaul, Russia.

Objectives

Evaluate nutritional status influence upon bone mineral density (BMD) of children with CP diagnosis after reconstructive hip joint surgery.

Methods

Eighteen children with CP diagnosis with III-V level Gross Motor Function Classification System took part in the research. All patients received reconstructive medical treatment in child traumatic-orthopedic unit of federal center after reconstructive hip joint surgery. Anthropometric measurement carried out using WHO Anthro, WHO AnthroPlus (2009) software. Following parameters were taken into account: weight/age, weight/height, height/age, body mass index (BMI)/age. Dual energy X-ray absorptiometry of L-1-L4 vertebrae of lumbar spine in Spine AP and Whole body was treated on EXCELL XR-46 apparatus (‘Norland’, USA) with estimation of Z-criterion according to WHO recommendations.

Results

The average age of children $8.8 \pm 2.6$ years old. Eight girls and ten boys. Anthropometric measurement of researched group of patients: average body weight $21.47 \pm 6.9$ kg, average body height $124.5 \pm 15.6$ sm., average body mass index $13.7 \pm 2.4$. Totally nutritional deficiency was detected in 15 (83.3%) surveyed children: mild malnutrition – 5 (27.7%), moderate – 3 (16.7%), severe – 7 (38.9%). According to the results of Whole body dual energy X-ray absorptiometry 15 patients (83.3%) showed a decrease in bone mass compared with the age norm (Z-criterion is less than -2.3 S.D.), the average value is $3.5 \pm 0.91$. At the same time, the average value of Z-criterion, BMD L1-L4 vertebrae were within the age norm and made – 0.91 $\pm 0.1$ a positive correlation between the Z-criterion of BMD at the Whole body and BMI ($r=0.662; P=0.02$).

Conclusion

A significant decrease in BMD is observed in children with severe cerebral palsy (GMFCS III-V), which is aggravated by malnutrition. Thus, the determination of BMD and nutritional support of these patients are required within the preparative preparation to reduce the risk extravegetal fractures.

Disclosure

The authors declared no competing interests.

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P034

Identification of bone remodelling alterations in Gorham-Stout disease

Michela Rossi1, Giulia Battafarano1, Paola Sabrina Buonuomo2, Alessandro Jenkner3, Ippolita Rana4, Rita De Vito5, Andrea Bartuli2 & Andrea Del Fattore1

1 Multifactorial Disease and Complex Phenotype Research Area, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; 2 Rare Diseases and Medical Genetic Unit, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; 3 Division of Immunology and Infectious Diseases Department of Pediatrics, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; 4 Department of Pediatric Hematology and Oncology, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; 5 Histopathology Unit, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy.

Objectives

Gorham-Stout disease (GSD) is a very rare disorder characterized by extensive angiomatic proliferation and progressive osteolysis without new bone formation. Only ~200 patients were reported. The quality of life is very poor since patients display pain, fractures, functional impairment and swelling of the affected regions. The etiology of GSD is unknown. We aim to investigate the bone phenotype and to identify molecular and cellular defects in GSD patients.

Methods

Eight patients were recruited for this study. Bone biopsy analysis was performed. Bone turnover’s markers were analysed by ELISA assay. In vitro osteoclast and osteoblast cultures were performed to evaluate alterations of differentiation, morphology and activity. Osteoclast and osteoblast’s gene expression was evaluated by Real-Time RT-PCR analysis.

Results

Bone biopsy analysis revealed fibrous tissue with dilated blood vessel as well as evidence of very active osteoclast resorption. A 10-fold increase of osteoclast number with high levels of serum ICTP was observed in patients. Osteoclast precursors (pOCs) isolated from patients showed a approximately twofold increased ability to differentiate into osteoclasts (412.6 ± 0.69.58 vs 887.7 ± 56.35; $P=0.03$), with higher number of nuclei per cell. About 75% of affected osteoclasts displayed a more motile phenotype. Real-Time RT-PCR expression analysis revealed that patients’ osteoclasts displayed a transcriptional increase of TICRG1, CSTK and MMP9 genes. pOCs from healthy donors treated with serum from patients showed an increase of oeclastoclastogenesis compared to pOCs treated with controls’ serum. Bone Marrow MSC isolated from a patient displayed the characteristic spindle-shaped morphology and the same immunophenotype as healthy donors (HD)-MSCs. After incubation with osteogenic medium, GSD- MSCs demonstrated reduced ALP activity and expression compared with HD-MSCs.

Conclusions

These results suggest that in Gorham-Stout disease the alteration of bone remodelling activity is related to bone cell autonomous defects and systemic factors. Understanding the molecular and cellular defects in GSD patients will allow to have a correct diagnosis and new therapeutic options for this rare disease.

Disclosure

The authors declared no competing interests.

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P035

Abstract withdrawn

P036

Decreased incidence of fracture after IV bisphosphonates in girls with Rett syndrome and severe bone fragility

Anne-Sophie Lambert1, Anya Rothenbühler2, Perrine Charles2, Elisabeth Celestin2, Nadia Bahri-Buisson1 & Agnes Léglise1


Background

Rett Syndrome (RS) is a disabling condition due to mutations in MECP2. Girls affected with RS are at risk of developing osteoporosis and fractures at a young age because of their lack of mobility and through a direct effect of MECP2 on bone mineralization. In these girls, bone fragility inflicts pain and may seriously impair the quality of life.

Objective

To retrospectively assess the effect of IV bisphosphonates on fracture, bone mineral density (BMD) and bone markers in RS girls with bone fragility.

Methods

Once diagnosed with bone fragility and fracture and/or bone pain, RS girls received either 1.5 mg/kg of pamidronate IV every 3 months ($n=16$) or 0.04 mg/kg of zoledronate IV every 6 months ($n=1$) for 2 years. Results are shown as median [min; max].

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Results
20 patients were studied (age: 12.5 years [6; 39]). 14/20 patients were non ambulatory. The incidence of fracture decreased from 37 fractures in 20 patients (6 months interval preceding the start of therapy), to 0 fracture in 20 patients during or after IV bisphosphonates (follow-up: 3.1 years [2; 4.2]). The spine BMD Z-score improved from $-3.2$ to $-5.6$ to $-0.1$ to $-2.05$ to $-3.8$ to 0.0, $P=0.0011$. Most parents reported a decrease in chronic pain; 2 patients started to walk around the end of the 2 years therapy. The urinary calcium excretion, decreased significantly from 0.68 [0.18; 1.5] to 0.2 [0.03; 0.67] μM/mM of creatinine ($P=0.0001$). Except for moderate hypocalcemia and fever, pamidronate was well tolerated in all girls.

Conclusion
Our results are in accordance with the beneficial effect of bisphosphonates in children with cerebral palsy. Impaired bone mineralization in RS girls should be screened and prevented through measures including vitamin D supplements, nutritional support and careful mechanical loading. In girls experiencing fractures, IV bisphosphonates appear to be a beneficial adjuvant treatment to diminish the risk of fracture and restore the bone density.

Disclosure
The authors declared no competing interests.

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P038
Hyophosphatasia associated with acute disseminated encephalomyelitis (ADEM): causal relationship or coincidence?

Benjamin Jacobs1, Angela Gall1, Daniela Pecova1, Sandrine Lacassagne2, Dinesh Talwar2, Emma L Wakeling2, Jair Tenorio3 & M Zulf Mughal4
1Royal National Orthopaedic Hospital, Stanmore, UK; 2Great Ormond Street Hospital, London, UK; 3Glasgow Royal Infirmary, Glasgow, UK; 4Northwick Park Hospital, London, UK; 5Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain; 6Royal Manchester Childrens Hospital, Manchester, UK.

Background
Hyophosphatasia is generally regarded as a disease of bone and teeth. Lack of tissue Non-Specific Alkaline Phosphatase (TNAP) leads to an accumulation of inorganic pyrophosphate and the Vitamin B6 metabolite pyridoxal 5′-phosphate (PLP), a reduction in pyridoxic acid (PA) and increased PLPPA ratio. Vitamin B6 deficiency in the brain impairs synthesis of neurotransmitters, and is a well-recognised cause of neonatal seizures. We have found no previous reports of ADEM as a feature of Hyophosphatasia beyond the neonatal period.

Presenting problem
A 12 year old girl with ADEM was noticed to have persistently low serum alkaline phosphatase activity. She had presented to her local hospital with a 1 week history of fever, drowsiness and difficulty walking. She developed increasing weakness, slurred speech and 2 days later respiratory failure requiring ventilation. Brain MRI and EEG showed signs of ADEM. She was born with a malformation of her left hand but never had dental or bone features of hypophosphatasia.

Clinical management
She was treated with intravenous antibiotics, antiviral therapy, steroids and plasmapheresis. It was later noticed that her serum Alkaline Phosphatase activity had been low since presentation (22–37 IU/L). Her plasma PLP was 302 nmol/d (range 20–140) with a PA of 39 nmol/l (9–60) giving a PLPPA ratio of 8 (normal non-supplemental subjects < 5.0) supporting the diagnosis of hypophosphatasia.

Genetic analysis showed a pathogenic heterozygous mutation in exon 5 of ALPL: c.346G>A, p.Ala116Thr. Review of her neonatal record, and that of her twin sister, revealed that both girls had low alkaline phosphatase activity on routine blood test at 4 days of age (47 and 58 IU/L, respectively). The twin has had no symptoms.

Discussion
TNAP is known to be expressed in the synapses of the cerebral cortex that are involved in neurotransmitter synthesis, synaptic stabilization, and myelin pattern formation. This case raises the possibility that that hypophosphatasia might be causally related to ADEM.

Disclosure
The authors declared no competing interests.

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P039
Cystinosin deficiency affects bone phenotype

Giulia Battafarano, Michelle Rossi, Gianna Di Giovamberardino, Anna Pastore, Anna Taranta & Andrea Del Fattore
Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy.

Objective
Cystinosis is a rare lysosomal storage disorder caused by loss-of-function mutations of the CTNS gene, encoding for cystinosin symporter that mediates cystine efflux from lysosomes. ~95% of cystinotic patients display nephropathic Fanconi’s syndrome, short stature, osteopenia and rickets. In this study we evaluated whether the absence of cystinosin primarily affects bone remodeling activity.

Methods
We analyzed bone phenotype of ctns±/- (KO) male mice lacking of nephropathy. Intra-lysosomal cystine accumulation in tissues was detected by HPLC. Bone phenotype was evaluated by µCT, transcriptomic and bone serum biomarkers analysis. In vitro study was performed to evaluate the differentiation and activity of osteoblasts.

Results
Intra-lyosomal cystine accumulates in Ctns±/- bones during life reaching higher levels than those observed in kidney. Cystinosin is expressed in human and murine osteoblasts and osteoclasts; interestingly its expression increases during osteoclastogenesis. µCT analysis showed a reduction of trabecular bone volume (BV/TV: 5% WT: 16.43±0.62; KO: 12.22±0.39; P<0.05) and bone mineral density in 1-month-old KO mice (BMD mgHA/cm³ WT: 194.01±4.04; KO: 158.02±6.00; P<0.05) with a decrease of trabecular number and thickness.

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P040

Atypical femoral fractures in 2 children treated with bisphosphonates
Benjamin Jacobs1, Caroline Brain2, Catherine DeVile3, Jeremy Allgrove1, Daniela Peeva1, Aresh Hashemi-Nejad1 & M Zulf Mughal1
1Royal National Orthopaedic Hospital, Stanmore, UK; 2Great Ormond Street Hospital, London, UK; 3Royal Manchester Childrens Hospital, Manchester, UK.

Background
Atypical Femur Fracture (AFF) has become widely reported as a complication of bisphosphonate therapy in adults since the first case report in 2005. A trend towards a similar pattern of fractures has been reported in children in Sheffield in 2012. A 13 year old boy was reported in 2014 with an ‘AFF’ of the tibia but that fracture did not meet the standard diagnostic criteria of AFF. Last year a 16-year-old girl treated with pamidronate for idiopathic juvenile osteoporosis was reported with an AFF and multiple AFF’s were reported in a teenage girl with OI type IV.

Presenting problem
A 13 year old girl started bisphosphonate treatment in 2009. The indication for treatment was unusual (heterozygous LRP5 mutation). She developed a classic AFF at 18 years of age. She had features recognised as AFF risk factors in adults: Asian origin, vitamin D deficiency and long term bisphosphonate treatment (6 years). A 10 month old baby girl presented with a fractured femur. She, and her older brother, were diagnosed with Osteogenesis Imperfecta (OI) Type I and found to have a COL1A1 c697-1 C>GT mutation. She started intravenous pamidronate treatment at 5 years of age, and presented at 7 years of age with an AFF.

Clinical management
Both girls had surgery to fix their AFF. The patient with heterozygous LRP5 mutation stopped bisphosphonate treatment as she is now an adult, but we plan to continue a lower dose treatment for the younger patient with OI.

Discussion
AFF must now be considered as a complication of bisphosphonate therapy in childhood. The risk of AFF should be considered when weighing up the clear benefits of this therapy in severe cases of OI and other more rare disorders. However it may be safer to withhold, or limit, bisphosphonate treatment in childhood. The risk of AFF should be considered when weighing up the clear benefits of this therapy in severe cases of OI and other more rare disorders.

Disclosure
The authors declared no competing interests.

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P041

A randomised double-blind placebo-controlled trial of vitamin D supplementation in juvenile-onset systemic lupus erythematosus: positive effect on trabecular microarchitecture using high resolution peripheral quantitative computed tomography
Juliane Paipitz, Glaucia Lima, Nadia Aikawa, Jackeline Alvarenga & Rosa Pereira
Faculdade de Medicina Universidade de Sao Paulo, Sao Paulo, Brazil.

Objectives
Vitamin D has an important effect on bone but there are no trials that directly address the boosting of serum levels of 25-hydroxyvitamin D (25OHVD) in bone microarchitecture in Juvenile-onset Systemic Lupus patients (JoSLE). The aim of this study was to evaluate the effect of vitamin D supplementation on bone microarchitecture parameters using HR-pQCT in JoSLE patients.

Methods
This study was a randomized double-blind placebo-controlled 24-week trial (Clinical Trial Registry NCT01892748) conducted at the Hospital das Clínicas da Universidade de Sao Paolo, Brazil. Forty female JoSLE patients were randomized (1:1) to receive oral cholecalciferol 50,000 IU/week (JoSLE-VitD) or placebo (JoSLE-PL). Medications remained stable throughout the study. Serum levels of 25OHVD were measured using radioimmunoassay. Bone microarchitecture and volumetric bone density were analysed using HR-pQCT at tibia site.

Results
At baseline, groups were similar regarding age, BMI, organ involvement, glucocorticoid dose, immunosuppressant use, serum levels of 25OHVD and HR-pQCT parameters. After 24 weeks, higher 25OHVD levels were observed in the JoSLE-VitD group compared to the JoSLE-PL [31.3(8.6) vs. 16.5(5.8) ng/ml, P < 0.001]. An increase in trabecular number [ΔTh.N: 0.16(0.24) vs. 0.03(0.19), P = 0.024] and a decrease in trabecular separation [ΔTh.Sp: -0.045(0.067) vs. 0.001(0.009), P = 0.017] were found in JoSLE-VitD group than in JoSLE-PL at tibia site. No differences were observed in other structural parameters (trabecular Tb.Th) or cortical thickness (Ct.Th), volumetric bone mineral densities, cortical porosity and biomechanical parameters (P > 0.05).

Conclusion
This study suggests that cholecalciferol supplementation for 24 weeks could be effective in improving bone microarchitecture parameters, mainly trabecular number in JoSLE patients. The authors have declared no conflicts of interest.

Disclosure
The authors declared no competing interests.

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P042

Osteogenesis imperfecta type VI presenting as suspected physical abuse – a report of two cases
Sivagamy Sithambaram1, Nick Bishop2,1, Lata Shankar1, Anaka C Offiah3, Rebecca C Pollitt1, Meena Balusabramanian5,6, Anand K Saggar2 & Paul Arundel1
1Academic Unit of Child Health, University of Sheffield, Sheffield, UK; 2Sheffield Children’s NHS Foundation Trust, Sheffield, UK; 3Community Paediatrics, Sherwood Forest Hospitals NHS Foundation Trust, Ashfield, UK; 4Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK; 5Sheffield Diagnostic Genetics Laboratory, Sheffield Children’s NHS Foundation Trust, Sheffield, UK; 6Mellanby Centre for Bone Health, Sheffield, UK; 7Sheffield Clinical Genetics Service, Sheffield Children’s NHS Foundation Trust, Sheffield, UK; 8St George’s University of London, London, UK.

Background
Osteogenesis imperfecta (OI) type VI is a rare recessive disease that may present with long bone fractures in early childhood. Bone in this condition is particularly brittle; the resulting pattern of long bone fractures and lack of distinct radiographic findings can make the diagnosis less obvious than in other types of OI. We report 2 unrelated children who presented with long bone fractures and were suspected of having suffered physical abuse with removal of parents’ custodial rights.

Presenting problem
Patient 1 was the second child of first cousins. He started walking at 12 m. His first fracture was of the clavicle aged 13 m. Humerus, fibula and multiple rib fractures had occurred by 2.5 yrs. He had off-white sclerae and ligamentous laxity. Patient 2 was born to healthy unrelated parents. He started walking at 12m. His first fracture was of the clavicle aged 13 m. He had white sclerae and ligamentous laxity. Both patients had multiple fractures, hearing loss and dentinogenesis imperfecta.

Disclosure
The authors declared no competing interests.

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fracture was of the clavicle aged 13 m. Femur, fibula, pars of C2 vertebra and multiple rib fractures had occurred by 2.0 yrs. He had off-white sclerae, ligamentous laxity and was relatively tall (91st percentile).

Management
Both had normal bone biochemistry (including 25OH-vitamin D). Skeletal surveys undertaken at presentation (2.3 and 1.8 yrs, respectively) did not provide any clear indication of underlying bone disease. Targeted OI exome panel was undertaken in both patients. Patient 1 was homozygous for c.499del (p.Arg167fs) SERPINF1 variant. Patient 2 was compound heterozygous for c.582_585dup (pThr196Valfs*8), c.272C > A (p.Ala91Asp) SERPINF1 variants. Patient 1 developed vertebral fractures aged 5 yrs. Pamidronate was started at 6 yrs. Patient 2 developed unequivocal vertebral wedging by 2.5 yrs, underwent bone biopsy and was started on zoledronic acid.

Discussion
OI type VI patients have commonly been reported to sustain their first fractures between 6 and 18 m. As in our two cases, such a presentation, together with a relative lack of clinical and radiological clues to the diagnosis, can lead to suspicion of physical abuse that may be difficult to refute without genetic screening. Our experience suggests that careful evaluation of fracture history, family history (including consanguinity), together with examination specifically for off-white sclerae, ligamentous laxity and vigilance for early radiographic signs of vertebral deformity can distinguish children with OI type VI.

Disclosure
The authors declared no competing interests.

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P044

Abstract withdrawn.

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P045

Abstract withdrawn.

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P046

A 16 years old follow up in one case of congenital multiple hormone deficiency: growth, sexual development and bone metabolism

Chiara Petrolini, Stefano Stagi, Chiara Rubino, Daniela Gioe, Luisa La Spina, Francesca Peluso, Matteo Della Monica & Maurizio de Martino

Health Sciences Department, University of Florence, Anna Meyer Children’s University Hospital, Florence, Italy.

Background
Congenital multiple hormone deficiency (CMHD) is a rare condition caused by mutations in transcription factors involved in pituitary ontogenesis1. Incidence of mutations in POU1F1 gene in results between 3.8 and 7.7% 2. POU1F1 gene mutations lead to somatolactotroph and thryotroph deficiencies. Brain MRI can be normal or shows pituitary hypoplasia without extrapituitary anomalies.

Presenting problem
We describe the growth, the sexual development and the bone metabolism of a patient with CMHD due to a de novo Q167K mutation in POU1F1 gene at the heterozygous level1.

Clinical management
After investigations for hypoglycemic hypotonia, deficit of growth and craniofacial dysmorphism in a 8 months patient already in replacement therapy for a congenital hypothyroidism we found TSH, FT4, basal PRL, IGF1 and IGFBP3 under normal range. GH and TSH stimulating tests showed total unresponsiveness, so substitutive GH therapy was begun. The anterior pituitary gland was hypoplastic at brain MRI. The patient underwent periodical clinical, biochemical and radiological controls. Replacement therapy was adjusted with the patient’s weight, speed of growth and blood exams. At 7 years old the patient presented precocious puberty so she started Gonadotropin-releasing hormone agonists until 11 years old. At 10 years old vomiting and weakness especially during stressful events happened: ACTH was undetectable so we started hydrocortisone with disappearance of symptoms. Parameters of bone metabolism showed hypovitaminosis D, for which we gave supplementation with low compliance of the patient. The quantitative bone ultrasonometry showed normal Z Score of BTT and AD-SoS. The patient reached the height of 157.4 cm (~0.75 SDS) inside her target zone and the weight of 57.4 kg (0.20 SDS).

Discussion
There are no informations in literature about bone metabolism in CHMD, besides the prematurity, the lack of endogenous hormones, the chronic use of drug (L-thyroxine at high doses, glucocorticoids, gonadotropin releasing hormone analogue) associated with this condition could interfere with bone metabolism. A good management of substitutive therapy could guarantee a normal growth, sexual development and bone densitometry together with supplementation of vitamin D. The lack of hypogonadism in POU1F1 mutation could be a protective factor for bone density. However an annual evaluation of bone metabolism could be useful in these patients.

Disclosure
The authors declared no competing interests.

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P047

Vitamin D status before and during treatment for childhood cancer
Eryk Latoch, Katarzyna Muszyńska-Roslan, Milena Osinski, Anna Pazik & Maryna Krawczuk-Rybak
Medical University of Białystok, Białystok, Poland.

Recent studies suggest the link between low vitamin D levels and the prevalence of cardiovascular disease, diabetes, hypertension and a number of different types of cancer. Nowadays, vitamin D deficiency is recognized as a pandemic health problem and pediatric cancer patients may be even at higher risk than the healthy children. Children suffered from malignancy are especially exposed to its deficiency, because of the potential impact of the disease and its treatment. However, there have been limited studies of the 25-hydroxyvitamin D (25(OH)D) status in children treated for childhood cancer and during the chemotherapy.

Methods
The study group consisted of 28 children with cancer (male: 19, female: 9). Mean age at diagnosis: 4.99 ± 3.91 years. Mean time between examinations: 5.94 ± 1.2 months. Children were treated for acute lymphoblastic leukemia (n=19), lymphoma (n=4), solid tumors (n=5). The 25(OH)D level was assessed using the immunochemical method. Statistical analysis was performed using Wilcoxon rank sum test and χ2 test.

Results
Analysis for paired observations showed that mean level of 25(OH)D was significantly higher during therapy (29.25 ± 14.5 ng/ml) than before the treatment (22.23 ± 11.9 ng/ml) P=0.022. The (25(OH)D) deficiency (20-30 ng/ml) was found in 11 (39%) children before and in 13 (46%) patients during chemotherapy. While the (25(OH)D) insufficiency (<20 ng/ml) was observed in 12 subjects (43%) before and in 7 (25%) patients during treatment. No statistical differences between the number of (25(OH)D)-insufficient and (25(OH)D)-deficient patients in both study points were found (P>0.05).

Conclusions
Vitamin D supplementation in children treated for cancer significantly increases the mean level of vitamin D during the anticancer treatment. However, the number of vitamin D-insufficient and vitamin D-deficient patients did not change in the course of treatment. Further studies in this field is needed.

Disclosure
The authors declared no competing interests.

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P048

A rare cause of rickets
Sophia Sakka1, Šumá Uday1, Tabitha Randell2, Justin H Davies3, Ved Bishan Arya4, Caroline Brain4, Jeremy Allgrove4, Wolfgang Högl5 & Nick J Shaw1,5

1Department of Paediatric Propaedeutics and Bone Metabolic Diseases, Birmingham Children’s Hospital, Birmingham, UK; 2Department of Paediatric Endocrinology and Diabetes, Nottingham Children’s Hospital, Nottingham, UK; 3Department of Endocrinology and Diabetes, University Hospital Southampton, Southampton, UK; 4Department of Paediatric Endocrinology, Great Ormond Street Hospital, London, UK; 5Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK.

Background
The development of hypophosphataemic rickets in infants fed with the elemental formula (EF) Neocate has been recently reported. We present seven cases of exclusively Neocate-fed babies who developed hypophosphataemic rickets. Presenting problem
Three patients (P1,3,4) had incidental findings of rickets on chest X-rays, two (P2,6) developed leg deformities and rickets was confirmed on X-rays, and two (P5,6) presented with femur fractures. Patient 7 was found to have low phosphate concentrations on routine blood testing and was further investigated. All patients (age 5 months – 3.2 years) were exclusively fed on Neocate at presentation and had normal serum calcium and parathyroid hormone concentrations, raised alkaline phosphatase and hypophosphataemia. Vitamin D deficiency and renal phosphate wasting were excluded (Table 1).

Clinical management
Following exclusion of other causes of rickets, reduced intestinal phosphate absorption due to EF was considered. Patients 1-6 were treated with phosphate supplements after diagnosis of rickets. Patient 6 was previously on long term steroids and received one course of bisphosphonates after the fracture. Formula was changed eventually in all patients and phosphate concentrations normalized after 1 week-4 months. Clinical and/or radiological improvement of rickets was noted in P2,3,4. No X-ray confirmation of improvement is available so far in the others.

Discussion
The fact that serum phosphate improved following weaning of Neocate supports its role in the causation of hypophosphataemia; poor intestinal absorption of phosphate is the assumed mechanism in infants exclusively fed with Neocate. Clinicians should exercise caution in the use of EF in the absence of clear clinical indications. Infants who are being exclusively fed on Neocate should have close clinical and biochemical monitoring of bone profile, in accordance with existing guidance.

Disclosure
The authors declared no competing interests.

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P049

Difficulties in diagnostics and clinical classification of osteogenesis imperfecta in Poland
Agnieszka Rusinska1,2, Izabela Michalusi1,2, Elzbieta Jakubowska-Pietkiewicz1,2, Karolina Beska1,2, Paulina Adamiecka1 & Danuta Chlebna-Sokoł1,2

1Department of Paediatric Propaedeutics and Bone Metabolic Diseases, Medical University of Lodz, Lodz, Poland; 2Central Clinical Hospital Medical Hospital of Lodz, Lodz, Poland.

Introduction
Osteogenesis imperfecta (OI) is a genetically determined bone dysplasia characterised predominantly by recurrent fractures, reduced bone mineral density and some clinical features connected with colagenopathy. However, not all patients have exhibit all these signs, and in this situation diagnosis may be difficult.

Aim
The aim of this work is to compare clinical symptoms of various types of osteogenesis imperfecta and to present diagnostic problems based on the analysis of patients in Poland supervised by our Department.

Patients and methods
Studies were performed in a group of 123 patients with the diagnosis of osteogenesis imperfecta (type I – 54 children, type II – 2, type III – 41, type IV – 26), aged between 1 week old and 18 years old, 58 girls and 65 boys. A survey regarding complaints present in patients, diagnostic tests performed so far and treatment applied as well paediatric and anthropometric examinations were performed. Moreover, a bone densitometry scan was performed using dual-energy X-ray absorptiometry (DXA).

Results
Recurrent bone fractures were observed in 100/123 (81%) subjects. The total number of fractures ranged between 0 and 40; however, there were no statistically significant differences regarding the absolute number of fractures between different types. Skeletal deformations were present in 70/123 (60%) subjects, and the most frequently in patients with type II and III. Bluish sclera were observed in 101/123 (82%) subjects. Dentinogenesis imperfecta was diagnosed only in 28/123 (22%) patients. Statistically significantly lower bone mineral density was demonstrated in patients with type III, and the best bone mineral density was observed in patients with type I (P<0.05).

Table 1 Clinical and bioc hemical characteristics of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gestational age</th>
<th>Diagnosis</th>
<th>TRP (%)</th>
<th>P047</th>
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<td>OI (II)</td>
<td>99.5</td>
<td></td>
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<tr>
<td>Patient 2</td>
<td>34w</td>
<td>OI (III)</td>
<td>99</td>
<td></td>
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<tr>
<td>Patient 3</td>
<td>31w term</td>
<td>OI (IV)</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>
| Patient 4 | 31w term | OI (V) | 97 | NA
| Patient 5 | 31w term | OI (VI) | 97 | NA
| Patient 6 | 31w term | OI (VII) | 97 | NA
| Patient 7 | 31w term | OI (VIII) | 97 | NA

Abbreviations: OI: osteogenesis imperfecta; P047: no aid available.

DOI: 10.1530/boneabs.6.P049

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Conclusions
- Osteogenesis imperfecta is a heterogeneous group of skeletal disorders associated with increased predisposition to fractures, and characterised by significant variation of symptoms in individual types the disease.
- Clear diagnosis and prognosis can be difficult in some patients, due to the “overlapping” of symptoms in some types of OI (I and IV, II and III, III and IV) and the modifying effect of implemented treatment.

Acknowledgements
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Disclosure
The authors declared no competing interests.

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P050
The prevalence of fragility fractures in children with cerebral palsy in Greater Manchester, UK-a cross-sectional survey
Ekta Patel, Anne Ferguson, Sattar Alshryda, Zulf Mughal & Raja Padidela
Royal Manchester Children’s Hospital, Manchester, UK.

Background
Cerebral Palsy (CP) is the most common physically disabling childhood motor disorder. Fractures in this group of children are common, however, prevalence and risk factors associated with fractures in children with CP in the UK is not known.

Aims
The aims of this cross-sectional survey were 1) to determine the prevalence of fractures in children with moderate-to-severe CP in Greater Manchester 2) to determine the common sites of fracture and 3) to identify risk factors associated with fractures.

Methods
This was a retrospective survey of a cohort of 96 children with CP and Gross Motor Functional Classification Score (GMFCS) levels III-V. Data were collected from Greater Manchester database of children with CP, clinical health records, radiograph imaging and central database of fragility fractures in children with developmental delay. Sex, age, seizures, seizure medications, nutritional status, presence of contractures, hip dislocations and fracture history were all collected and statistically analysed.

Results
Twelve children were found to have fractures, with a total of 23 fracture episodes, providing a prevalence of 12.5%. The median age of fractures was 6 years. Sixty six per cent of the fractures were found to occur in children with a GMFCS level of V, with a 66% of fractures occurring in a child who was fed via a gastrostomy (χ² = 7.14, df = 1, P < 0.008). The most common fracture site was around knee joint. Thirty per cent (GMFCS-5, n = 3; GMFCS-3, n = 1) of the children had multiple fractures.

Conclusion
The prevalence of fractures in children with CP was found to be consistent with the figures in literature. Of the risk factors studied, the use of a gastrostomy-feeding device was the only variable found to be associated with an increased fracture risk. However, the presence of a gastrostomy may be a marker of the severity of the child’s CP, predisposing them to fractures. Healthcare professionals and carers should be aware of the increased risk of non-traumatic fragility fractures in children with CP.

Disclosure
The authors declared no competing interests.

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P051
Low bone density and fragility fractures in unbalanced translocation T(9;11)
Silvia Vai, Francesca Broggi & Maria Luisa Bianchi
Bone Metabolism Unit, Experimental Laboratory for Children’s Bone Metabolism Research, Istituto Auxologico Italiano IRCCS, Milano, Italy.

Background
Trisomy 9p is a rare abnormality caused by duplication of the short arm of chromosome 9. Translocation (9;11) is a rarer variant. Both anomalies are compatible with long survival. Clinical manifestations are very variable, and include short height, mental retardation, hypertelorism, strabismus, foot/ankle anomalies, delayed bone maturation. Low bone mineral density (BMD) or fragility fractures have never been reported.

Presentation
A 12-year-old boy and a 9-year-old girl with unbalanced translocation t(9;11) were referred to our Center: the boy for two forearm fractures after minimal trauma, the girl for kyphosis. Blood exams, including calcium, phosphorus and magnesium, were normal for age in both subjects. The 24-hour urine collection revealed much reduced calcium (boy: 56 mg/24 h; girl: 58 mg/24 h; normal range 100-250 mg/24 h) with normal phosphaturia. 25OHD vitamin D levels were normal in both children, but the boy had moderately elevated parathyroid hormone levels (77 pg/ml). In both children, bone resorption turnover markers were moderately increased for age. Other metabolism studies (including thyroid activity, intestinal malabsorption, liver and kidney function, inflammatory markers, electrolytes and urine analysis) revealed no abnormalities, thus excluding other causes of low bone density. Dual energy X-ray absorptiometry (DXA; by Hologic Discovery) was performed at lumbar spine (LS) and on total body less head (TBLH). In both cases, at both sites, BMD was severely reduced (LS BMD Z-score: boy −3.8, girl −4.2; TBLH BMD Z-score boy −3.2, girl −3.4).

Clinical management
In the girl only, spine X-rays revealed fractures of 5 thoracic vertebrae. For this reason, she was put on treatment with i.v. pamidronate (0.5 mg/kg per day for 3 consecutive days every 4 months). After 3 years of treatment no new vertebral or peripheral fractures were observed. DXA revealed a significant increase in BMD (spine Z-score −1.9).

Discussion
The rarity of this genetic alteration does not allow controlled studies. Our finding of reduced BMD and fractures in two cases of unbalanced translocation t(9;14) justifies DXA BMD evaluation, search for undiagnosed vertebral fractures, and adequate therapeutic measures as necessary, in similar cases.

Disclosure
The authors declared no competing interests.

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P052
Hypophosphatasia - from symptom to diagnosis - case report
Izabela Michulak, Agnieszka Rusinska, Gabriela Orzechowska & Danuta Chełbna Sokol
Department of Pediatric Propedeutic and Bone Metabolic Diseases Medical University of Lodz, Lodz, Poland.

Introduction
Hypophosphatasia is a rare genetic disease caused by a mutation in the tissue-nonspecific alkaline phosphatase gene. TNSALP gene is located on the short arm of chromosome 1 (p36.1-34). Over 200 point mutations have been described for this gene so far. Hypophosphatasia is inherited in an autosomal recessive or dominant way, which is related to the severity of symptoms. Pathophysiology of this disease is associated with the impaired function of osteoblasts that do not incorporate calcium into the matrix of the newly formed osseous tissue. Low alkaline phosphatase activity disturbs hydroxyapatite formation. The clinical picture of the disease depends on the time of symptom emergence. Depending on the severity, 6 hypophosphatasia sub-types may be identified: perinatal, infantile, childhood, adult hypophosphatasia, odontohypophosphatasia, and pseudohypophosphatasia.

Aim
The aim of this study was to present the case of a girl with calcium-phosphate disorders, diagnosed with hypophosphatasia.

Method
Child's medical records from birth to diagnosis and treatment administration were subject to analysis. The reason why the diagnosis was delayed in our female patient and other children described in the literature was the omission of alkaline phosphatase test interpretation; this enzyme levels have always been lowered, even with a poor clinical picture.

Conclusions
1. Hypophosphatasia diagnosis is difficult due to a variety of forms and clinical symptoms, as well as to its occasional occurrence.
2. The diagnostics of disorders with skeletal system lesions should consider both lower and upper reference ranges of biochemical parameters, because their thorough analysis is the base for the differential diagnostics.
3. The introduction of enzyme replacement therapy leads to clinical, biochemical and radiological improvement in patients with hypophosphatasia.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P052
Determinants of bone density in Duchenne muscular dystrophy

Francesca Broggi1, Silvia Val1, Giovanni Baranello2, Ksenia Gorni3, Grazia D’Angelo3, Marika Pane3, Gianluca Vita4 & Maria Luisa Bianchi1
1Bone Metabolism Unit, Experimental Laboratory for Children’s Bone Metabolism Research, Istituto Auxologico Italiano IRCCS, Milano, Italy; 2Child Neurology Unit, Istituto Nazionale Neurologico C. Besta IRCCS, Milano, Italy; 3Centro Clinico Nemo, Ospedale Niguarda, Milano, Italy; 4Istituto Medea IRCCS, La Nostra Famiglia, Bossio Parini (LC), Italy; 5Dipartimento Neurologia Pediatrica, Policlinico Universitario “Agostino Gemelli” Università Cattolica, Roma, Italy; 6Centro Clinico Nemo Sud, U.O.C. di Neurologia e Malattie Neuromuscolari, Policlinico Universitario di Messina, Messina, Italy.

Objective
Low bone mineral density (BMD) and increased frequency of peripheral and vertebral fractures have been reported in boys with Duchenne muscular dystrophy (DMD), but studies on the determinants of low BMD are still very few. We are currently carrying out a multicenter, prospective study aimed to identify the characteristics of DMD boys with a higher risk of bone loss and fractures.

Methods
Forty-two DMD boys (mean ± standard deviation: age 9 ± 3.3 years) underwent BMD evaluation by dual energy X-ray absorptiometry (DXA), with calculation of bone mineral apparent density (BMAD), evaluation of bone turnover markers (plasma osteocalcin (OC); serum bone-specific alkaline phosphatase (BSAP) and C-terminal telopeptide (CTX)), serum osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B ligand (RANKL), interleukin 6 (IL-6), and (for the first time in DMD) serum Dickkopf related protein 1 (Dkk1). All subjects were on long-term glucocorticoid (GC) treatment.

Results
At baseline DXA evaluation, 32/42 (76.2%) patients had Z-score spine BMD <2.0. 9/42 (21.4%) patients had sustained at least one vertebral fracture (all at thoracic spine; eight of nine patients with fractures were aged ≥11 years); and 10/42 patients (23.8%) had serum 25-OH-vitamin D levels <20 ng/ml. Bone formation markers (OC, BSAP) were within normal range for age, while bone resorption markers (CTXs) were increased (P < 0.05). The RANKL/OPG ratio was significantly higher than normal (78.2 ± 37.4 vs 28 ± 11 in normal controls; P < 0.001), while Dkk1 was lower than normal (25.3 ± 19 pg/ml vs 37 ± 18.3 pg/ml in normal controls; P < 0.02). BMAD Z-scores were significantly correlated (inversely) with age (P < 0.01) and duration of GC treatment (P = 0.02), and also (directly) with 25-OH-vitamin D levels (P < 0.01). Significant inverse correlations were found between BMAD Z-scores and Dkk1 levels (P < 0.01) and between BMAD Z-scores and IL-6 (P < 0.05).

Conclusion
In our study, spine BMD in DMD children was influenced by age and steroids (i.e. higher age or prolonged GC treatment corresponded to lower BMD). Other relevant determinants were 25-OH-vitamin D status, IL-6 levels, imbalance between RANKL and OPG with insufficient compensation due to Dkk1 reduction.

Disclosure
The authors declared no competing interests.

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Conservative management of metabolic derangements in osseous tissue among patients with vitamin D-dependent rickets type 2

Svetlana Martsenyka & Tamara Kincha-Polischuk
Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences, Kyiv, Ukraine.

Objectives
To determine the influence of conservative management upon genetically-determined metabolic derangements in osseous tissue among patients with vitamin D-dependent rickets type 2.

Methods
A series of experiments were undertaken to investigate the effects of immune cells on the growth and alkaline phosphatase activity of the osteosarcoma cell line Saos-2. Peripheral blood mononuclear cells were isolated from healthy volunteers, and a CD4+ lymphocytes enriched population generated. These two populations were co-cultured with Saos-2 cells studying the effects of immune cell number, their activation status, and the role of cell contact.

Experiments
The experiments showed that increasing numbers of activated CD4+ lymphocytes reduced Saos-2 cell number by 48% (P < 0.001) with a clear dose response effect. In contrast, resting CD4+ lymphocytes increased Saos-2 cell number by 27% (P = 0.013). The presence of a transwell insert increased the number of Saos-2 cells by 106% in the activated condition (P < 0.001) and by 7% in the resting condition (P = 0.466). In addition, Saos-2 altered expression of activation markers by CD4+ lymphocytes, increasing expression of CD25 (3.5% vs 27.5%; P = 0.002) and CD69 (0.7% vs 24%; P = 0.029) by resting cells and decreasing their expression by activated cells. Introduction of immune cells after Saos-2 adhesion abrogated the observed effect on their growth. PBMC effects were similar to that of the CD4+ lymphocytes.

Conclusion
The findings outlined support the paradigm that activated CD4+ lymphocytes inhibit the growth of osteoblasts. The additional findings of immune cells supporting growth of Saos-2 cells and two-way signaling suggest a potentially more complex relationship. Observations in the published literature describe antigen experienced lymphocytes maintained within the bone, and osteoimmune interactions supporting both the immune system and bone metabolism. Therefore we need to consider bone not only as a primary, but also a secondary lymphoid organ.

Disclosure
The authors declared no competing interests.

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The influence of immune dysregulation on bone metabolism in children with inflammatory bowel disease: the potential for bone as a secondary lymphoid organ

Gareth Pennman1,2, David Campbell3, A. Graham Pockley1 & Nicholas Bishop1
1Academic Unit of Child Health, University of Sheffield, Sheffield, South Yorkshire, UK; 2Department of Paediatric Gastroenterology, Sheffield Children’s Hospital, Sheffield, South Yorkshire, UK; 3Bone Metabolism Research, Istituto Auxologico Italiano IRCCS, Milano, Italy.

The influence of immune dysregulation on bone metabolism in children with inflammatory bowel disease: the potential for bone as a secondary lymphoid organ.

Background
Whilst their clinical relevance in terms of fracture may be questioned, systemic inflammatory disorders in children impacts on their bone metabolism and reduces bone mineral density. Similar observations in adults are in part explained by interactions between lymphocytes and osteoclasts via the receptor activator of nuclear factor kappa-B ligand/osteoprotegerin pathway, but in a child’s growing bone it is necessary to look at the effects of lymphocytes on osteoblasts.
stage, then down to 45000 U/month) and alfalcacidol (up to 1 µg/month at the onset of treatment) for activation of receptors to vitamin D (VDR). Subsequently, the management does not require high doses of vitamin D. For the achievement of therapeutic effect in treatment of orthopedic manifestations inrickety process level of the hormonal form of vitamin D (calcitriol) should be limited within range 250–350 mg/ml (P < 0.05). There is no reason to use alfalcacidol for pharmacological treatment of VDDR type 2.

Disclosure
The authors declared no competing interests.

Table 1 Average measures of osseous metabolism among examined patients with VDDR type 2.

<table>
<thead>
<tr>
<th>Indices of bone metabolism</th>
<th>Stage of treatment</th>
<th>M ± m</th>
<th>Stage of treatment</th>
<th>M ± m</th>
<th>Stage of treatment</th>
<th>M ± m</th>
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<th>M ± m</th>
<th>Stage of treatment</th>
<th>M ± m</th>
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</thead>
<tbody>
<tr>
<td>Ca (~)</td>
<td>1.24 ± 0.0097</td>
<td>1.28 ± 0.104</td>
<td>1.28 ± 0.0154</td>
<td>1.32 ± 0.0087</td>
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<tr>
<td>P</td>
<td>1.71 ± 0.0099</td>
<td>1.837068 ± 0.0583</td>
<td>1.704 ± 0.0006</td>
<td>1.79 ± 0.1079</td>
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<tr>
<td>Ca (~)</td>
<td>2.51 ± 0.0158</td>
<td>2.57 ± 0.0557</td>
<td>2.59 ± 0.0000</td>
<td>3.09 ± 0.4082</td>
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<tr>
<td>25(OH)D</td>
<td>34.51 ± 3.3962</td>
<td>69.05 ± 4.9992</td>
<td>77.60 ± 6.3518</td>
<td>73.16 ± 30.2903</td>
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<tr>
<td>1,25(OH)2D</td>
<td>140.92 ± 6.6843</td>
<td>270.79 ± 20.5888</td>
<td>306.63 ± 24.7801</td>
<td>306.52 ± 33.7730</td>
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<tr>
<td>PTH</td>
<td>32.87 ± 2.6910</td>
<td>25.25 ± 3.3325</td>
<td>23.42 ± 0.9011</td>
<td>24.15 ± 4.0938</td>
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<tr>
<td>Osteocalcin (~)</td>
<td>33.95 ± 4.3689</td>
<td>16.15 ± 2.0541</td>
<td>15.03 ± 0.2647</td>
<td>15.45 ± 4.1944</td>
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<tr>
<td>Urine calcium (daily)</td>
<td>1.74 ± 0.1288</td>
<td>2.047950 ± 0.3555</td>
<td>1.4 ± 0.2638</td>
<td>1.4285 ± 4.3751</td>
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<tr>
<td>Urine phosphorus (daily)</td>
<td>11.16 ± 0.1625</td>
<td>14.7205 ± 1.8063</td>
<td>11.55 ± 0.8004</td>
<td>8.05 ± 0.0305</td>
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<tr>
<td>B-CTx</td>
<td>885.81 ± 41.2451</td>
<td>665.98 ± 46.7807</td>
<td>587.66 ± 35.3047</td>
<td>583.29 ± 31.7597</td>
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</table>

* = significant difference of the parameter comparing to the 1st stage of treatment (P < 0.05) / * = trend close to significant difference of the parameter comparing to the 1st stage of treatment (0.1 > P > 0.05).

Table 2 Correlation between blood and urine parameters among the patients with VDDR type 2 (before the treatment).

Table 3 Correlation between blood and urine parameters among the patients with VDDR type 2 (after the 1st therapeutic stage).

Objective
Hypophosphatasia (HPP) is a rare hereditary disease, leading to deficits in bone and tooth mineralization, muscular as well as neurological abnormalities due to decreased enzymatic activity of the tissue-nonspecific alkaline phosphatase (TNAP, encoded by the alpl gene). In this project, the zebrafish (Danio rerio) will be established as a new and valuable animal model for HPP research. Consequently, endogenous TNAP expression should be analyzed in different zebrafish samples and a set of staining methods should be established for future analysis of TNAP in zebrafish. In addition, first functional experiments should clarify consequences of gain- and loss-of-function variants in the zebrafish model.

Methods
In order to analyze the endogenous and tissue-specific TNAP function in zebrafish, Alkaline Phosphatase (AP) activity-assays (CSPD-assays) were performed in various tissue lysates. In parallel, ELF® 97 endogenous phosphatase staining was established on cryosections of different zebrafish tissues and stages to clarify spatio-temporal distribution. Additional functional experiments were performed by RNA microinjections into one-cell stage zebrafish embryos in order to establish short-time TNAP overexpression as well as TNAP Morpholino knockdown.

Results
CSPD-assays revealed AP activity in all analyzed tissues, with the highest detectable levels in the eyes, skin, and heart. The TNAP-specific inhibitor levamisole was able to diminish the detectable signals dependent on the respective concentration in all analyzed tissues apart from gut and liver, indicating the expression of other AP isozymes in these tissues. Furthermore, ELF® 97 staining showed tissue restricted TNAP activity in cornea, bones, musculature, gills, kidneys, and brain of zebrafish. For functional investigations either RNA were derived from patient specific TNAP isoforms were microinjected into zebrafish embryos to establish short-time overexpression or a TNAP Morpholino knockdown was performed. This set of experiments clearly showed TNAP’s influence on early developmental processes and clarified the functional consequences of different TNAP mutations.

Conclusions
TNAP activity was detected and could be localized in different zebrafish tissues using either CSPD-assay or ELF® 97 staining. Furthermore, short-time overexpression and knockdown of TNAP could be established and illustrate the feasibility of zebrafish for functional TNAP analyses. Taken together, the zebrafish seems to be a promising in vivo model for HPP, but further refinements are required to fully establish it as a new animal model for the investigation of new therapies for HPP.

1 S.G. and D.L. contributed equally to this work.
2 F.J. received honoraria for lectures and advice from Alexion.

Acknowledgment
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P057
Zebrafish as a model for hypophosphatasia
Stephanie Graser1, Daniel Liedtke2, Barbara Geidner2, Yvone Heppenstiel1, Franz Jakob1 & Eva Kloocki1
1Orthopedic Department, Orthopedic Center for Musculoskeletal Research, University of Würzburg, Würzburg, Germany; 2Institute of Human Genetics, Biocentre University of Würzburg, Würzburg, Germany.

The relationship between maternal and child bone density in Nigerian children with and without nutritional rickets
Tanner Bommersbach1, Philip Fischer1, John Pettit2 & Tom Thacher1,3
1Mayo Clinic, Rochester, MN, USA; 2University of the Witwatersrand, Johannesburg, South Africa; 3Jos University Teaching Hospital, Jos, Plateau State, Nigeria.

Objective
To determine the relationship between maternal and child bone density in a cohort of mothers and their children with and without rickets.

Methods
Using a case-control design without matching, areal forearm bone mineral density (aBMD) was measured in 52 and 135 Nigerian children with and without rickets, respectively, and their mothers. The metaphyseal site was located at the site of minimal bone density of the distal radius and ulna. The diaphyseal site was located at 1/3 the distance from the wrist to the elbow. Active rickets was confirmed or excluded in all children radiographically. We performed multi-variate linear regression analyses to assess the relationship between maternal and child aBMD z-scores and to assess if nutritional rickets modified this relationship.

Results
Mothers of children with rickets had an earlier age of menarche (14.5 ± 1.4 vs 15.1 ± 3.0 years, P = 0.05), were taller (161.3 ± 5.5 vs 159.2 ± 6.3 cm, P = 0.04), and had a shorter duration of breast feeding (16.3 ± 6.5 vs 19.0 ± 3.1 months, P < 0.001) than mothers of children without rickets. Children with rickets were younger (3.3 ± 1.9 vs 5.2 ± 2.4 years, P < 0.001) and more likely male (57.0% vs 42.3%, P = 0.04) than children without rickets. In a regression analysis adjusted for the presence of rickets in the child, child’s age and sex, height-for-age z-score, weight-for-age z-score, child forearm aBMD z-scores were associated with
maternal Z-scores at both metaphyseal (effect estimate 0.23 (95% CI 0.08 to 0.37)) and diaphyseal (effect estimate 0.16 (0.01 to 0.30)) sites of the forearm. In the adjusted model, the presence of rickets was inversely associated with child’s aBMD Z-score at the diaphyseal site (effect estimate −0.45 (−0.65 to −0.24)) but not at the metaphyseal site. The positive relationship of maternal aBMD Z-score with the child’s aBMD Z-score was marginally greater in children with rickets (r = 0.46) than in those without rickets (r = 0.20) at the diaphyseal site (P = 0.06 for interaction) but not at the metaphyseal site (P = 0.46).

Conclusion

In Nigerian children with and without rickets, forearm aBMD Z-scores were positively associated with maternal Z-scores at metaphyseal and diaphyseal sites. Rickets in the child marginally modified the relationship at the diaphyseal site only.

Disclosure

Dr. Thacher is a consultant for Biomedical Systems, Inc.

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P059

Retrospective evaluation of serum alkaline phosphatases (ALP) in Italian children referred to a tertiary children’s hospital

Stefano Stagi1, Chiara Rubino1, Chiara Petrolini1, Elena Sandini1, Chiara Maggioni2, Roberto Schiatti2 & Maurizio de Martino2

1Health Sciences Department, University of Florence, Anna Meyer Children’s University Hospital, Florence, Italy; 2Central Laboratory, Anna Meyer Children’s University Hospital, Florence, Italy.

Objectives

To evaluate the frequency of low serum alkaline phosphatase (ALP) activities in patients referred to a tertiary children’s hospital. Another item was to explore potentially missed diagnoses and to evaluate the role of laboratory screening for hypophosphatasia.

Study design

A retrospective evaluation over an 6-year period (between December 2010 and December 2016) carried out to identify children and adolescents, referred to Anna Meyer Children’s University Hospital in Florence, younger than 16 years old with low ALP activity for sex, age, and pubertal stage.

Results

Of 16,896 patients and 26,724 analyzed samples in our Hospital, 523 (3.1%) patients had low ALP activity for sex, age, and pubertal stage.

Missed diagnoses of hypophosphatasia are frequent in a tertiary children’s Hospital. However, patients with persistently low ALP activity require clinical, biochemical, and radiological assessment for hypophosphatasia, even in the absence of obvious clinical symptoms. Our data is important also because early detection of cases of hypophosphatasia is of utmost importance for early and appropriate treatment and genetic counselling.

Disclosure

The authors declared no competing interests.

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P060

Abstract withdrawn.

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P061

Renal tubular acidosis with an elevated urinary β2 microglobulin in a boy presenting with sporadic hypophosphatemic rickets and intellectual disability (Dent’s Disease)

Justin Brown1,2, Lilian Johnston3, Alison Yeung1,4 & Christine Rodda1,3,4

1Department of Paediatrics, Monash University, Clayton, Victoria, Australia; 2Department of Paediatrics, Parkville, Victoria, Australia; 3AIMSS, University of Melbourne, Sunshine Hospital, St Albans, Victoria, Australia; 4Monash Genetics, Monash Health, Clayton, Victoria, Australia.

Background

X linked hypophosphatemic rickets is the commonest cause of renal phosphate wasting, however sporadic cases may warrant additional investigations to exclude less common causes, as exemplified by our case.

Presenting problem

A 3 year 7 month boy was referred for assessment and ongoing management of rickets and short stature (height less than 1st %). He originally presented with leg bowing and waddling gait from the age of 12 months. His parents were non-consanguineous and there were no other affected relatives. He had been treated with routine cholecalciferol supplementation without biochemical or clinical improvement. Subsequently he was also diagnosed with a moderate intellectual disability (FSIQ 60). Initial investigations showed normal serum calcium, 25 Vitamin D and PTH, with a low serum phosphate (1.06 mmol/l), raised alkaline phosphate (524 u/l), and a decreased tubular reabsorption of phosphate (TRP) of 85%.

Clinical management

With a clinical picture suggestive of X linked hypophosphatemic rickets, he was commenced on calcitriol and phosphate with initial clinical improvement. Measurement of ionised calcium lead to the recognition that he also had a persistently marked metabolic acidosis with a pH of 7.16. Further investigations revealed a raised protein:creatinine ratio of 0.17 g/mmol (0-0.03) and a low molecular weight (LMW) proteinuria (urine β2 microglobulin 22700 μg/l (0-300)) without evidence of a generalised tubulopathy. Renal ultrasound also demonstrated early nephrocalcinosis.

Discussion

LMW proteinuria is pathognomonic of Dent’s Disease which is defined by the additional clinical features of hypercalciuria with one of the following nephrocalcinosis/nephrolithiasis, haematuria, chronic kidney disease or suggestive family history. Dent’s disease is a heterogeneous group of X linked recessive disorders of variable phenotype associated with proximal tubular dysfunction and is typically associated with inactivating mutations of CLCN5. Extra renal manifestations, including intellectual disability are seen in Dent-2 disease associated with OCR1 mutations. Whole exome sequencing is awaited. We recommend careful assessment of acid base status and renal tubular function in all children (particularly male) presenting with sporadic hypophosphatemic rickets, as early recognition of Dent’s disease is important to ensure appropriate treatment and genetic counselling.

Disclosure

The authors declared no competing interests.

Reference


DOI: 10.1530/boneabs.6.P061

P062

Lysinuric protein intolerance presenting with short stature and osteoporosis

Gülay Karagüzel1, Halil Aydin1 & Begum Erginer1

1School of Medicine, Karadeniz Technical University, Trabzon, Turkey; 2Ankara Hospital, Baskent University, Ankara, Turkey.

Background

Lysinuric protein intolerance (LPI) is a rare autosomal recessive multisystemic metabolic disorder, caused by defective transport of cationic amino acids at the basolateral membrane of epithelial cells.

Presenting problem

A 14-year-old boy was referred to our clinic for short stature. He was the second child of consanguineous healthy parents. He had previously suffered for 4 fractures occurred secondary to minimal trauma in the upper limbs. He reported that he did not like to eat meat and dairy products. His weight was 30 kg (< 3p), height 135 cm (< 3p), height SDS -3.28, Tanner 2 pubertal stage, bone age 11.5 years, target height 171.5 cm, 1 cm hepatomegaly, no splenomegaly On laboratory; his hemoglobin, hepatic and renal function tests, serum calcium, phosphorus, and parathormone were normal. His serum alkaline phosphatase was

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Clinical management

His osteoporosis had treated with intravenous pamidronate infusion cycles, vitamin D and calcium supplements. After two years, repeated amino-acid analysis revealed high urinary concentrations of lysine, arginine and ornithine and low blood concentration of lysine compatible with the diagnosis of LPI. The blood was separated for genetic testing and treatment with a low-protein diet and supplementation with citrulline and nitrogen-scavenging drugs was started.

Discussion

The diagnosis of LPI is difficult due to nonspecific clinical features. Classic symptoms of protein intolerance may remain unnoticed because of subconscious avoidance of dietary protein. Over time, patients may present delayed growth, osteoporosis, hepatosplenomegaly, and life-threatening complications such as alveolar proteinosis, haemophagocytic lymphohistiocytosis and macrophage activation syndrome. SLCA7A1 is the only gene in which mutation is currently known to cause LPI.

Disclosure

The authors declared no competing interests.

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**P064**

**Extensive periosteal new bone formation secondary to copper deficiency in a 2 year-old boy with arterial tortuosity syndrome**

Sasigarn Bowden, Brent Adler, Ala Shaiikhkhalil, Kan Hor, Kim McBride & Katherine Steingass

Nationale Children’s Hospital, Columbus, OH, USA.

Background

Periosteal reaction can be a manifestation of various underlying medical conditions, including tumor, infection, trauma, metabolic or genetic diseases. Presenting problem

A 2-year-old male presented for evaluation of periosteal bone formation in symmetrical distribution of proximal humerus, radius, ulna, femur, and clavicles, noted after having persistent fussiness, irritability and inability to bear weight and use arms for 3 weeks. He had a complex medical history: 30 week prematurity, hiatal hernia (post repair), intestinal malrotation, and mild cardiac left outflow tract obstruction with mild left ventricular hypertrophy. His feeding was elemental formula via gastrojejunostomy tube.

Clinical management

He had normal serum calcium, phosphorus, alkaline phosphatase, CRP, LDH, vitamin D, and PTH levels. Vitamin C was elevated at 138 μmol/L (normal 23–114), thus ruling out scurvy. Coagulation studies and vitamin A were normal. Abdominal and chest CT scan obtained due to concern for underlying malignancy demonstrated tortuosity and ectasia of aorta. This finding in addition to his past medical history raises suspicion of arterial tortuosity syndrome (ATS). DNA sequencing revealed homozygous mutation in the SLC2A10 gene, confirming ATS. He had very low serum copper (<10 μg/dl; normal 75–153) and ceruloplasmin levels (<7 mg/dl; normal 21–53), indicating copper deficiency.

He was treated with one intravenous copper infusion followed by daily enteral copper. Serum copper and ceruloplasmin levels repeated one month later were normal, while gradual clinical improvement in bone pain was noted, suggesting that the skeletal changes were attributed to copper deficiency. Repeat bone radiograph 1 month after treatment showed a more exuberant maturing periosteal new bone formation along the proximal to mid femur and tibia, suggesting bone healing.

Discussion

Copper deficiency impairs bone collagen and elastin integrity, thereby manifesting as metabolic bone disease, the work up of which led to diagnosis of ATS in this patient. Periosteal reaction has not been reported in ATS, a rare connective tissue disease that can have skeletal abnormalities. It is unclear whether copper deficiency is solely secondary to malabsorption in the jejunum or may be associated with ATS due to loss of glucose transporter 10 that may have an indirect role in copper metabolism.

Disclosure

The authors declared no competing interests.

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**P065**

A novel form of congenital rickets due to a recurrent gain of function mutation in CYP3A4

Christine P Rodda1, Michael A Levine2, Jeffrey D Roizen2, Muhammad K Javadi,1 Peter R Ebeling1,1 Hahn Nguyen1, Peter Dewez2 & Nicholas J Shaw6

1Australian Institute for Musculoskeletal Science (AIMSS), University of Melbourne and Paediatric Department, Sunshine Hospital, St Albans 3021, Australia; 2Division of Endocrinology and Diabetes, The Children’s Hospital of Philadelphia, Philadelphia, PA, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, PA, USA; 3National Institute for Health Research (NIHR) Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, UK; 4Monash University, Monash Medical Centre, Clayton 3168, Australia; 5Paediatrician, 44 Docker Street, Wangaratta 3677, Australia; 6Birmingham Children’s Hospital and University of Birmingham, Birmingham, UK.

Disclosure

Hogler: travel, consulting fees from Ultragenyx; Portale: travel fees, advisory panel from Ultragenyx; Carpenter: grant support, travel fees from Ultragenyx; Imel, Boot, Linglart, van’t Hoff: travel, consulting fees from Ultragenyx; Paleide: consulting f.

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Although Vitamin D deficiency is the most common form of rickets worldwide, when there is a failure to respond to cholecalciferol, inborn errors of vitamin D metabolism should be considered. We describe two unrelated individuals who presented with early onset rickets characterised by reduced serum levels of 25(OH)D and 1,25(OH)2D, and a deficient response to Vitamin D2/D3 and calcitriol. Case 1: A Caucasian Australian girl with non-consanguineous parents was referred aged 2.5 years with refractory rickets. Bow legs and an unsteady gait were noted from aged 20 months. She was otherwise healthy, with regular direct sun exposure. Cholecalciferol 5000 IU daily and calcitriol 0.25 μg orally daily for 1 month, resulted in no clinical improvement. Cholecalciferol 600 000 IU was given nasogastrically as a vitamin D generation test. Five days later, her serum calcium increased to near normal to 2.02 mmol/l and her 25 (OH)D increased to 97 nmol/l. She was commenced on cholecalciferol syrup 50 000 IU daily, which resulted in healing of her rickets radiologically and biochemically. Case 2: A girl born to non-consanguineous parents in Jordan developed bowed legs in infancy, with delayed walking at 4.5 years. Calcitriol was commenced in early childhood, with no improvement. She came to the UK in 2009, aged 16 years. Despite large doses cholecalciferol doses, she was unable to achieve a satisfactory level of 25(OH)D or normalise PTH. Using whole exome sequencing (WES), an identical heterozygous single nucleotide change in exon 10 of the CYP3A4 gene (c.902T>C) resulting in replacement of serine by threonine at codon 301 (p.I301T) was identified in both girls (1). This change was not present in other first-degree relatives, nor in any public databases searched. The mutant CYP3A4 (p.I301T) was nearly 10-fold more active than the wild type enzyme. In summary, a recurrent missense mutation in CYP3A4 resulted in increased CYP3A4 activity with enhanced inactivation of vitamin D metabolites. This gain-of-function mutation of CYP3A4 causes a novel form of congenital rickets, (Vitamin D dependant rickets Type 3) responsive to large doses of oral cholecalciferol.

Disclosure
The authors declared no competing interests.

Reference

Table 1 Biochemical findings of case 1 and case 2.

<table>
<thead>
<tr>
<th>Laboratory investigation</th>
<th>Case 1 Initial</th>
<th>Case 1 Follow-up 9 months</th>
<th>Case 1 Follow-up 16 months</th>
<th>Case 2 Initial</th>
<th>Case 2 Follow-up 9 months</th>
<th>Case 2 Follow-up 16 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (2.05–2.60 mmol/l)</td>
<td>2.13</td>
<td>2.20</td>
<td>2.33</td>
<td>2.24</td>
<td>2.49</td>
<td>2.53</td>
</tr>
<tr>
<td>Phosphate (1.04–1.8 mmol/l)</td>
<td>1.15</td>
<td>1.45</td>
<td>1.51</td>
<td>0.97</td>
<td>1.89</td>
<td>1.77</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>454</td>
<td>382</td>
<td>324</td>
<td>623</td>
<td>311</td>
<td>314</td>
</tr>
<tr>
<td>Parathyroid hormone (pg/ml)</td>
<td>5.1</td>
<td>1.6</td>
<td>2.1</td>
<td>3.0</td>
<td>2.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.7</td>
<td>12.3</td>
<td>12.3</td>
<td>11.5</td>
<td>11.5</td>
<td>11.7</td>
</tr>
<tr>
<td>MCV (71–91 fl)</td>
<td>79.4</td>
<td>80.5</td>
<td>80.5</td>
<td>74.7</td>
<td>73.7</td>
<td>73.7</td>
</tr>
<tr>
<td>MCH (25.8–31.7 pg)</td>
<td>14.3</td>
<td>25.2</td>
<td>25.2</td>
<td>21.4</td>
<td>21.4</td>
<td>21.4</td>
</tr>
<tr>
<td>Tetanic KCl score</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Both cases were treated with oral phosphate supplements, one alpha vitamin D and ferrous gluconate. The rickets healed and anemia was resolving after 9 months on the above mentioned treatment and after another 9 months of ferrous gluconate only (Table 1).

Discussion
These two clinical cases highlight the association in children between iron deficiency anemia secondary to pica and hypophosphataemic rickets. The biological intersection of iron and phosphate homeostasis through FGF23 is unknown and complex. Although, FGF23 levels were not measured in these children, it is possible that elevated FGF23 levels as a consequence of chronic iron deficiency associated with pica were responsible for hypophosphataemia and rickets. The association between pica, iron deficiency and FGF23 needs further investigation.

Disclosure
The authors declared no competing interests.

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P067

Mid-gestation anomaly scan cannot be relied upon for screening for severe perinatal hypophosphataemia

Amish Chinoy1, Chibueke Iruoloh2, Bronwyn Kerr2, Robert Yates1, Zulf Mughal1 & Raja Padidela1
1Royal Manchester Children’s Hospital, Manchester, UK, 2St Mary’s Hospital, Manchester, UK.

Background
Hypophosphatasia (HPP) is a disorder of bone mineralisation caused by deficiency of alkaline phosphatase (secondary to ALPL gene mutations), causing accumulation of inorganic pyrophosphate (PPi) thus inhibiting bone mineralisation. The perinatal form presents with severe manifestations at birth. Most severe skeletal manifestations are detectable by 20 weeks gestational age (GA) anomaly scan, and antenatal care within the UK practices routine detailed anomaly ultrasound scan (USS) at 20 weeks GA, with no further detailed scans until delivery if this is satisfactory. We describe a patient where there was a family history of HPP and antenatal scan at 20 weeks failed to detect any skeletal abnormalities.

Case history
Parents were known to be carriers of ALPL mutation. They had had a previous termination of an affected foetus. Detailed USS at 16 and 20 weeks GA were normal and therefore family decided to continue with pregnancy (Fig. 1A). Because of poor foetal growth a further USS was performed at 31 weeks GA, which showed poor skeletal mineralisation, micromelia and limb fractures (Fig. 1B). Genetic testing at 32 weeks GA confirmed HPP. This infant was born with severe skeletal manifestations of HPP (Fig. 1C). Asfotase alfa was initiated neonatally, resulting in survival of the infant and improvement of bone mineralisation (Fig. 1D).

Figure 1 Right tibia and fibula: A: Normal at 20 weeks GA; B: Fractures and rickets-like changes at 31 weeks GA; C: At birth, shows shortened and hypomineralised bones; D: Improvement in bone mineralisation following 5 months treatment with asfotase alfa.

Discussion
Skeletal features of perinatal HPP may not be apparent at 20 weeks USS. We speculate that foetal accumulation of PPi during pregnancy results in progressive skeletal demineralisation, which may only become apparent on USS in later pregnancy.

Conclusion
In the presence of a family history of HPP, we recommend antenatal genetic testing should be offered to families, as early USS cannot be relied upon for confirming diagnosis of perinatal HPP.

Disclosure
The authors declared no competing interests.

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Craniosynostosis can occur in children with nutritional rickets
L Forestier-Zhang1, P Arundel1, R Gilby Cross1, M Z Mughal1, A C Offiah1 & M S Cheung2
1 Evelina London Children’s Hospital, London, UK; 2 Sheffield Children’s Hospital, Sheffield, UK; 3 Royal Manchester Children’s Hospital, Manchester, UK.

Background
Severe vitamin D deficiency (VDD), is a common disorder, which has complications including rickets, hypocalcaemia, hypotonia, delayed development and cardiomyopathy. Although nutritional rickets associated craniostenosis has been reported, there is little awareness of this or knowledge about its clinical course or severity. We present five cases of late onset craniosynostosis in association with nutritional rickets.

Clinical presentation
The diagnosis of craniosynostosis was made between the age of 16 months and 3 years (N=5). All children had clinically evident scaphocephaly and radiological evidence of previous rickets. All children had risk factors for severe VDD: African Caribbean or Asian ethnic backgrounds with darker skin pigmentation (N=5); multiple food intolerances (N=2) and prolonged breast feeding withicky eating habits (N=2). They presented in two ways: Group 1 (N=3) presented with clinical and radiological signs of severe rickets after a long period of untreated severe VDD. Serum 25OH vitamin D concentrations were <20nmol/l, alkaline phosphatase and parathyroid hormone (PTH) concentrations were elevated and serum calcium and phosphate concentrations were low. They were managed with treatment doses of vitamin D and calcium supplementation where necessary. In two patients, treatment had been completed and clinical signs resolved when the craniosynostosis was diagnosed. Group 2 (N=2) presented with sagittal suture ridging and scaphocephaly associated with resolving rickets on radiology. Clinically there were few other signs of VDD and serum 25OH vitamin D concentrations were 33–44nmol/l with normal PTH and bone profiles. CT in all cases showed fusion of the sagittal sutures. Three of the children also had multiple suture fusion. All in Group 1 were managed conservatively but Group 2 patients had raised intracranial pressure and both underwent surgical cranial vault remodelling.

Conclusions
All the patients had nutritional rickets associated with craniosynostosis. Patients with late presentation and sagittal suture ridging went on to have emergency cranial vault remodelling. It is important to recognise this complication early and refer to the neurosurgeons and so prevent raised intracranial pressure. It is important to collect detailed data on this and study a larger cohort to raise awareness, establish the pathophysiology and try to prevent this complication.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P069

Multiple fractures that begun in utero in a pre-adolescent child with low ALP levels and nephrocalcinosis: clinical aproximation for the differential diagnosis of hypophosphatasia (HPP)
Maria Isabel González Fernández, Berta López Montesinos, Miguel Marti & Inmaculada Calvo
Hospital Universitario Politécnico La Fe, Valencia, Spain.

Objectives
Differential diagnosis vs. Osteogenesis Imperfecta (OI).

Methods
The parents were Moroccan origin, consanguineous. The patient is an 8 year old girl, who was visited for a first time in our hospital in October 2016, presenting a fracture of the left femur, with dramatic bone deformations, with important disability, unable to walk and with growth retardation (weight 15 kg, length: 92 cm). The first registered fracture is at birth, consisting on a femoral fracture. To differentiate hypophosphatasia (HPP) from OI, the following studies were performed: radiological bone series, MRI, complete bone metabolism biochemical profile including calcium, phosphorus, ALP (alkaline phosphatase), PLP (pyridoxal-5’-phosphate), PTH (parathyroid hormone), and vitamin D and the genetic molecular analysis for bone dysplasia and OI. The evaluation of the renal impairment included 24 h urine sediment evaluation, renal ultrasound, creatinine and BUN (blood ureic nitrogen).

Results
The radiological bone series and the MRI revealed a severe osteopenia, with long bone deformation, elbows in varus, a wide thorax and xiphoid process in vertebral level with platyspondyly. All these radiological findings are compatible with a bone dysplasia. Renal ultrasound showed nephrocalcinosis. A low bone mineral density with DXA lumbar 0.280 g/cm². Z-Score: −5.8 s.d. The laboratory results included serum calcium in the upper normality range. Vitamin D deficiency and PTH remained normal. The ALP levels were low considering the presence of the fractures. The results of genetic molecular analysis are pending.

Conclusion
In this patient it should be consider the differential diagnosis between HPP and OI. The nephrocalcinosis and low ALP levels with the presence of the fractures led us to possibility of HPP.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P070

The abnormally high and heterogeneous bone matrix mineralization after childhood solid organ transplantation is not further increased by bisphosphonate treatment
Nadja Fratzl-Zelman1, Helena Valtu2, Renata C Pereira3, Barbara M Misof4, Paul Roschger1, Hannu Jalanko2, Kathrine Wesseling-Perry5, Klaus Klauschofer5 & Outi Mäkitie2,4
1 Ludwig Boltzmann Institute of Osteology at Hanusch Hospital of WGGK and AUVA Trauma Centre Meidling, 1st Med. Department Hanusch Hospital, Vienna, Austria; 2 Children’s Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland; 3 Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, California, USA; 4 Center for Molecular Medicine, Karolinska Institute and Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden.

Background
Chronic renal, liver and heart failure in children associate with multiple skeletal complications. Increased fracture incidence often persists after transplantation and might be related to alterations in bone material properties. Moreover, it is not clear whether bisphosphonate therapy (BP) alters bone matrix mineralization in these patients.

Methods
In the present study we evaluated bone mineralization density distribution (BMDD) by quantitative backscattered electron imaging (qBEI) in transilic bone biopsies obtained from kidney (n=9), liver (n=9) and heart (n=5) transplant recipients (aged 7.6–19.7 years, 6.0±2.6 years post-transplantation) with suspected osteoporosis and in two kidney and four liver recipients after 2 years’ BP treatment (paired biopsies). Furthermore, we related the BMDD parameters with clinical and bone histomorphometric outcomes.

Results
Compared to healthy children, qBEI analyses in transplant recipients prior BP revealed an increase in the most frequently occurring calcium concentration (+2.9%, P=0.001; +3.5%, P=0.014), in the portion of fully mineralized bone (livefold; 10-fold, both P<0.0001), in heterogeneity of mineralization (+26.5% and +7.4% both, P<0.0001 and P=0.001), in cancellous and cortical bone respectively. Moreover, we observed a strong inverse correlation between the average calcium content of the bone matrix and patients’ biochemical ALP levels, histomorphometric indices of bone formation and resorption. BP did not significantly alter the average mean calcium content of the bone matrix (cancellous bone: 21.6% post BP vs 22.2% prior BP, P=0.47; cortical bone: 21.4% after BP vs 22.0% prior BP, P=0.56) except in one patient with abnormally low bone matrix mineralization and increased indices of bone turnover that normalized after treatment. Accumulation of mineralized cartilage was observed in the bone tissue after BP.

Conclusions
The abnormally high bone matrix mineralization in transplant recipients, consistent with serum and histomorphometric outcomes, indicates a history of low bone turnover with accumulation of fully mineralized bone packets. The increased heterogeneity of mineralization suggests local alterations in mineralization kinetics. This may be linked to dysfunctional osteocytes that accumulate within the bone matrix during organ failure and concomitant glucocorticoid and immunosuppressive medication. BP does not further increase bone matrix mineralization, however the presence of mineralized cartilage warrants further clarifications.

The authors have no conflicts of interest.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P070
PO71
In search of hypophosphatasia: a need to establish normative data for low alkaline phosphatase in pediatric population
Pawel Abramowicz1, Jerzy Konstantynowicz2, Beata Zelazowska-Rutkowska1 & Bogdan Cylwik3
1Department of Pediatrics, Rheumatology, Immunology and Metabolic Bone Diseases, Medical University of Bialystok, Bialystok, Poland;
2Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok, Bialystok, Poland.

Background
Hypophosphatasia (HPP) is a rare inborn error of metabolism caused by mutations in the gene encoding tissue-nonspecific alkaline phosphatase gene (TNSALP), leading to low alkaline phosphatase (ALP) activity. At least 6 clinical forms of HPP have been reported. Certain benign or asymptomatic presentations of HPP in older children may remain undiagnosed, in contrast to severe perinatal and infantile types. The underlying reason of this diagnostic inconsistency may result from unawareness and neglects in labelling and traceability of low ALP levels in laboratories.

Objective
This single center retrospective study was aimed to determine the prevalence of low ALP in children referred to a pediatric hospital throughout one year, relative to medical diagnoses and clinical symptoms. Given the low reference range for ALP may have been omitted, we hypothesized that some individuals with clinical HPP remained underdiagnosed.

Methods
In 853 individuals (382 girls, 471 boys) aged 1 month – 18 years, ALP level was measured using the standard colorimetric automatic method with Roche Cobas Integra800 analyzer. As there were no customary algorithm for low reference range for tissue nonspecific ALP levels in our laboratory, we applied the age- and sex-adjusted normative data derived from the previously published reference (1, 2).

Results
The mean level of ALP was 255 ± 192 IU/L (range: 22–2093). Low ALP was found in 43 children (5%), of which 32 were those aged 4–11 years. There was no hypophosphatasaemia in neonates/infants within the registry. The main diagnoses associated with low ALP were: acute lymphoblastic leukemia and solid tumors (n = 14), newly diagnosed or exacerbated juvenile idiopathic arthritis (n = 9), infections (n = 4), traumatic fractures (n = 2), while the diagnosis remained undetermined in three cases. Based on the retrospective medical records, four children may have demonstrated symptoms suggesting the features of benign HPP. No case of severe disorder was identified with regard to low ALP.

Conclusions
There is an urgent need to flag the laboratory results of ALP below the normal reference range, although the clinically apparent HPP is regarded a rare disease. Most cases with low ALP are non-characteristic. However, both clinicians and the laboratory staff should be aware that the scoring of low ALP may help identifying individuals with HPP. Further nationwide survey is warranted to determine true and reliable lower cut-off values for ALP in children.

Disclosure
The authors declared no competing interests.

References

DOI: 10.1530/boneabs.6.P071

PO72
Raised intracranial pressure in a boy with Pycnodysostosis with open fontanelles
Laila Al Hashmi, Raja Padidela, Mars Skae & M Zulf Mughal
Royal Manchester Children’s Hospital, Manchester, UK.

Background
Pycnodysostosis (PDO) is a rare autosomal recessive high bone mass disorder caused by absence of active cathepsin K, which is a lysosomal cysteine protease that plays an important role in degrading the organic matrix of bones. In spite of open fontanelles, raised intracranial pressure has been reported in children with PDO.

PO73
Spectrum of paediatric hypophosphataemic rickets in a tertiary centre
Emily Cottrell & Talat Mushtaq
Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Background
Hypophosphataemic rickets is a rare form of rickets characterised by hypophosphatamaemia and hyperphosphaturia. Children can present with bowed legs, gait abnormalities or persisting rickets. Occasionally the clinical and biochemical features may be mild. It is most commonly caused by a mutation in the phosphate-regulating endopeptidase homolog, X-linked (PHEX) gene which leads to an elevated FGF23.

Objectives
We wished to review our cohort of children with hypophosphataemic rickets, both their clinical and biochemical findings and their surgical management.

Methods
All current patients in our service were identified and their notes, results and correspondance retrospectively reviewed.

Results
Ten children (six female, four male) (three Asian, seven Caucasian) with hypophosphataemic rickets were identified. Four had a family member with hypophosphataemic rickets leading to a diagnosis within the first year. Six of the other index cases had a confirmed diagnosis at median age 4.6 years (range 3.7–15 years). All children had low or low normal plasma phosphate levels. FGF23 was available for seven children and was raised in all but one child: Range 85–618 RU/ml (normal range <100). The 1,25 Vit D levels were inappropriately normal or marginally raised. Genetic confirmation was obtained for five children. Four of them had a mutation in the PHEX gene and one child had an Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) mutation. One child had craniosynostosis, one had coeliac disease. All children were treated with a combination of phosphate sandoz and alfalcacidol. Four (two female) children had orthopaedic surgery to correct their bowed legs, half of whom had a family history of hypophosphataemic rickets and the other half were index cases. One adolescent was eligible for surgery but refused.
Conclusion
Half of our children were eligible for orthopaedic surgery, with no clear sex bias. ENPP1 mutations can be associated with generalised arterial calcification thus pursuing a genetic diagnosis is important. The biochemical abnormalities may be mild and thus delay a diagnosis, so it is imperative that those with low plasma phosphate levels and persistence of gait abnormalities or bowed legs are investigated thoroughly for hypophosphataemic rickets.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P074

P074
High bone turnover markers and disturbances of bone mineral density in children with hypophosphataemic rickets
Agnieszka Rusinska1, Izabela Michal1, Izabela Woch2, Paulina Adamiecka2 & Danuta Chlebna-Sokol1,2
1Department of Paediatric Propaedeutics and Bone Metabolic Diseases, Medical University of Lodz, Lodz, Poland; 2Central Clinical Hospital Medical University of Lodz, Lodz, Poland.

Introduction
Hypophosphataemic rickets belongs to genetically determined rare disorders characterised by bone deformations, including varus deformity of the lower limbs and short stature. This type of rickets is related to renal phosphate wasting and hypophosphataemia. Less is known about bone turnover abnormalities and bone mass in this disease entity.

Aim
The aim of this study was to analyse bone turnover markers and bone mineral density in patients suffering from hypophosphataemic rickets being treated in our department.

Patients and methods
This study involved five children with hypophosphataemic rickets (two girls and three boys) aged 1–15. Levels of urine and serum calcium and phosphate, tubular reabsorption of phosphate, levels of hepatic and renal vitamin D3 and parathormone metabolites were assessed in patients. Bone turnover markers, such as alkaline phosphatase, as well as osteocalcin and pyridylnolin were analysed. Bone mineral density was determined by densitometry with dual X-ray absorptiometry (DXA) using total body and spine programmes. A quantitative ultrasound assay (QUIS) of the tibia and radius was also performed.

Results
Hyperphosphaturia was observed in all cases (66%), while hypophosphataemia was present in 4/5 patients. Concentrations of vitamin D and PTH metabolites were normal, except for one girl, who did not follow therapeutic recommendations. We observed an increase in the bone formation markers in 4/5 patients, while an increase in bone resorption rates in 3/5 cases. Bone mineral density was reduced in one patient, whereas increased Z-score were observed in two children. A significant decrease in indicators of quantitative ultrasound examination for the tibia and/or radial bone were observed in all analysed patients.

Conclusions
- Typical biochemical symptoms in hypophosphataemic rickets may be accompanied by accelerated bone turnover. Therefore, it is advisable to monitor bone formation and resorption markers in this disease.
- Disorders of mineral metabolism in hypophosphataemic rickets may reveal both an increase and reduction in bone mineral density.
- The reduction in parameters of the quantitative ultrasound in all investigated patients indicates worse bone quality in this disease.

Acknowledgements
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Disclosure
The authors declared no competing interests.

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P076
Abstract withdrawn.

P077
The role of bone age in the evaluation of trabecular bone score (TBS) of children and adolescents 5–19 years old
Miguel Angel Guagnelli1, Regina Ambrosi1, Desiree Lopez-Gonzalez1, Renaud Winzenith2, Luis Del Rio1 & Patricia Clark2
1Clinical Epidemiology Unit, Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico; 2R&D Department, Med-Imaps, Pessac, France; 3CECIR Medical Centre, Barcelona, Spain.

Rationale
Trabecular Bone Score (TBS) is a texture-based tool analyzing DXA images in order to assess bone microarchitecture in the lumbar region. In pediatric population, definition of normative values has remained elusive due to the disparities of results in normal population, probably link to uncontrolled factors which impact bone microarchitecture and the nonlinear behavior of bone growth. Our objective was to evaluate TBS in healthy Mexican children and adolescents using chronological age or bone age (BA) taking into account skeletal maturation and puberty onset as confounding variables.

Method
DXA acquisitions from 269 boys and 296 girls aged 5–20 years were included. Bone age was evaluated according to Greulich and Pyle method. Pseudo volumetric BMD (3D BMD) was calculated based on cylindrical model proposed by Kroeger et al. (Bone Mineral, 1992). TBS assessment was evaluated using a custom version of TBS (Med-Imaps SASU, France) that includes a soft tissue correction for pediatric subjects. The LMS statistical method proposed by Cole and Green (Stat Med, 1992) was used to construct abMD, vBMD and TBS age-related curves using LMSchartmaker 2.0.

Conclusion
Children and adolescents with eating disorders are at risk of reduced bone mass and bone mineral density. Indeed, 60% higher risk of fracture has been reported in childhood/adolescent eating disorders compared to healthy controls. However, few longitudinal studies have been carried out to examine size adjusted changes in bone health over time, and the relationship with anthropometry and growth.

Methods
A retrospective audit of 25 female eating disorder patients who had attended a paediatric DXA service on two occasions (baseline and follow-up) was carried out. Each patient had received total body (TB), lumbar spine (LS) scans and 15 patients had a lateral spine for vertebral fracture assessment (VFA). TB results were converted to percent predicted BMC for bone area (BA) LS DXA results were adjusted using BMAD as recommended by the ISCD. Measurements of lean mass (LM) and fat mass (FM) were derived from the TB DXA scan, and height, weight and BMI SDS were calculated using the 1990 British Growth Reference data.

Results
Mean age at baseline was 14.3(11.1–17.1) years and 16.3(12.1–19.8) years at follow-up. Bone area (BA) increased at TB from 1760(1288–2285) cm² at baseline to 1875(1510–2321) cm² at follow-up (P < 0.001), and LS BA increased from 36.3(28.1–44.2) cm² at baseline to 37.7(31.8–47.2) cm² at follow-up (P = 0.002). TB percent predicted BMC for BA decreased from 98.2(89.1–108.6) to 95.9(88–111) at follow-up (P = 0.029). LS BMAD SDS decreased from −1.2 (−2.6 to 0.3) at baseline to −1.3 (−3.0 to −0.3) at follow-up (P = 0.041). Height SDS was −0.2 (−2.2 to 1.2) at baseline and −0.5 (−3.8 to 1.1) at follow-up. There was no correlation between change in any of the bone parameters and change in any of the body composition parameters. However, there was a positive correlation = 0.507 (P = 0.012) between change in height SDS and change in LS BMAD. No vertebral fractures were reported in this patient group.

Conclusion
While bone size increased in childhood/adolescent eating disorder, size adjusted bone density decreased. This decrease was not associated with change in weight, LM, FM or BMI, but was associated with change in height SDS. Failing growth in this patient group is likely to be a risk factor for low bone mass, hence potential increase in fracture risk.

Disclosure
The authors declared no competing interests.

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Results
When chronological age was used, girls’ curve showed decreasing phase delineating a ‘U’ shape. However, when evaluated with bone age, both graphs show constant TBS until 9 years in girls and 12 years in boys with accordance to the age of puberty, the onset of which is different between genres, a well-known phenomenon.

Discussion
Bone age, better related to puberty onset than chronological age, may be more useful to interpret TBS. This preliminary data need to be reproduced by other groups in healthy children and adolescents as well as in groups with different pathologies affecting this population.

Figure 1 (a) False positive SpineAnalyzer result. Wedging of T7 and T8 as indicated by SpineAnalyzer was reported by the consensus expert panel as physiological, rather than pathological wedging. (b) False negative SpineAnalyzer result. T11, T12 and L2 were reported by the consensus expert panel as fractured but were scored normal by SpineAnalyzer.

Conclusion
In contrast to adults, the six-point technique assessing anterior, middle and posterior vertebral height ratios is neither satisfactorily reliable nor sensitive for VF diagnosis in children. Training of the software on pediatric images is required, in order that a pediatric standard is developed which incorporates not only specific vertebral body height ratios but also the age-related physiological changes in vertebral shape that occur throughout childhood.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P079

P080
Bone age assessment using Greulich and Pyle and Tanner-Whitehouse methods: a systematic review
Khalaf Alshamrani1 & Amaka Offiah1,2
1The University of Sheffield, Sheffield, UK; 2Sheffield Children’s NHS Foundation Trust, Sheffield, UK.

Objectives
To have a better understanding of the applicability of the Greulich and Pyle and Tanner Whitehouse methods of bone age estimation in children who are of a different ethnicity from those of the original standards.

Method
A systematic search of the Medline database was conducted to include studies published between 1st January 1959 and 1st May 2016 (keywords ‘Greulich Pyle’; ‘Greulich and Pyle’) and 1st January 2001 and 1st May 2016 (keywords ‘Tanner Whitehouse’, ‘TW3’). Each study’s title and abstract were screened. The full text was retrieved when the reviewer could not decide on the study’s eligibility from the title and abstract alone. The following exclusion criteria were then applied, participants with developmental disorders or subjected to nutritional supplementation, studies which used modified methods of G&P or TW3 and/or used other imaging modalities, full text not available within the resources available to the reviewer, full text not in English and review articles.

Results
376 studies were identified, of which 40 were eligible for inclusion. Five additional studies were identified from the reference lists. In relation to the use of the G&P atlas in Caucasian children, of the 24 studies reviewed, 9 (38%) were unequivocally in support, while 12 (50%) concluded that some modification was required. 72% and 26% of the studies related to African and Asian populations respectively, and concluded that new standards were required. In Hispanic populations, 3 studies (50%) concluded that the standard is applicable, while 3 studies (50%) reported that some modification was required. Socio-economic status was only reported in 8 studies (17.7%). Within these 8 studies, children from lower socioeconomic status tend to be skeletally delayed and show major deviations between bone age and chronological. Seven studies identified were in relation to TW3, of which 1 study suggests poor applicability of TW3 in Hispanics.

Conclusion
The G&P standard may be used in Caucasians, but caution is required when applied to Asian and African populations. There is a complex inter-relationship between the impacts of socioeconomic status and ethnicity on bone age using the G&P atlas, which no study to date has clearly set out to address.
Bone age determination using dual-energy X-ray absorptiometry
Khalaf Alshamrani1, Amaka Ofihai2 & Elzene kruger3
1Department of Oncology & Metabolism, The University of Sheffield, Sheffield, UK; 2Sheffield Children’s NHS Foundation Trust, Sheffield, UK.

Objective
To assess whether hand-wrist dual-energy x-ray absorptiometry (DXA) can replace radiographs for bone age assessment using the Greulich & Pyle (G&P) and/or Tanner & Whitehouse (TW3) methods.

Methodology
Purposive sampling was used to include a total of 20 patients identified from an Endocrine Clinic; two males and two females from each of 5 age groups (< 5; 5 to 7; 8 to 10; 11 to 13; 14 to 16 years). Bone age as determined from DXA and radiographs performed on the same day were compared for each child. Two observers independently assessed all radiographs and DXA scans on two occasions. For each observer, there was a minimum interval of two weeks between the two reads. Adequacy of hand positioning and image quality were assessed using paired t tests. Interclass correlation coefficient and Bland Altman plots were used to evaluate agreement between the observers and correlation between the two imaging modalities.

Findings
The mean chronological age was 9.04 (s.d. ± 3.8) and 9.8 (s.d. ± 3.2) years for girls and boys respectively. A significant difference between DXA scans and radiographs (P < 0.001) was observed in terms of hand positioning. The ulna was excluded from the scan field in 15% of the left hand DXAs. The overall image quality assessment showed a significant difference (P < 0.001) between left hand DXA scans and left hand radiographs. Visibility of soft tissue and fat planes was poor in all DXA scans compared to radiographs. Despite the reduced image quality of DXA, inter-observer agreement for bone age determination was 0.987 for radiographs and 0.980 for DXA using the G&P technique. For Observer 1, intra-observer agreement for radiographs and DXA was 0.993 and 0.983 respectively, and 0.995 and 0.994 respectively for Observer 2. Poor DXA image quality did not allow bone age determination using the TW3 method.

Conclusion
Bone age can be determined from left hand/wrist DXA scans using G&P. However, limited DXA image quality prohibits its use for bone age assessment using the TW3 method.

Acknowledgement
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Disclosure
Dr Amaka Ofihai is Convenor of the Skeletal Dysplasia Group for Teaching & Research but consistent with the Society’s Terms of Reference had no input in the grant review process for this study.

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Impact of age, sex, location of injury, physical activity, vitamin D and calcium intake on the injury outcome of wrist and ankle in children
Hassan A Alshamrani1,2, Hana Alloub3, Derek Burke1 & Amaka C Ofihai1,3
1University of Sheffield, Sheffield, UK; 2Najran University, Najran, Saudi Arabia; 3Sheffield Children’s NHS Foundation Trust, Sheffield, UK.

Objectives
The aim of this study was to assess the impact of age, sex, location of injury, physical activity, dietary intake of calcium and vitamin D on injury outcome in otherwise healthy children.

Materials
This study was a cross-sectional prospective study. Children aged 6 to 15 years who presented to the Emergency Department of a single tertiary paediatric referral hospital were recruited. Children were included if they were known not to have underlying disease or long-term medication. Children were not eligible if they had been involved in high-energy trauma as we hypothesised that fracture is more likely to happen regardless of other factors. Patients’ age, sex and injury location were retrieved from their emergency notes. Physical activity were assessed using validated recall questionnaire. Vitamin D and calcium intake were assessed using validated food frequency questionnaire. All participants had a radiograph of their injured limb reported by a consultant radiologist. The patients were classified into two outcome groups (fracture or no fracture) based on the imaging findings. Data analysis include descriptive and inferential statistic. Logistic regression was performed to assess the impact of a number of factors on the injury outcome.

Results
130 patients were recruited and of these, 53 (40%) patients sustained a fracture, 119 (91%) and 127 (98%) children did not consume the recommended daily dietary amount of calcium and vitamin D, respectively. Patients’ age, physical activity, vitamin D and calcium intake were not significantly associated with fractures. Sex was found to have a significant effect on injury outcome with males being at higher risk of fracture. Injury to the wrist was significantly associated with an increased risk of fracture. The logistic regression showed that some factors were significant predictors of the injury outcome when controlling for other factors in the model.

Conclusion
Children’s lifestyle in this study was poor in terms of dietary intake of vitamin D and calcium as well as their engagement in physical activities. We failed to show any association between these modifiable factors and injury outcome, probably because of the high prevalence amongst the population of low dietary vitamin D and calcium.

Disclosure
The authors declared no competing interests.

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Schmorl’s node and vitamin D deficiency: cause or coincidence
Ilknur Surucu Karσ1, Altan Çalmasur2, Zerrin Orbak3, Erdal Karavas4 & Mehmet Soyturtk4
1Department of Pediatrics, Medical Faculty, Erzincan University, Erzincan, Turkey; 2Department of Radiology, Regional Training and Research Hospital, Erzurum, Turkey; 3Department of Pediatric Endocrinology, Medical Faculty, Ataturk University, Erzurum, Turkey; 4Department of Radiology, Medical Faculty, Erzincan University, Erzincan, Turkey.

Pathologist Christian Georg Schmorl described a specific type of vertebral lesion, which is now known as Schmorl’s node. A Schmorl’s node or intrasub- chondral herniation is herniation of nuclear pulposus through the cartilaginous and bony end plate into the body of an adjacent vertebra. Schmorl nodes are seen primarily in the thoracolumbar spines in an elderly population. Schmorl nodes are associated with moderate but not advanced degenerative changes. An 11-year-old presented with severe pain in the low back. He had neither history of injury, trauma. Neither sensory nor motor deficit of his lower extremities was apparent.
He had very low serum vitamin D level. Other laboratory values were normal, including the erythrocyte sedimentation rate and C-reactive protein, autoantibodies. Magnetic resonance imaging (MRI) revealed a Schmorl’s node in the superior endplate of L5 vertebra without disc degeneration, spinal deformity (Figure 1). Vitamin D plays an important role in bone health because of its effect on calcium metabolism. The vertebral bones are linked with the intervertebral discs. The Schmorl’s node can occur because of low strength of vertebrae bone tissue due to a vitamin D deficiency. We conclude that the lack of vitamin D is one of the risk factors on developing of Schmorl’s node.

Disclosure
The authors declared no competing interests.

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P084
Chronological age, height adjusted age and bone age: Which of them correlates better to bone mineral density in kidney transplant recipient children?
Regina Ambrosi1, Miguel Angel Guagnelli1, Alma Almiray1, Ana Hernandez2, Mara Medeiros2 & Patricia Clark1
1Clinical Epidemiology Unit, Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico; 2Nephrology Research Laboratory, Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico.

Chronic kidney disease in children causes multiple bone alterations, particularly renal osteodystrophy, which affects both bone quality and size, in turn causing short stature, bone deformities and brittleness. Once they get a transplant, this process starts to revert, and although mineral alterations improve, short stature often requires growth hormone supplementation but bone fragility requires evaluations in order to revert the disease’s effects. DXA is a valuable tool for its measurement in children affected with chronic diseases but it requires adjustment for their proper evaluation.

Objective
To evaluate height adjusted age (according to 50th percentile in CDC growth charts) and bone age (according to Greulich and Pyle charts) correlation to bone mineral density in kidney transplant recipient children.

Methods
DXA measurements were made for 31 (16 girls) pediatric kidney transplant recipients. Lumbar and total body less head (TBLH) bone mineral density (BMD) were obtained. Within the same measurement, non-dominant hand image was obtained to evaluate bone age. BMD for chronological age was obtained directly, height adjusted age and bone age were calculated and the results graphed into a scatterplot. R² values were obtained.

Results
R² correlation of lumbar and TBLH were higher in both bone age and height adjusted age than in chronological age. Height adjusted age shows the best correlation in both boys and girls with values up to 0.76 in girls for TBLH and 0.68 for girls in lumbar region.

Conclusion
Height adjusted age seems to show a better correlation with BMD in children recipient of kidney transplant. Follow up of these patients is in progress to evaluate long term evolution of bone mineral density, response to transplant and long term fracture risk.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P085

P085
Cumulative radiation exposure from diagnostic imaging and associated lifetime cancer risk in children with Osteogenesis Imperfecta
Amy Thorby-Lister1, Wolfgang Höglèr1,2, Kirsten Hodgson3, Nicola Crabtree1, Nick Shaw1,2 & Vrinda Saraff1
1Department of Endocrinology and Diabetes, Birmingham Children’s Hospital, Birmingham, UK; 2Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK; 3Radiation Protection Services (RPPS) University Hospitals Birmingham, Birmingham, UK.

Background and objectives
Fracture rate in Osteogenesis Imperfecta (OI) is highest between 0 and 19 years, and associated radiation exposure also carries the highest lifetime cancer risk. Here, we investigate the cumulative effective radiation dose (E) and lifetime cancer risk from diagnostic imaging in OI children. We also explore the hypothesis that negative family history of OI will increase injury-related, fracture-negative X-rays due to parental anxiety.

Methods
We reviewed all X-ray imaging (X-ray, CT & DXA scans) conducted from 2003 to 2016 in children with OI (0–19 years) with a minimum observation period of 5 years, at Birmingham Children’s Hospital, UK. E was estimated individually and compared across five age groups (0–2, 2–5, 5–9, 9–14, 14–19 years). Lifetime cancer risk was calculated using cumulative E and organ, sex and age specific risk coefficients.

Results
We present preliminary results from 47 children (51% females, 3200 images) with OI Type I (n=26), III (n=6) and IV (n=15). The median (range) observation period was 12.5 years (5.2–14). The number of X-rays per year for Type I was 3.9 (0.14–11.86), Type IV 4.9 (0.86–14.78) and Type III 19.5 (5.66–32.42). Cumulative E was similar for Type I versus Type IV (P=0.132), but higher in Type III compared to Type I and IV (P<0.05), which was consistent across age groups. The additional lifetime cancer risk is 1 in 21,740 (1,083–625,000) for Type I, 1 in 7,180 (1,181–57,964) for Type IV and 1 in 1,130 (383–13,155) for Type III. The lifetime cancer risk for Type III is higher than Type I (P<0.05) but not different to Type IV (P=0.059). Across OI types, fracture-negative X-rays and cumulative E were not influenced by family history of OI.

Conclusions
When compared to baseline lifetime cancer risk (1 in 2) the additional cancer risk from diagnostic imaging is small but not negligible, broadly falling under the categories of ‘very low’ (1 in 10,000–100,000), ‘low’ (1 in 1,000–10,000) and ‘low-moderate’ (1 in 100–10,000) for Type I, IV and III, respectively. Hence it remains important to exercise caution to minimize radiation exposure. Family history of OI does not impact injury-related presentations where X-rays are fracture-negative.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P086

P086
Feasibility and reproducibility using HRpQCTII in children and adolescents
Kyla Kent1, Jessica Whalen1, Ariana Strickland1, Mary Leonard1 & Andrew J. Burghardt2
1Stanford University School of Medicine, Stanford, California, USA; 2University of California, San Francisco, California, USA.

Feasibility and reproducibility using HRpQCTII in children and adolescents

KH Kyla Kent1, Jessica Whalen1, Ariana Strickland1, Mary Leonard1 & Andrew J. Burghardt2
1Stanford University School of Medicine, Stanford, California, USA; 2University of California, San Francisco, California, USA.

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We recruited 60 healthy volunteers ages 5 to 21 to perform scan-rescan precision tests on the XtremeCT II. Participants were positioned in a carbon fiber immobilization cast. iPad-based video content was used to facilitate motion-free compliance. Distal radius and tibia scans were acquired starting 2 mm proximal to the proximal margin of the growth plate or growth plate remnant. Diaphyseal radius and tibia scans were centered at an offset from the same landmark, corresponding to 30% of limb length. Repeat scans were performed following complete repositioning of the participant. Scans were assessed for movement and the image quality was on the standard 5-point scale. The manufacturer’s image analysis pipeline was optimized for pediatric distal and diaphyseal scans to measure bone density and structure, and to estimate bone strength by micro-finite element analysis (μFEA). Precision errors were calculated from the test-retest measurements using root mean square of the coefficient of variation (CV%). The success rates for acceptable quality scans based on extreme (image grade ≤ 1), strict (≤ 2), and moderate criteria (≤ 3), are reported in Table 1 by age group and scan site. Precision errors measured from paired scans meeting the moderate quality criteria (≤ 3) by scan site are reported in Table 2. These data demonstrate that performance of HR-pQCT scans is feasible in the majority of children and adolescents. The performance in younger children was improved with the use of a video to provide distraction. With the exception of distal cortical porosity, precision was outstanding and greater than reported in prior XtremeCT I reproducibility studies in adults. Diaphyseal measurements of cortical porosity offer superior precision to measurements immediately adjacent to the growth plate.

Disclosure

The authors declared no competing interests.

Table 1 Probability to Satisfy Image Quality Criteria by Age (Image Grade: ≤1 ≤2 ≤3).

<table>
<thead>
<tr>
<th>Age</th>
<th>≤1 %</th>
<th>≤2 %</th>
<th>≤3 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-10</td>
<td>50%</td>
<td>55%</td>
<td>52%</td>
</tr>
<tr>
<td>11-15</td>
<td>55%</td>
<td>57%</td>
<td>54%</td>
</tr>
<tr>
<td>16-21</td>
<td>57%</td>
<td>59%</td>
<td>56%</td>
</tr>
</tbody>
</table>

Table 2 Test-Retest Precision Data (RMS CV%).

<table>
<thead>
<tr>
<th>BMD</th>
<th>CL.BMD</th>
<th>CL.Th</th>
<th>CL.Po</th>
<th>Tb.BMD</th>
<th>Tb.N</th>
<th>Tb.Th</th>
<th>Failure Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal Radius</td>
<td>0.40%</td>
<td>0.27%</td>
<td>1.27%</td>
<td>16.3%</td>
<td>0.55%</td>
<td>1.35%</td>
<td>0.86%</td>
</tr>
<tr>
<td>Distal Tibia</td>
<td>0.20%</td>
<td>0.18%</td>
<td>0.38%</td>
<td>5.59%</td>
<td>-</td>
<td>-</td>
<td>0.38%</td>
</tr>
<tr>
<td>30% Radius</td>
<td>0.18%</td>
<td>0.20%</td>
<td>0.38%</td>
<td>5.59%</td>
<td>-</td>
<td>-</td>
<td>0.38%</td>
</tr>
<tr>
<td>30% Tibia</td>
<td>0.46%</td>
<td>0.50%</td>
<td>0.94%</td>
<td>5.07%</td>
<td>-</td>
<td>-</td>
<td>0.70%</td>
</tr>
</tbody>
</table>

L. Based on a movement score of 1–5 where 1–2 is optimal and 3 is possible. (Burghardt, A.)

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P087 Cranial synostosis and Chiari 1 malformation in X-linked hypophosphatemic rickets

Aanya Rothenbuhler1, Justine Bacchetta2, Yahya Debza5, Catherine Adamsbaum3 & Federico Di Rocco4

1Pediatric Endocrinology Department, Bicêtre Hospital, APHP, Reference Center for Rare Disorders of the Calcium and Phosphate Metabolism, Filière OSCAR and Platform of Expertise for Rare Diseases Paris, Le Kremlin Bicêtre, France; 2Pediatric Nephrology Department, Reference Center for Rare Renal Diseases and INSERM 1033, Lyon, France; 3Pediatric Radiology Department, Bicêtre Hospital, APHP, Le Kremlin Bicêtre, France; 4Pediatric Neurosurgery Department and INSERM 1033, Lyon, France; 5INSERM U1169, Le Kremlin Bicêtre, France.

Background

X-linked hypophosphatemic rickets (XLHR) represents the most common form of hypophosphatemia.

Aim

The aim of this prospective study was to describe and analyze the incidence of cranial and cervico-occipital junction (COJ) anomalies in a series of children with XLHR.

Patients and methods

Seventeen children (13 girls, 4 boys, mean age 7.3 years) followed for XLHR at the French national reference center for rare diseases of the calcium and phosphate metabolism were included. All patients consented to the CT scan of the skull and MRI of the COJ. On CT, the patency of the sutures was noted. The cranial index was calculated as biparietal diameter divided by the occipitofrontal diameter and multiplied by 100. On MRI, the position of the cerebellar tonsils was analyzed. A Chiari type 1 malformation was considered when the cerebellar tonsils were lower than 5 mm from the foramen magnum edge on sagittal slices. The volume of the subarachnoid spaces and of the ventricles was also assessed.

Results

All children had a dolichocephalic deformation. In 12/17 children, a full or partial cranial synostosis of the sagittal suture was found. In two children, the right coronal suture was also partially affected with a plagiocephalic deviation of the forehead and of orbital bandeau. In one child, a closure of both lambdoids was associated to the loss of the sagittal suture. In nine children, we found a Chiari 1 malformation. All of them presented also a full or partial fusion of the sagittal suture. No child with an overt patent sagittal suture had a Chiari malformation. Children with Chiari 1 malformation had a smaller cranial index compared to those without (P = 0.021).

Conclusions

This study highlights that cranial synostosis and Chiari 1 malformation are more frequent in XLHR than previously thought and related to the impaired growth of the cranial bones. It is still necessary to investigate further the clinical consequences of these findings and the modalities for their radiological follow-up to adjust indications for neurosurgery.

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P087

P088 Normative data for lateral distal femur bone mineral density in children from 3 to 18 years of age using Lunar Prodigy absorptiometry

Melissa Fiscaliatti1, Frank Rauch2,3, Bethany Foster1 & Nathalie Alos1,4

1Sainte Justine University Health Center, Montreal, Quebec, Canada; 2Shriners Hospital For Children, Montreal, Quebec, Canada; 3McGill University Health Center, Montreal, Quebec, Canada; 4Sainte Justine Research Center, Montreal, Quebec, Canada.

Objectives

Children with compromised weight bearing and limited mobility are particularly at risk for fractures with minimal trauma. Unfortunately, they can also present several obstacles that can impede proper dual X-ray absorptiometry (DXA) scan assessment of whole body or spine bone mineral density (BMD). Since the distal femur is the most common site of fracture in those patients, the lateral distal femur (LDF) represents the site that best addresses the challenges of BMD assessment in children with neuromuscular impairments, however, normative data for the Lunar Prodigy DXA Scan are lacking. This study provides paediatric normative data for the LDF in children aged 3–18 years of age using the Lunar Prodigy DXA scan.

Methods

We assessed cross-sectionally 230 healthy Canadian children (49% males, near 90% Caucasian) ranging in age from 3 to 18 years of age. LDF, lumbar spine and whole body BMD were measured using the Lunar Prodigy DXA scan. Sex-specific reference curves for each of the 3 LDF regions were generated using LMS-ChartMaker. LDF Z-scores were calculated then compared to lumbar spine and whole body BMD by correlation analysis.

Results

LDF BMD increased steadily with age and was highly correlated with height and weight (R² ≥ 0.66) as well as whole body and lumbar spine BMD measurements (R² ≥ 0.72).

Conclusion

This is the first study to provide normative data for LDF BMD for children between the ages of 3 and 18 years of age using the Lunar Prodigy DXA scan. In addition, it confirms the feasibility of measuring LDF BMD in children who are unable to undergo DXA assessment at other anatomical sites.

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P088
**P089**

Cross Calibration of GE Lunar DPX Pro and GE Lunar iDXA

Rubina Mandlik, Utkarshini Kirtikar, Veena Ekbote, Anjali Jaiswal, Nidhi Kadam, Vaman Khadilkar, Shashi Chiplonkar & Anuradha Khadilkar

Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India.

Objective

The objective of this study was to enable migration of DXA data of patients following replacement of the GE Lunar DPX-Pro, pencil beam densitometer with GE Lunar iDXA, fan beam densitometer.

Methods

Scans of total body were conducted for 67 subjects, aged 3.0 to 19.9 years, on both machines. A sub-set of six subjects (3 boys) were randomly selected and reserved as a validation sample. Differences between the values obtained on the two machines were tested using the Student’s T-Test. Regression Analyses were applied to the data of the remaining 61 subjects (33 boys) to obtain regression equations for predicting DPX values of Total Body Less Head (TBLH) Bone Mineral Content (BMC) and TBLH Bone Area (BA) using corresponding iDXA values for both genders. Values were log transformed before generating regression equations.

Results

Regression equations for TBLH BMC and TBLH BA were generated with DPX values for both genders. Values were log transformed before generating regression equations.

Conclusions

The authors declared no competing interests.

**Figure 1**

(a) $y = 0.855x + 1.301$, $R^2 = 0.990$

(b) $y = 1.229x - 1.541$, $R^2 = 0.985$

**Figure 2**

(a) $y = 1.136x - 0.896$, $R^2 = 0.982$

(b) $y = 1.229x - 1.541$, $R^2 = 0.985$

**P090**

Intra-observer precision of vertebral height measurements using spine X-Rays And DXA in boys with Duchenne Muscular Dystrophy

R Morrice, S Joseph, I Horrocks, S Shepherd, SF Ahmed & SC Wong

1 Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK; 2 Paediatric Neurosciences Research Group, Department of Paediatric Neurology, Royal Hospital for Children, Glasgow, UK.

Background

The role of untrained observers in evaluating vertebral height and therefore detection of vertebral fracture (VF) from spinal radiographs (SXR) and dual energy-absorptiometry (DXA) images in children with concerns about osteoporosis is currently unknown.

Objective

To assess intra-observer agreement of morphometric measurements of vertebral height by an untrained observer using SXR and DXA in boys with Duchenne Muscular Dystrophy (DMD).

Methods

Vertebral height from SXR and DXA were measured on two separate occasions using the 6-point quantitative method. Relative technical error of measurement (rTEM) and bland-altman analysis were performed to evaluate intra-observer agreement of vertebral height measurements. Weighted kappa scores ($\kappa$) were calculated to assess intra-observer agreement of VF grading based on measurements of vertebral height.

Results

Fourteen boys with no history of VF were selected from a prospective study of bone health in DMD, median age 9.5 years (range 7.1 to 14.9). Both SXR and DXA provided highly readable images for vertebral assessment with 97.1% and 96.4% of vertebrae readable in the L4-T5 region. Intra-observer error of vertebral height measurements (T3-L4) on SXR and DXA is low (SXR rTEM = 2.5%, DXA rTEM = 2.9%) and was comparable across vertebrae levels. Intra-observer error was lowest in lumbar spine using SXR (rTEM 1.9%) and highest in mid-thoracic region using DXA (rTEM 3.1%). Bland altman plots for vertebral height ratios were closely distributed around zero. Limits of agreement of anterior: posterior ratio 8.0 and 11.0% on SXR and DXA, whilst limits of agreement of middle:posterior ratio 7.9 and 9.7% on SXR and DXA. Intra-observer agreement of VF grade from was substantial in both imaging modalities’ (DXA, $\kappa$ = 0.61; SXR, $\kappa$ = 0.76).

Conclusion

This study showed for the first time that an untrained observer can precisely perform vertebral height measurements. However, the accuracy of VF detection may require confirmation by comparison with a trained radiologist.

Disclosure

The authors declared no competing interests.

**Figure 1**

(a) $y = 0.855x + 1.301$, $R^2 = 0.990$

(b) $y = 1.229x - 1.541$, $R^2 = 0.985$

**Figure 2**

(a) $y = 1.136x - 0.896$, $R^2 = 0.982$

(b) $y = 1.229x - 1.541$, $R^2 = 0.985$

**P091**

Reliability and validity of DXA based images for measurement of height in children

R Macdonald, N Capaldi, S Joseph, A Mason & SC Wong

1 Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK; 2 Paediatric Neurosciences Research Group, Department of Paediatric Neurology, Royal Hospital for Children, Glasgow, UK.

Background

Height is required for interpretation of bone mineral density in children and is often challenging in non-weight bearing children. Monitoring of linear growth in non-weight bearing children as part of assessment of bone health is also important.

Objective

To investigate the feasibility of a novel method of using DXA images to measure height (Ht), sitting height (SH) and leg length (LL) in children.

Methods

Ht and SH were measured on DXA digital images performed for clinical monitoring of bone health on three separate occasions in 125 children by one single observer (RM). Twenty five children had Ht and SH measurements performed in clinic on the same day as DXA scans. Intra-class correlation (ICC) was used to assess reliability (three readings). Bland-altman plots were used to evaluate validity of DXA based measurements. Accuracy of DXA Ht SDS, SH SDS and LL SDS was pre-determined as agreement with clinical measurements of within ± 0.3 SD.

Results

ICC of DXA Ht and SH were 0.999 (95% CI 0.998 to 0.999), respectively and ICC of DXA LL was 0.997 (95% CI 0.996 to 0.999), indicating almost perfect reliability. Mean difference of DXA Ht SDS with clinic measurements was 0.099, with upper limit of agreement of 0.3 SD.
limits of agreement of 0.072 (95% CI 0.058 to 0.082) and lower limits of agreement of \(-0.270\) (95% CI \(-0.258\) to \(-0.282\)). Mean difference of DXA SH SDS with clinic measurements was \(-0.395\), with upper limits of agreement of 0.366 (95% CI 0.317 to 0.423) and lower limits of agreement of \(-1.153\) (95% CI \(-1.097\) to \(-1.203\)). Mean difference of DXA LL SDS with clinic measurements was \(-0.207\), with upper limit of agreement of 0.938 (95% CI 0.791 to 1.089) and lower limits of agreement of \(-0.524\) (95% CI \(-0.371\) to \(-0.669\)).

Conclusion
This study demonstrated for the first time that measuring height, sitting height and leg length using DXA images is feasible and highly reproducible. However, only height measurements showed good agreement with the gold standard of clinic measurements, suggesting that height measurements of non-weight bearing children undergoing DXA scan for monitoring of bone health can be performed.

Disclosure
The authors declared no competing interests.

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P092
Bone mineral density and quantitative ultrasound in the longitudinal monitoring of bone status in patient with Neurofibromatosis Type 1

Carla Caiffarelli, Valentina Francolini, Maria Dea Tomai Pitinca, Ranuccio Nuti & Stefano Gonnelli
University of Siena, Siena, Italy.

Objectives
Neurofibromatosis 1 (NF1) is one of the most common autosomal dominant diseases. Skeletal involvement such as short stature, kyphoscoliosis, tibial bowing and pseudarthrosis are common osseous manifestations of NF1. Moreover, there is a growing evidence that reduced bone mineral density (BMD), is a common feature of NF1 subjects. The aim of the study was to evaluate the usefulness of Bone Mineral Density and Quantitative Ultrasound (QUS) at phalanges in the assessment and monitoring of bone status in NF1 subjects.

Methods
We studied 51 patients (age: 8.4±5.2 years) with NF1 and 41 age and sex-matched controls. In all subjects bone mineral density at lumbar spine (BMD-LS), at femoral neck (BMD-FN) and at total femur (BMD-T) was measured by using a DXA machine (Hologic QDR 4500). Moreover QUS parameters at phalanges by Bone Profiler-II (AMPS amplitude dependent speed of sound: AD-SoS and bone transmission time: BTT) were assessed. A subgroup of 34 patients with NF1 and 27 controls were followed longitudinally (follow up period: 3.5±2.7 years).

Results
In NF1 subjects the values of BMD were significantly lower than in controls at all skeletal sites. In NF1 subjects BMD values, expressed as \(Z\)-scores, were \(-0.270±1.03\); \(-0.699±1.06\) and \(-0.577±1.11\) for lumbar spine, femoral neck and total femur, respectively. All QUS parameters were significantly reduced in NF1 subjects; the lowest values were observed for BTT (\(Z\)-score \(-0.315±0.8\) and AD-SoS (\(Z\)-score \(-0.470±1.2\)). The NF1 subjects were separated in groups with (27.5%) and without (72.5%) skeletal abnormality. The subjects with skeletal abnormality had a decrease in densitometric parameters that reached the statistical significance for BMD-FN and BMD-T respectively (\(p<0.05\), but not for BMD-LS. During the longitudinal study the NF1 subjects presented a worsening of either BMD values, expressed as \(Z\)-score or QUS parameters namely BTT and AD-SoS \(Z\)-score.

Conclusions
Our findings indicate that young NF1 patients have statistically significant decreased BMD and QUS values compared with controls and that the presence of skeletal abnormalities seems to be associated with a greater impairment in bone status. The longitudinal study has shown that in NF1 subjects both BMD and QUS remained significantly lower with respect to age-matched controls.

Disclosure
The authors declared no competing interests.

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P093
A boy with spondylo-epiphyseal dysplasia tarda

Gu¨lay Karagu¨zel & Emel Ac¸ikgo¨z
Karadeniz Technical University, School of Medicine, Trabzon, Turkey.

Background
Spondylo-epiphyseal dysplasia tarda (SEDT) is a rare late-onset X-linked recessive osteochondrodysplasia that mainly involves the epiphyses and vertebral bodies. Patients usually have short stature and early development of degenerative joint disease.

Presenting problem
A 12-year-old boy was referred to our hospital for evaluation of short stature. His height was 125 cm (~3.03 SDS), weight was 22 kg (~0.5 SDS), arm span 130 cm. The short stature became apparent at approximately 6 years of age. He had short neck, slight torticollis, x-bain deformity, and enlarged knees without joint pain. His motor and cognitive functions, and findings of other system examinations were normal. His mother was 141 cm. No other family members had a history of degenerative joint disease or hip joint replacement.

Clinical management
His serum concentration of insulin-like growth factor-1, calcium, phosphorus, alkaline phosphatase, parathormone, and thyroid hormones were normal. Serum acute-phase reactants were negative. Radiographs of patient showed flattening of the vertebral bodies and widening of the epiphyses of the knees. He did not reported back and hip pain or morning stiffness. He was in a milder clinical condition and a physiotherapy program was planned.

Discussion
Both radiological findings and family history are important for the diagnosis of X-linked SEDT. If the patients do not have a relevant family history or a clear radiographic findings, genetic analysis for confirmatory diagnosis of X-linked SEDT are important. A radiograph of the lateral lumbar vertebrae is helpful for screening of SEDT especially in boys with short stature after 6–8 years of age. SEDT should be taken into consideration in the differential diagnosis of JRA, because it gives rise to articular changes resembling JRA.

Disclosure
The authors declared no competing interests.

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P094
Bone health assessment in children with thalassaemia major

Sophia Sakka1, Nicola Crabtree1, Aswath Kumar3, Mark Velangi3, Wolfgang Högler1,2 & Nick J. Shaw1,4
1Department of Endocrinology and Diabetes, Birmingham Women’s and Children’s Hospital NHS Foundation Trust, Birmingham, UK; 2Department of Paediatrics, Royal Stoke University Hospital, Stoke, UK; 3Department of Clinical and Laboratory, Haematology Women’s and Children’s Hospital NHS Foundation Trust, Birmingham, UK; 4Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK.

Objectives
Bone disease is a long-term complication in patients with thalassaemia and therefore current UK guidelines recommend bimannual bone density assessment from the age of 10 years. The aim of this study was to evaluate bone health in children with thalassaemia major.

Methods
Twenty-nine patients (11 boys) with a mean (s.d.) age of 13.07 year (2.29) with thalassaemia major had measurement of lumbar spine BMAD (L2-L4) and of total body less head BMD (TBLH BMD) using a GE Lunar iDXA. The presence of vertebral fractures (VF) was assessed by lateral spine DXA images, and vertebrae were graded using morphometric X-ray absorptiometry (MXA) in conjunction with the Genant scoring system. Detailed medical history and assessment of pubertal status was obtained from medical records. 25-OH-Vitamin-D (25OHD), ferritin and haemoglobin levels before transfusion were measured around the time of the scan.

Results
Mean (SD) BMAD \(Z\)-score of patients was \(-0.9\) (1.0) with only three subjects (10.3%) having a \(Z\)-score below \(-2.0\), and TBLH BMD \(Z\)-score was \(-1.6\) (0.6) with 10 subjects (34.5%) having a \(Z\)-score below \(-2.0\). In total, 377 vertebrae in 29 subjects were assessed. Eight subjects (27.6%) had 22 VF, six of whom had normal BMAD \(Z\)-scores. Seventeen VF were mild (20–25% vertebral height reduction) and five were moderate (25–40% vertebral height reduction). Two patients had \(>5\) VFs one of whom was started on bisphosphonate treatment. Although there was a trend towards lower BMAD \(z\)-score and 25OHD levels in children with VF, there were no significant differences in lumbar BMAD \(z\)-score, TBLH BMD \(z\)-score, 25OHD, ferritin and haemoglobin between subjects with or without VF.

Conclusion
This study has shown that despite normal lumbar spine BMAD in the majority of subjects, VFs were present in 27.6%. Therefore, current guidelines for bone health monitoring in thalassaemia should be revised to ensure spinal imaging is included in order to detect vertebral fractures.

Disclosure
The authors declared no competing interests.

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P095

Legg Calvé Perthes disease and growth hormone treatment
Alina Daniela Belceanu, Ioana Armasu, Mirela Timrovan, Anamaria Bursuc, Mariana Cabac, Iulia Crumpe, Georgiana Constantinescu, Cristina Preda, Maria Christina Ungureanu & Carmen Vulpoi
Department of Endocrinology, University of Medicine and Pharmacy Gr. T. Popa, IASI, Romania.

Background
Current extension in the usage of growth hormone therapy (GHT) has increased the prevalence of bone complications. Legg Calvé Perthes disease (LCPD) is characterized by idiopathic avascular necrosis of the proximal femoral epiphysis. More frequently in boys than girls, 80% of cases of LCPD is of unknown etiology. An increased incidence has been stated in cases of GHT deficiency. There is increasing data that children with LCPD may have a more widespread skeletal disorder involving short stature, disproportionate growth retardation and delayed bone age.

Case reports
Case 1: 7 years old male, presented in January 2014 height deficit (97 cm, −2.5 S.D.), underweight, with small and triangular facies, flat feet, lower limb length asymmetry (right < left with 2 cm), bilateral genu recurvatum, clinodactyly of the 5th finger. He had delayed bone age of ~7 years. Somatotropin axis investigations revealed low IGF-1 (48 ng/ml, N: 64–345). GHT was considered contraindicated due to the higher risk of contralateral LCPD. Case 2: 7 years old male, presented in January 2014 height deficit (97 cm, −2.5 S.D.) and significantly delayed bone age (2 years) and GHT was started: Somatotropin 0.23 mg/kg per week, with good evolution (height velocity > 0.6 cm/month). In August 2016, his growth velocity decreased to 0.35 cm/month and he complained of pain in his left hip, increasing with activity. Limping and a deficit in internal rotation of the left hip were noted. Radiography of the lower extremity was conducted and left LCPD was diagnosed. GHT was interrupted.

Conclusion
Orthopaedic complications associated with GHT are rare, but severe. Whatever the causal association, the presence of LCPD imposes caution in children with GHT. To our knowledge there have been only a few reported cases of LCPD in children with GHT. Before beginning GHT, it is essential to take into account all the risk factors of the individual patient. Close monitoring with clinical and radiographic check-ups is required to allow early diagnosis and treatment of these complications, but no published guidelines exist to date.

Disclosure
The authors declared no competing interests.

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P096

Chronic intermittent torticollis in a toddler: a rare case of axis (C2) Ewing sarcoma presentation
Vassilios Papadakis1, Elpis Vlachopapadopoulou2, Kondylia Antoniadi1, Vassiliki Tzotzola1, John Nikas1, Kalliopi Stefanak1, George Sfakianos2 & Sophia Polychnronopoulou1
1Agia Sofia Children’s Hospital, Athens, Greece; 2Children’s Hospital P. K. Kyriakou, Athens, Greece.

Background
Torticollis in toddlers is most frequently a manifestation of traumatic atlantoaxial rotatory displacement or oropharyngeal inflammation, and rarely due to retropharyngeal abscesses and pyogenic cervical spondylitis. Rarely, intermittent torticollis may be caused by posterior fossa tumors. A very rare case of chronic intermittent torticollis due to upper spine Ewing sarcoma is presented.

Presenting problem
A 2.2 year-old girl suffered four episodes of painful torticollis, over a 9 month period. Anti-inflammatory drugs helped relieving the symptoms. Three months later, a very painful torticollis episode lasted for 15 days and then proceeded to full immobilization of the neck in torticollis. Following evaluation by a pediatric orthopedic at age 3.4 years, a MRI scan was suggested and revealed a solid mass occupying lesion arising from the body of the C2 vertebra, extending posteriorly, causing the neck and the pedals of the axis (C2) and extending to the spinous process. The mass extended to the superior and inferior joint and the spinal canal, resulting in spinal cord compression. Maximal length of the intracanal mass was 3.2 cm and was causing spinal cord displacement and compression by imaging, interestingly without significant neurologic deficits clinically.

Clinical management
The patient was transferred to our neurosurgery service, where steroids were initiated. CT guided biopsies were suggestive of Ewing Sarcoma, the diagnosis of which was established with an open biopsy. Full staging confirmed only local pathology, without metastases. The patient was started on treatment according to the EURO-Ewing Protocol, while taking extreme care for the stability of the upper cervical area.

Discussion
Chronic intermittent, relapsing remitting torticollis is rare in toddlers, as acute torticollis is caused by trauma or inflammation. Rare causes to be considered are early childhood myopathies and myasthenia, juvenile cerebral palsy, phentoin- zine-induced acute dystonic reactions, juvenile-onset Wilson or Huntington disease, gastroesophageal reflux, anterior-horn disease, radiculopathy and C1-C2 pathologies. This patient had intermittent chronic torticollis, without neurological deficits yet, despite the significant spinal cord compression, due to a very lingering chronic course. Early appropriate imaging could have revealed the exact pathology to the patient’s benefit.

Disclosure
The authors declared no competing interests.

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P097

Evaluation of the use of body quantitative computed tomography for the assessment of the tibia in children with Neurofibromatosis 1
Alex Ireland1, Zulf Mughal2, Judith Adams3,4, Keenan Brown5, Judith Ell Foo1 & Kate Ward1,2
1Manchester Metropolitan University, Manchester, UK; 2Royal Manchester Children’s Hospital, Manchester, UK; 3Academic Health Science Centre, Manchester University Hospital NHS Foundation Trust, Manchester, UK; 4Division of Informatics, Imaging & Data Sciences, Faculty of Biology, Medicine & Health, Manchester, UK; 5Mindways Software, Inc., Texas, USA; 6St. Mary’s Hospital, Manchester, UK; 7MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK; 8MRC Elsie Widdowson Laboratory, Cambridge, UK.

Assessment of metaphyseal bone growth in children by peripheral quantitative computed tomography (pQCT) is limited by use of a thin (2.3 mm) 2D slice to represent the ~20 mm metaphyseal region within which there is substantial local variation in bone properties (Marjanovic et al., 2009). In addition, scan positioning is performed by manual visual growth plate identification from which a region located proximal to the growth plate at 4% of tibia length is scanned. An image analysis protocol was developed by the Children’s Hospital of Philadelphia (CHOP) to assess whole-bone quantitative computed tomography (QCT) of the whole metaphysis using QCT-Pro (Mindways Software). Image properties are used to define the metaphysis region of interest (ROI), from metaphyseal onset defined from peak BMD in medullary bone profiles along limb length, to the proximal end of the metaphysis defined as the point where trabecular BMD reaches 0 g/mm. We hypothesise that use of a larger ROI, and scan site selection by a more objective reference placement method will lead to improved precision in tracking longitudinal bone change in growing
children. Twenty children with neurofibromatosis type I (NF1) had QCT scans of their whole tibia using Mindways phantom and software and at 4% distal-proximal tibia length by pQCT (Stratec XCT2000). Changes were assessed using tibial bone mineral content (BMC), cross-sectional area (CSA) and trabecular bone mineral density (BMD) using XCT CHOP protocol and pQCT standard analysis baseline (age 8.0 ± 1.2 years) and follow-up (age 11.4 ± 1.1 years). Results of the two methods correlated highly (all > 0.7 and P < 0.001), although there was a mean offset in values attributable to the 0.06 g/cm² density offset employed in the pQCT scanner. Bland-Altman plots showed good agreement with no measurement bias. In conclusion, CHOP assessment may offer higher precision for metaphyseal bone change assessment, with good agreement with low-dose 2D-pQCT. QCT scan speed (~1 mm for whole tibia) and the ability to assess bone properties along the whole bone length may offer advantages over established methods, and is more feasible in children with disabilities such as Duchenne Muscular Dystrophy and cerebral palsy.

Disclosure
Dr Keenan Brown is a employee of Mindways, in addition to holding stock in the company.

Reference

DOI: 10.1530/boneabs.6.P097

P099
Bone strength and microarchitectural deficits in children with cystinosis Andrew Burghardt1, Kyla Kent2, Jin Long2, Jessica Whalen2, Maira Phelps2 & Mary Leonard2
1University of California, San Francisco, California, USA; 2Stanford University, Stanford, California, USA.

Children with cystinosis have numerous risk factors for impaired bone accrual. We used state-of-the-art quantitative imaging of bone microarchitecture (HR-pQCT) to measure trabecular and cortical microstructure and bone strength in children and adolescents (5-20yrs) with cystinosis. We enrolled 20 cystinosis patients and recruited 34 healthy age- and gender-matched controls. Distal radius and tibia HR-pQCT scans (XtremeCT II, Scanco Medical) were acquired 2 mm proximal to the proximal margin of the growth plate or remnant. Diaphyseal radius and tibia scans were centered at an offset from the same landmark, corresponding to 30% of limb length. Bone volumetric density and microstructure were measured using an automated image analysis pipeline optimized for pediatric scans. Microfinite element analysis (μFEA) was used to estimate bone strength. One-way ANOVA regression, adjusting for age and sex was used for test for differences in bone measures between cystinosis and healthy control groups.

After correcting for age and sex (Table 1 and 2), cystinosis patients had significantly lower bone strength, most prominently at the distal tibia (p < 0.008) where smaller cross-sectional area, thinner cortices, and deficits in trabecular architecture were all significant. Smaller bone size was observed at all sites, suggesting a systemic lag in bone development. Our findings indicate cystinosis subjects have significantly impaired bone strength and abnormal architecture at the weight-bearing tibia, and will consequently have an elevated lifetime risk of sustaining a fragility fracture.

Disclosure
The authors declared no competing interests.

Table 1

<table>
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<th>Distal Radius</th>
<th>Distal Tibia</th>
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<tbody>
<tr>
<td>N</td>
<td>CYST 18</td>
<td>CTRL 32</td>
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<tr>
<td>Total-Area</td>
<td>172 ± 81</td>
<td>193 ± 67</td>
</tr>
<tr>
<td>BMC</td>
<td>310 ± 53</td>
<td>313 ± 53</td>
</tr>
<tr>
<td>CT.Ar</td>
<td>40 ± 14</td>
<td>46 ± 17</td>
</tr>
<tr>
<td>CT.BMC</td>
<td>788 ± 76</td>
<td>763 ± 83</td>
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<tr>
<td>CT.Po</td>
<td>2 ± 9</td>
<td>0 ± 5</td>
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<tr>
<td>CT.Th</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.3</td>
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<tr>
<td>Tb.Ar</td>
<td>134 ± 71</td>
<td>149 ± 54</td>
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<tr>
<td>Tb.BMC</td>
<td>153 ± 32</td>
<td>167 ± 34</td>
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<td>Tb.Th</td>
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<tr>
<td>Tb.Th</td>
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<td>Tb.1/SD</td>
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<tr>
<td>AppMod</td>
<td>1505 ± 394</td>
<td>1660 ± 315</td>
</tr>
<tr>
<td>FailureLoad</td>
<td>2695 ± 1076</td>
<td>3135 ± 1684</td>
</tr>
<tr>
<td>CLIF.Dist</td>
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<td>39 ± 10</td>
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Table 2

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<th>Diaphyseal Tibia</th>
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<tbody>
<tr>
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<td>CYST 17</td>
<td>CTRL 31</td>
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<tr>
<td>Total-Area</td>
<td>65 ± 20</td>
<td>81 ± 24</td>
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<tr>
<td>BMC</td>
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<td>CT.Ar</td>
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<tr>
<td>CT.BMC</td>
<td>991 ± 76</td>
<td>1017 ± 52</td>
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<tr>
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<td>0.02 ± 0.01</td>
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<td>CT.Th</td>
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<td>Tb.Ar</td>
<td>13 ± 8.4</td>
<td>13 ± 8.4</td>
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<tr>
<td>AppMod</td>
<td>7904 ± 877</td>
<td>8442 ± 691</td>
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<tr>
<td>FailureLoad</td>
<td>3219 ± 1031</td>
<td>4543 ± 1578</td>
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DOI: 10.1530/boneabs.6.P099
P100
Decreased bone turnover in HIV-infected children on antiretroviral therapy
Stephanie Shiau1,2, Michael Yin1, Renate Streblau2, Faezeh Patel2, Ndielka Mbete1, Donald McMahon1, Louise Kuhn1,2, Ashraf Coovadia1,2 & Stephen Arpad1,2
1Columbia University, New York, New York, USA; 2University of the Witwatersrand, Johannesburg, South Africa.

Introduction
Lower bone mineral content (BMC) has been reported in HIV-infected children, as well as those on ritonavir-boosted lopinavir (LPV/r)-based antiretroviral therapy (ART). Older studies of children with HIV report increased bone resorption, but data with current antiretroviral regimens are limited.

Methods
This analysis presents data from the CHANGES Bone Study (Johannesburg, South Africa). Whole body (WB) BMC was assessed by dual-energy X-ray absorptiometry. BMC Z-scores adjusted for sex, age, and height were generated. C-telopeptide of type-1 collagen (CTX), pro-collagen type-1 N-terminal propeptide (PINP), and osteocalcin were analyzed. Outcomes were compared between HIV-infected (N=219) and HIV-uninfected children (N=180), as well as between HIV-infected children previously randomized to switch to efavirenz (N=106) versus remain on LPV/r (N=113) (intent-to-treat).

Results
The 219 HIV-infected children (49% male) and 180 HIV-uninfected children (55% male) were 5–9 years of age (mean 6.7 years). HIV-infected children were on treatment for a mean of 5.7 years and mean (SD) viral suppression (HIV-1 RNA < 40 copies/ml). Mean WB BMC Z-score was lower in HIV-infected than uninfected children (−0.95 vs −0.79, P=0.05) as well as in children on LPV/r versus efavirenz (−1.20 vs −0.68, P<0.01). CTX (1.72 vs 2.05 ng/ml, P<0.01) and PINP (584 vs 634 ng/ml, P<0.01) concentrations were lower in HIV-infected than uninfected children, although not significantly (64.1 vs 69.2 ng/ml, P=0.50). CTX (1.70 vs 1.75, P=0.5) and PINP (585 vs 583, P=0.9) were similar in HIV-infected children on LPV/r versus efavirenz, but osteocalcin was higher in children on LPV/r than efavirenz (72.4 vs 55.6, P<0.01). Bone turnover markers were not strongly correlated with bone mass.

Conclusions
Compared to uninfected controls, HIV-infected children in South Africa had lower BMC and lower markers of bone resorption (CTX) and bone formation (PINP) in contrast to older studies. Although children on LPV/r had higher bone formation (osteocalcin), bone mass was decreased compared those on efavirenz. Longitudinal studies with broader measures of bone turnover are needed to understand the impact of HIV and antiretroviral medications on the dynamics of bone modeling and remodeling during childhood.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P100

P102
Do measures of adiposity and muscle cross-sectionally predict the weight of height-bearing bones in 11–12 year old Australian children?: A population-based study
Najmi Ismail1,2, Peter Simm3,4, Kate Lyckett4, William Osborn1,2 & Melissa Wake1,2
1The University of Melbourne, Parkville, Victoria, Australia; 2Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 3Department of Endocrinology and Diabetes, The Royal Children’s Hospital, Parkville, Victoria, Australia; 4Centre for Community Child Health, The Royal Children’s Hospital, Parkville, Victoria, Australia.

Objectives
To investigate whether bone health outcomes (volumetric bone mineral density, geometry and strength) is associated with adiposity and muscle in late childhood.

Methods
Design: Population-based cross-sectional study nested within the Longitudinal Study of Australian Children. Participants: 11–12 year-olds attending the Child Health CheckPoint physical module. Exposures: Measures of adiposity (BMI z-score, fat mass (kg), waist circumference (z-score)) and muscle (lean mass (kg), skeletal muscle mass (kg), lower leg muscle cross-sectional area (CSA)). Outcomes: Measures of bone health assessed via peripheral quantitative computed tomography, yielding tibial bone density (trabecular and cortical), geometry (endosteal and periosteal circumference) and strength (polar stress–strain index (SSI)). Statistical analysis: Multivariable linear regression models adjusted for age, sex, height, puberty, neighbourhood disadvantage and moderate to vigorous physical activity. In addition, models of adiposity were further adjusted for skeletal muscle mass and models of muscle were further adjusted for fat mass. Interaction tests also assessed the effect of sex. We present the fully adjusted models below.

Results
Of the 3,764 eligible children, 1,220 (32%) had bone and body composition data available. On average, children were aged 11.4 years (s.d.: 0.4), half were male, and one quarter were overweight or obese. Bone health outcomes showed differential associations with adiposity and muscle for females and males. In females, better bone health was most strongly associated with higher BMI z-scores and higher waist circumference. For example, each unit higher in BMI z-score was associated with better bone geometry (endosteal and periosteal circumference) and strength (SSI), with effect sizes ranging from 0.17–0.31 (all P<0.001). Whereas in males, better bone health was most strongly associated with skeletal muscle mass and lower leg muscle CSA, with effect sizes ranging 0.13–0.34 (all P<0.001). Across both sexes fat mass showed little association with the bone measures. Dialogue

Conclusions
By late childhood, adiposity and muscle show differential associations with bone health for male and females, consistent with previous reports of gender dimorphism for bone health outcomes. These findings point to the complexity of looking at each exposure measure separately and highlight the need to consider body composition as a whole (i.e. composition analyses).

Conflict of Interest
All authors declare no conflict of interest.

Funding
The Child Health CheckPoint has been supported to date by the Australian National Health and Medical Research Council (NHMRC) (1041352, 1109355), The Royal Children’s Hospital Foundation (2014-241), Murdoch Childrens Research Institute and The University of Melbourne. Research at the Murdoch...
Femoral fractures in infants – comparison of a population-based and an osteogenesis imperfecta-cohort

Hilkka Ryhänen1, Ilkka Vuorimies1, Sanna Toiviainen-Salo1,2, Pentti Kalio1, Oui Makitie1 & Mervi Mäyränpää1,2
1Children’s Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland; 2Department of Radiology, Children’s Hospital, HUS Medical Imaging Center, University of Helsinki and Helsinki University Hospital (HUH), Helsinki, Finland.

Objectives
Fractures in older children are common, often related to physical activity. In contrast, fractures in infants are rare and especially those involving the femur (upper leg) are infrequent. Femoral fractures in young children are highly suspicious for non-accidental trauma, and screening for possible child abuse should be urgently carried out. However, some metabolic bone diseases, like osteogenesis imperfecta (OI), may predispose to fractures already in infancy and should be differentiated from child abuse. The aim of this study was to characterize and compare the femoral fracture pattern in the overall infant population and in infants with known OI.

Methods
We carried out two retrospective studies on children with femur fractures before the age of one year. The population-based cohort (I) comprised all fractured infants from the city of Helsinki, Finland, during the years 1998–2012. The other cohort (II) involved all children with severe or moderate OI from Finland who sustained a femoral fracture during infancy in 1990–2012. Details regarding patient demographics, fracture type, and trauma mechanism were collected. All fractures were confirmed radiographically.

Results
In total there were 55 femoral fractures in 51 infants, of whom 14 were infants with OI. Four children had two separate fractures before the age of one year, all with OI. The girls to boys ratio was 1:0.9. The annual incidence for Helsinki was 4 per 10 000. Median age for fracture was 7 months for cohort I and 5 months for cohort II. The most common cause for the femoral fracture was a fall from height for cohort I whereas for OI infants in most cases no clear trauma could be identified. Location of the fracture was more proximal for OI and distal for non-OI patients. In six cases the OI diagnosis was set only after the femoral fracture.

Conclusion
We observed overlap but also some differences in femoral fracture pattern for infants with and without OI. The role of non-accidental trauma as fracture mechanism, more detailed trends, and other contributing factors remain to be elucidated in further analysis of the data.

Discussion
Since one of the criteria for reporting cases of NR to the BPSU is a serum 25OHD <25 nmol/l, some cases in which DCA and vitamin D deficiency (DCaD) may have contributed to the causation of NR may have been missed. Provision of vitamin D along with recommended dietary intake of calcium for the age of the child is important for prevention of NR.

Conclusion
We conclude that DCaD, along with inadequate vitamin D status (serum 25OHD <25 but >50 nmol/l), contributes to the causation of NR in the UK. The authors declared no competing interests.

Discriminant analysis of serum osteocalcin and markers of bone turnover (Alp, P1NP) in patients with established osteogenesis imperfecta

Benjamin Jacobs1
1Royal Manchester Children’s Hospital, Manchester, UK.

Objective
To explore whether a simple discriminant analysis of serum osteocalcin and markers of bone turnover (Alp, P1NP) in patients with established osteogenesis imperfecta (OI) can identify phenotypes.

Methods
Subjects from the UK and Ireland (n=137) were included in the study. Bone turnover markers, osteocalcin, Alp and P1NP were measured in addition to other OI markers such as procollagen type I N-terminal propeptide (PIIINP) and osteocalcin. OI phenotype was defined on the basis of phenotype at the referring centre. OI type was defined by OI classification.

Results
A simple discriminant analysis model was constructed using only osteocalcin and P1NP. The model predicted OI type with a sensitivity of 65% and specificity of 81%. This analysis was repeated for each phenotype separately and a model for severe OI was constructed.

Conclusion
A simple discriminant analysis model was constructed using serum osteocalcin and P1NP to predict OI type. This model could be refined for severe OI.

Authors declared no conflicts of interest.

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Does growth hormone and estrogens prevent girls with Turner syndrome from increased fracture rates?

Ondrej Soucek1, Zdenek Havlak1, Jan Lebl2 & Zdenek Sumnik1
12nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic; 2Faculty of Physics and Mathematics, Charles University in Prague, Prague, Czech Republic.

Objectives

Turner syndrome (TS) affects 1:2000 girls and is mainly characterised by short stature and ovarian failure. Increased fracture rate has been reported in historical cohorts of women with TS and it was linked to their decreased bone mineral density (BMD). Nowadays, girls with TS are treated with growth hormone and substituted with estrogens, of which role in optimal bone mass accretion has been confirmed. Whether increased fracture rate is still of concern in these patients remains to be elucidated.

Methods

The extremity long bone and vertebral fractures were recorded from the National Registry of Hospitalised Patients and the demographic data were obtained from the National Institute of Health Information and Statistics. Fracture data were also recorded from the largest children hospital in the country to calculate the age-specific proportions of hospitalised patients. The age-specific fracture incidence in girls up to 20 years of age was calculated. Data for years 2008-2014 were averaged. Monte Carlo simulation was used to estimate the probability of certain number of fractures. The incident fractures were recorded in 32 girls with TS over 6 year observation period. All patients were treated with growth hormone and, if appropriate, substituted with estriodol.

Results

Among the 32 girls with TS, three sustained new fracture during the 6 year follow-up. The fracture rate in the healthy population increased form birth up to age 11 years with a peak incidence of 20%. Then the incidence decreased and was 2% at age 20. Based on the age-specific fracture rates in healthy population calculated from the same years as was the follow-up of girls with TS, the lower limit for the expected number of fractures was 4.4 over the six year observation period. The upper limit for the probability of observing maximum 3 new fracture cases over the six-year follow-up was 0.34.

Conclusion

Our study demonstrates that girls with TS getting currently recommended standard hormonal treatment do not present with increased fracture rates compared to those in otherwise healthy girls. We encourage similar studies in other developed countries to confirm this finding.

Disclosure

The authors declared no competing interests.

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P108

Genetic transmission of osteogenesis imperfecta type V by a healthy mosaic carrier father

Sofie Symoens1, Kathrin Maurer2, Gisela Schweigmann3 & Elisabeth Steichen-Gersdorf1
1Center for Medical Genetics, Ghent University, Ghent, Belgium; 2Department of Radiology, Medical University, Innsbruck, Austria; 3Department of Pediatrics I, Medical University, Innsbruck, Austria.

Background

OI-V is an autosomal dominant type of OI, which is characterized by recurrent fractures, hyperlastic callus formation and forearm interosseous membrane calcification. Less than 5% of OI patients are diagnosed with OI-V. The 5’-UTR ITITM5 mutation is a single recurrent heterozygous mutation reported in the majority of these patients.

Presenting problem

The 2 years old girl was born at term, BW 2880g(P25-50), L 48 cm (P25-50), OFC 33 cm(P3). Motor development was delayed, sitting age 15 months, standing without support at 2 years, whereas cognitive development seemed to be normal. At the age of 7 months the girl complained with pain after bending sitting at the mothers womb. X-ray revealed a fracture of the right femur. Spinal X-ray after acute back pain revealed vertebral fractures. A second low impact femur fracture occurred at 13 months, suggesting a clinical diagnosis of Osteogenesis imperfecta (OI) type 1. However, molecular analysis of the type 1 collagen genes (COL1A1 and COL1A2) was normal. There was no history of fractures in the family.

Clinical Management

Fracture healing was noticed to be abnormal with delayed and hypertrophic callus formation. The child was treated with Nerdronate 2 mg/kg every 3 months with good response. In follow up care a limitation in forearm supination/pronation was noticed at 1½ years. Molecular analysis of the ITITM5 gene in the proband revealed the presence of the recurrent heterozygous mutation (c.-14C>T) in the 5’ untranslated region (exon 1). Segregation analysis showed that the ITITM5 mutation was absent in the mother but was present in the father, albeit at a lower amount. Subsequent deep sequencing by NGS confirmed the mosaic state of the mutation in the father, revealing a mutation load of 34% in the paternal blood. Bone density in the father was normal.

Discussion

OI-V caused by the 5’-UTR ITITM5 mutation was confirmed in our patient. There are few reports of families with autosomal dominant inheritance from an affected parent. To our best knowledge a transmission from an unaffected parent was not reported before. In view of the implication of families and recurrence risk genetic investigation of healthy parents is warranted.

Disclosure

The authors declared no competing interests.

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P107

A case of a novel de novo PLS3 deletion, presenting with vertebral fractures and mild dysmorphism

A. Dougeraki1, A. Çöstantini1, A. Kämpfe2, E. Karavitakis3, N. Jantti4, P. Kralli5, H. Athanassopoulou5, A. Xiaedara5 & O. Mäkinen4
1Department of bone and mineral metabolism, Institute of child health, Athens, Greece; 2Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; 3Department of Paediatrics, General Hospital of Chania, Chania, Greece; 42nd Paediatric Orthopaedic Clinic, Agia Sophia Children’s Hospital, Athens, Greece; 51st Paediatric University clinic, Agia Sophia Children’s Hospital, Athens, Greece.

Background

Mutations in the PLS3 gene, encoding plastin 3, cause X-linked osteoporosis. Osteoporosis is characterized by low bone mineral density (BMD) and increased susceptibility to fractures. Here we describe a 7-year-old boy with osteoporosis due to a novel PLS3 deletion.

Presentation problem

The patient, born to non-consanguineous parents, had a history of one low-energy long-bone fracture, three vertebral fractures (T5, T6 T8) and kyphosis. DXA scan showed decreased BMD, both at the lumbar spine (Z-score −3.5) and for the whole body less head (Z-score −2.2). His bones had normal length but they were thin and the whole body BMC/LTM ratio was low. He had had surgery for pyloric stenosis and right-sided cryptorchidism. His growth was normal but he had blue sclerae, joint hypermobility and epicanthus, narrow external ear canals, mild micrognathia and high-arched palate. There was no family history of osteoporosis.

Clinical management

Genetic tests for COL1A1 and COL1A2 were negative. Sanger sequencing of PLS3 detected a novel de novo hemizygous deletion in exon 10 (c.1057_1101delACT- TA). This deletion causes a stop codon at p.Ala371* and is likely to lead to a lack of protein due to nonsense-mediated mRNA decay. Both parents were negative for the deletion. The patient is currently on oral treatment with bisphosphonate (alendronate, 35 mg/week), swims regularly and receives adequate calcium and vitamin D through his diet. He is reviewed every six months (clinical examination and metabolic bone profile).

Discussion

We identified a novel PLS3 deletion causing bone fragility in our index patient. This finding emphasizes the importance of screening of the PLS3 gene for mutations in cases of early-onset osteoporosis not caused by mutations in type I collagen. It needs to be addressed in future studies whether our patient’s other features are related to the PLS3 mutation.

Funding

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Disclosure

The authors declared no competing interests.

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Microdeletion of 12p11.22-p11.21 resulting in a skeletal dysplasia characterized by significant metaphyseal abnormalities and osteosclerosis

Jennifer Harrington, Andrew Howard, Malte Spielmann & Peter Kannu

1University of Toronto, Hospital for Sick Children, Toronto, ON, Canada; 2University of Washington, Seattle, Washington, USA.

Background:
Parathyroid hormone-like hormone (PTHLH) is an important regulator of endochondral bone development. Mutations of the PTHLH gene can cause a variety of different skeletal dysplasias, with duplications of the PTHLH gene resulting in a phenotype characterized by endochondromatosism, metaphyseal dysplasia and osteosclerosis.

Presence of problem:
Our patient presented at the age of 4 months, given concerns regarding smaller limb deformities and ribcage asymmetry. A skeletal survey revealed multiple rib abnormalities, metaphyseal concavities of the long bones and osteolytic changes. Bone histomorphometry demonstrated increased bone turnover with significantly increased osteoid, areas of woven osteoid and bone marrow fibrosis; features in keeping with hyperparathyroid-bone disease. Serum calcium, phosphate, ALP, PTH and PTHrP concentrations however were all normal. Microarray demonstrated a de novo chromosomal microdeletion on chromosome 12 (12p11.22-p11.21), located near the PTHLH gene. No other known causative mutations or mutations involving the PTHLH pathway were found on exome sequencing.

Clinical evaluation:
Our patient’s clinical course was complicated by significant progressive deformities, of both upper and lower limbs. He had bilateral tibial pseudarthroses and went on to develop other deformities requiring long bone rodding as well as restrictive lung disease secondary to limited chest wall growth. He was commenced on bisphosphonate treatment. Subsequently there has been a significant improvement in ambulation, in the non-operated forearm deformities as well as the operated lower limb deformities. Using qPCR, PTHLH expression in fibroblasts was increased in our patient compared to controls. Analysis of the microdeletion demonstrated that it includes PTHLH regulatory sites and results in a distal limb-enhancer being brought into close proximity to the PTHLH gene.

Discussion:
We hypothesize that this novel chromosome 12 microdeletion results in a combination of loss of normal PTHLH regulators and gain of a foreign enhancer, resulting in a phenotype similar to patients with PTHLH duplications.

P110
Case report: a potentially new skeletal dysplasia with autosomal recessive inheritance

Caroline Lekszas, Barbara Vona, Indrajit Nanda, Reza Maroofian & Reza Maroofian

1Institute for Human Genetics, Julius Maximilians University, Würzburg, Germany; 2Monogenic Molecular Genetics, University of Exeter Medical School, Exeter EX1 2LU, UK.

Background:
In this case report, we introduce a patient presenting a potentially unknown syndrome with skeletal involvement.

Presenting problem:
At the time of physical examination, the boy was 10 years old, displaying short stature (z-score -2.4), hearing loss, visual impairment, delayed eruption of teeth and severe dental caries, dysmorphic facial features, micrognathia, mild platyspondyly and genu valgum. Although he appears to have normal intelligence, he answers questions with delay and his school performance is poor, which may be linked to the hearing impairment. The patient is the only affected living child from a consanguineous Iranian couple. He has one unaffected sister. His parents have a history of multiple pregnancies that resulted in a spontaneous abortion, neonatal death or preterm termination due to skeletal malformations detected by ultrasound screening.

Clinical management:
We performed whole exome sequencing (WES) using genomic DNA from the patient, one deceased affected sibling, as well as both parents using the Nextera Rapid Capture library preparation protocol and the NextSeq500 desktop sequencer (Illumina). Data analysis was conducted with GeneSearchNGS, Exomiser and Phenomerizer. By now, no putative causative variants in genes associated with initially suspected diseases like Stickler Syndrome and Schwartz-Jampel Syndrome were observed.

Discussion:
Since our patient’s symptoms resemble a collagenopathy affecting the formation and maintenance of cartilage, we have primarily focused on analysing collagen type II, IX and XI genes. A holistic analysis aided through WES data from four family members will ensue. Following this strategy, we will be able to rapidly investigate genes associated with other collagenopathies and skeletal dysplasias in general, as well as their protein interaction partners (STRING). Notably, an article published in 2005 describes two affected brothers of consanguineous Turkish descent with remarkable phenotypic overlap with our patient. The authors performed mutation analysis of type I collagen genes, but were unable to identify a genetic cause for their patients’ disease. Due to the family’s clinical history and the negative result from the molecular genetic analysis that has been currently performed, we suspect a new collagenopathy with autosomal recessive inheritance and aim to identify a novel candidate gene from ongoing analyses.

Disclosure:
The authors declared no competing interests.

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Vitamin D-dependent rickets type 1 due to a novel mutation in CYP27B1

Yael Levy-Shraga, Ben Pode-Shakked, Naoré Pode-Shakked, Ortal Barel, Jeffrey Jacobson, Gideon Paret & Amnick Raas-Rothschild

1Pediatric Endocrinology Unit, Edmond and Lily Safra Children’s Hospital, Sheba Medical Center, Tel-Hashomer, Israel; 2Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; 3The Institute for Rare Diseases, The Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel; 4Department of Pediatrics A, Edmond and Lily Safra Children’s Hospital, Tel-Hashomer, Israel; 5Sheba Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel; 6Pediatric Imaging Unit, Edmond and Lily Safra Children’s Hospital, Sheba Medical Center, Tel-Hashomer, Israel; 7Department of Pediatric Intensive Care, Edmond and Lily Safra Children’s Hospital, Sheba Medical Center, Tel-Hashomer, Israel.

Background:
Vitamin D-dependent rickets type 1 (VDDR-1) is a rare autosomal recessive disorder caused by mutations in CYP27B1. This gene encodes the 25-hydroxyxylase enzyme which converts 25-hydroxyvitamin D to the active form 1,25-dihydroxyvitamin D.

Objective:
To describe a case of VDDR-1 due to a novel CYP27B1 mutation.

Presenting problem:
A 27-month-old female was admitted to the Pediatric Intensive Care Unit due to respiratory failure. She was born at term to consanguineous parents of Arab Muslim descent. Up until 8 months of age she was reportedly healthy, at which time hypotonia, distended abdomen and developmental delay were noted. She was hospitalized due to failure to thrive, clinical evidence of rickets and recurrent pneumonia episodes, the latest of which brought to respiratory failure and required mechanical ventilation. Due to failed repeated extubation attempts, she was transferred for further evaluation and treatment to our medical center. At admission, her weight was 7 kg (-4SD), height 72 cm (-4SD), and physical examination revealed a large anterior fontanel and palpable rachitic rosary. Blood tests: Calcium-6.9 mg/dl, phosphorus-1.3 mg/dl, magnesium-1.7 mg/dl, alkaline phosphatase-539 IU/l, PTH-521 pg/ml, 25hydroxyvitamin D-36.3 ng/ml, 1,25dihydroxyvitamin D-11 pg/ml. Urine amino acid profile showed significant aminoaciduria, suggestive of renal Fanconi. Radiographs of the skeleton revealed severe demineralization of the skeleton with multiple long bone fractures and vertebral compression fractures along with elongated frayed metaphyses of long bones and wide calvarial sutures. In order to pursue a molecular diagnosis, DNA was extracted from whole blood for whole exome sequencing.

Clinical management:
Sequence analysis revealed a novel homozygous missense mutation, c.383C>T (p.T128I) in the CYP27B1 gene, confirming the diagnosis of VDDR-1. This mutation has not been previously reported and is predicted to be deleterious. The girl was treated with alfalcaldicol, calcium and phosphor supplements and finally was extubated successfully.

Disclosure:
The authors declared no competing interests.

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P112
A novel mutation of CYP27B1 in two siblings with vitamin D-dependent rickets type 1A
Zerrin Orbak
Ataturk University, Medical Faculty Department of Pediatric Endocrinology, Erzurum, Turkey.

Rickets can occur due to vitamin D deficiency or defects in its metabolism. Mutations in the CYP27B1 gene, which encodes vitamin D 1α-hydroxylase, are the genetic basis of vitamin D-dependent rickets type 1A (VDDR1A, OMIM 264700). Vitamin D dependent rickets type 1 is inherited in an autosomal recessive pattern. We report here a new mutation in CYP27B1, which lead to vitamin D dependent rickets type 1. Two boy siblings from a consanguineous Turkish family presented to endocrinologist with short stature and classic features of rickets. We investigated the CYP27B1 gene. A genetic analysis identified a novel homozygous mutation (CYP27B1: Homozygous c.574A>T, p.K192E)). According to the Human Gene Mutation Database, the homozygous mutation identified in our patients is novel and has not yet been reported in the literature. This mutation provides a new basis for further research on VDDR1A and for clinical diagnostics.

The authors declared no competing interests.

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P113
A challenging case of hyperphosphatemic tumoral calcinosis
Virginie Ribault, Philippe M. Campeau, Marie Laberge-Malo, Patricia Olivier, Carine Nyalendo & Nathalie Alos
CHU Ste-Justine, Montreal, Quebec, Canada.

Hyperphosphatemic tumoral calcinosis (HTC) is a rare autosomal recessive metabolic disorder characterized by ectopic calcifications due to progressive deposition of bony-like calcium phosphates in soft tissues. The biochemical hallmark of HTC is hyperphosphatemia caused by increased renal absorption of phosphate due to loss-of-function mutations in three genes: in the fibroblast growth factor-23 gene (FGF23) coding for a potent phosphaturic protein, in GALNT3, gene which encodes a glycosyltransferase responsible for FGF23 O-glycosylation or in KL encoding Klotho, which serves as a co-receptor for FGF23. Only one case of tumoral calcinosis due to a homozygous mutation in KL and FGFRI were recently reported. We report the case of an 8-month-old infant who presented a large painful subcutaneous calcified lesion of the forearm with no history of trauma. His blood work showed elevated fasting serum phosphate levels (Ph: 2.6 mmol/l), inappropriately elevated tubular maximum phosphate reabsorption per unit glomerular filtration rate (TmP/GFR), associated with increased levels of intact FGF23 (SN). Renal function and ionized calcium level were normal with a normal PTH level. Because of progression of the lesion interfering with elbow movements, naproxen therapy was started. The naproxen appeared to be effective on pain and was well tolerated. Elbow function improved in a few weeks. We observed a regression of the calcifications on the radiograph after 8 months of treatment. Six months after stopping the therapy, he is asymptomatic. However, FGF 23 levels remain high with slightly increased Phosphate and normal calcium levels. Persistently elevated FGF23 levels associated with hyperphosphatemia highly suggest a KL mutation. Nevertheless no mutation was found in KL, neither in FGF23 and GALNT3 analyzed by FGF23 analysis and direct sequencing. However, the first 99 amino acids of the KL gene are rare and this analysis is not clear whether genetic factors determine susceptibility to bone fractures in children with normal BMD. In order to further explore the genetic background we screened a cohort of 69 young Finnish patients with mild to severe skeletal fragility for novel pathogenic copy-number variants (CNVs).

The authors declared no competing interests.

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P114
Rare copy number variants in array-based comparative genomic hybridization in early-onset skeletal fragility
Alice Skärp1, Anders Kärr1, Marise Persson1, Riikka Mäkitie2, Minna Männikkö3, Hong Jiao1, Fulya Taylan1, Anna Lindstrand1 & Outi Mäkitie1,3
1Karolinska Institutet, Stockholm, Sweden; 2University of Oulu, Oulu, Finland; 3University of Helsinki, Helsinki, Finland.

Objectives
Early-onset osteoporosis is characterized by low bone mineral density (BMD) and reduced bone strength since childhood or young adulthood. Although several monogenic forms have already been identified, the spectrum of mutations and genes behind this condition remain inadequately characterized. Furthermore, it is not clear whether genetic factors determine susceptibility to bone fractures in children with normal BMD. In order to further explore the genetic background we screened a cohort of 69 young Finnish patients with mild to severe skeletal fragility for novel pathogenic copy-number variants (CNVs).

Methods
We used a custom-made high-resolution 400K comparative genomic hybridization array (array-CGH) with enriched probe density in over 300 genes important for bone metabolism and over 800 genes involved in ciliary function. Findings were validated with breakpoint PCR or whole genome sequencing.

Results
The study cohort included 15 subjects with primary osteoporosis before age 30 years and 54 subjects with a pathological fracture history before age 16 years but mostly normal BMD. Overall, we identified three novel likely pathogenic CNVs: a 4.6-kb deletion involving exons 1-4 of COL1A2 (NM_000089.3), a 11-kb duplication of exon 3 in PLS3 (NM_005302.6) and a 1.6-Mb deletion affecting the entire ETV1 gene and in part DGR8. Mutations in COL1A2, encoding the α2 chain of type 1 collagen, and PLS3, encoding plastin 3, have already been linked to monogenic forms of osteoporosis but deletions in COL1A2 are rare and duplications have not been described in PLS3. Both CNVs were identified in subjects with significant osteoporosis and were present also in other affected members in the two families. Mutations in the transcription factor ETV1, which plays a role e.g. in Ewing sarcoma, and in the diacylglycerol kinase beta (DGKB), have not yet been associated with skeletal fragility. This third CNV therefore needs to be further investigated.

Conclusion
Our study expands the number of CNVs currently known to cause bone fragility and underscores the validity of this method in finding novel candidate genes for early-onset osteoporosis. This study has been supported by the Swedish Research Council. The authors declare no conflict of interest.

The authors declared no competing interests.

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P115
Expanding the genotype-phenotype correlation of osteogenesis imperfecta with a novel mutation in COLA2 gene
Mariomona Al Qanoobi, Harriet Ryan & Ciara McDonnell
Children’s University Hospital, Temple St., Dublin, Ireland.

Background
Osteogenesis imperfecta (OI) is a disorder of bone fragility with a variable spectrum of severity and poor correlation of antenatal findings with postnatal outcome. We present two antenatal diagnosed cases with a mild postnatal course significant for the absence of fractures and progressive remodelling of the long bones. A novel heterozygous pathogenic mutation predicted to replace glycine with aspartic acid at position 913 in exon 42 of the COL1A2 gene has been identified in both cases.

Presenting problem
Case 1: A female proband born at term by C-section was identified on scan at 20-weeks’ gestation to have bowed femur. Postnatally, she had marked curvature of her lower limbs, left talipes, frontal bossing, flat feet, hypermobile joints and white sclerae. Her height is <0.4th percentile. Her head circumference was persistently > 99th centile. Routine CT identified mild dilatation of the lateral
ventricles and evidence of basilar invagination. She has no neurological symptoms.

Case 2: A male proband born at 36-weeks gestation by normal vaginal delivery was identified on antenatal scan to have short long bones and angulation of his femurs. Postnatally he had curved lower limbs, a right parietal skull fracture, frontal bossing, flat feet and white sclerae. His height is 9–25th centile.

Clinical management

Both children [female: born 6y, male: 2.4 years] attend endocrine clinic for regular monitoring for complications of osteogenesis imperfecta. In both cases, motor development, dentition and hearing are normal and neither has had further fracture. Both have normal height velocity with mid-parental height on the 2nd centile. Neither is indicated for bisphosphonate therapy. Both children are monitored for symptoms of basilar invagination. Both attend local intervention services for allied health resources. In both cases the lower limbs have demonstrated progressive remodelling without intervention. Both children have healthy unaffected siblings but the mother of the male proband mother shares the clinical phenotype.

Discussion

Published genotype-phenotype correlations suggest that C-terminal mutations involving glycine substitution with aspartic acid would be suggestive of a higher risk of lethality or a more severe outcome. Phenotyping of antenatal cases based on early ultrasound findings and genotype remain fraught with uncertainty and can result in milder outcomes.

Disclosure

The authors declared no competing interests.

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P116

Classical SATB2-associated syndrome with severe osteoporosis, recurrent bony fractures and tibial bowing

Benjamin Jacobs1, Anna M. Rose2, M. Zulf Mughal3, Richard Keen1 & Emma L. Wakeing2

1Royal National Orthopaedic Hospital, Stannum, UK; 2Northwick Park Hospital, London, UK; 3Royal Manchester Childrens Hospital, Manchester, UK.

Background

Mutations in SATB2 have been described in association with a unique phenotype known as SATB2-associated syndrome (SAS). This condition is characterised by severe intellectual disability affecting speech development, behaviour, facial features and dental anomalies. Skeletal features and osteoporosis have been reported in older individuals (aged 15–36), in association with point mutations. We report a 24-year-old man with a SATB2 missense mutation and classical SAS phenotype, with osteoporosis, fractures and tibial bowing from early childhood.

Presenting problem

The patient developed anterior bowing of the tibiae at 4 years of age. He had 17 fractures, the first at six years of age. Osteoporosis was diagnosed on DEXA scan at age 9.

Clinical management

He was treated with intravenous bisphosphonates for 10 years. Radiological examination aged 12 showed lateral bowing of both femora and anterior bowing of the tibiae and fibulae, in association with diaphyseal widening and cortical thickening. DEXA showed normal bone density 6 months after stopping bisphosphonate therapy. Bone chemistry, including ALP level, is normal. He has severe intellectual disability. He has also required treatment for multiple fractures, the first at six years of age. Osteoporosis was diagnosed on DEXA scan at age 9.

Discussion

This case provides further evidence for the association between SATB2 mutation, osteoporosis and tibial bowing, presenting younger in this patient than previously reported. In patients with intellectual disability of unknown cause, the presence of these distinctive skeletal features may aid clinical diagnosis of SAS. The optimal bone density surveillance and treatment strategy remains to be determined for patients diagnosed with SAS.

Disclosure

The authors declared no competing interests.

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P117

Uptake of influenza vaccine in UK patients with fibro dysplasia ossificans progressiva

Nataliya Gak, Jacqueline Vinton, Benjamin Jacobs & Richard Keen

Royal National Orthopaedic Hospital, London, UK.

Fibrodysplasia ossificans progressiva (FOP; OMIM #135100) begins in childhood and leads to irreversible restriction of movement, functional impairment, and shortened life-span. Individuals with FOP develop progressive limitations in chest expansion, resulting in restrictive lung disease. Current management guidelines published in 2011 (1) highlight that Influenza may be a causative factor for FOP flares-ups, and can also cause potentially deadly cardiopulmonary complications, especially in individuals with severe restrictive chest wall disease. It is therefore recommended that patients with FOP should consider receiving influenza immunisations annually, and that unaffected household members of patients with FOP should also consider annual immunisations to decrease the risk of spreading the influenza to highly susceptible FOP patients. The aim of this study is to investigate the vaccination rates of UK patients with FOP and their household contacts. Telephone interviews were conducted with patients with FOP or their parents/guardians. All patients were resident in the UK and attended a single specialist centre. We interviewed 17 of 20 patients (85%). Of these 10 were children (age < 18 years) and 7 adults (age ≥ 18 years). Seasonal influenza vaccination rates were 23.5% (n=4) in children and 11.7% (n=2) in adults. The main reasons for not having the vaccine were: anxiety about the injection causing a flare of the disease (n=9, 52.9%), recurrent flares of the disease (n=3, 17.6%), and previous administration of influenza vaccine via the intramuscular route rather than subcutaneous, resulting in an acute flare (n=2, 11.7%). Influenza vaccination rates among household contacts (n=39), including parents, partners, children or siblings were 71.4% (n=5) in children and 31.2% (n=10) in adults. This study highlights the need to provide education to individuals with FOP and their close contacts regarding the importance of influenza vaccination. There may also be an added role for education of the primary care/family physician involved in their care. In FOP individuals who are not vaccinated, consideration should be made to have access to antiviral medication if they develop influenza.

Disclosure

The authors declared no competing interests.

Reference


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P118

Genome-wide association study identifies five novel genetic determinants of dental maturatio

Olga Gregic1,2, Strahinja Vucic1,2, Carolina Medina-Gomez1,2, Katerina Trajanoska1,2, Brumilda Dhamo1,3, Vincent Jaddoe1,3, Edwin Ongkosuwito1,3, Marijo-Riutta Jarvelin1,2, Nicholas Timpson2, David Evans2,3, Eppo Wolvius1,2 & Fernando Raviele1,3

1The Generation R Study Group, Rotterdam, The Netherlands; 2Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; 3Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus MC, Rotterdam, The Netherlands; 4NFBC1966 LifeCourse Epidemiology, Imperial College, London, UK; 5ALSPAC, IMRC Integrative Epidemiology Unit, Bristol University, Bristol, UK; 6Diamantina Institute, University of Queensland/AU, Queensland, Australia.

Objectives

Advanced or delayed physiological age may influence significantly health and disease processes. Physiological age can be estimated using several parameters including dental age (DA). Previous meta-analyses studying “Number of Teeth at 15 Months” (NT15M) and “Age at First Teeth Eruption” (AFTE) have identified 15 loci. We performed a genome-wide association study (GWAS) meta-analysis to identify genetic determinants of in children of school age.

Methods

Discovery GWAS of DA was performed in the Generation R study, a multiethnic pregnancy cohort in Rotterdam, The Netherlands. We included 2,793 children or siblings with mean age 9.82 (S.D. = 0.34) years. DA was determined from dental panoramic radiographs using the Demirjian method. Participants were genotyped with the HumanHap 610K platform, imputed to the 1000GP reference panel. Analysis was adjusted for age, sex, height, BMI and 20 genomic principal components; genome-wide significance (GWS) was set at $P<5\times 10^{-8}$. Replication of signals associated with DA was pursued using summary-level results from the published GWAS meta-analysis of the ALSPAC and NFBC1966 studies (n = 12,012) studying NT15M and AFTE. Fisher’s combined probability
test weighted by sample size, implemented in METAL, was used for the combined meta-analysis.

Results
Top signals mapped to 16q12.2 (IRX5: \(P = 1.1 \times 10^{-5}\)) and 17p11.2 (SREBF1; \(P = 9.1 \times 10^{-5}\)) loci associated with advanced DA. Significant evidence for replication of both GWAS signals was observed in the previous NT15M meta-analysis (IRX5: \(P = 2.7 \times 10^{-3}\) and SREBF1; \(P = 0.001\)). In the combined meta-analysis, the top-associated marker in the IRX5-region reached GWS (\(P = 2.1 \times 10^{-5}\)). Also, alleles of these markers associated with higher DA were nominally associated with earlier tooth eruption in the AFTE meta-analysis (IRX5: \(P = 1.5 \times 10^{-3}\) and SREBF1: \(P = 0.002\)). Furthermore, after genome-wide meta-analysis we identified variants in three novel loci: 1q23.1 (ASCL5; \(P = 3.2 \times 10^{-5}\)), 7p15.3 (GDF5; \(P = 2.87 \times 10^{-5}\)) and 14q13.3 (PAX9; \(P = 3.5 \times 10^{-5}\)); on top of replicating seven previously reported loci: 2q13 (EDAR), 10q22.2 (ADK), 12q14.3 (HMGA2), 14q22.2 (BMP4), 14q24.1 (RAD51B), 17q21.32 (G2P2B1), 17q22 (TEX5) and 17q25.3 (intergenic).

Conclusion
We describe here five novel loci associated with dental development. These findings provide further understanding into the process of dental maturation in children from early infancy to late school age. Further, these novel loci implicate diverse pathways related to BMI, lipid metabolism, growth hormone/insulin-like growth factors and craniofacial development.

Disclosure
The authors declared no competing interests.

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P120 Vitamin D-dependent rickets – a rare form of rickets – diagnostics and therapeutic problem
Izabela Michalina, Agnieszka Rusinska, Anna Lupinska & Danuta Chlebna Sokol
Department of Pediatric Propedeutics and Bone Metabolic Diseases Medical University of Lodz, Lodz, Poland.

Introduction
Pseudovitamin D deficiency rickets type I is inherited in an autosomal recessive pattern and forms usually as a result of mutation of CYP27B1 gene localised at chromosome 12. It leads to the deficiency of 1,25-dihydroxycholecalciferol (25(OH)D3) which is crucial for calcium and phosphate transport and metabolism. The diagnosis is made on the basis of history, physical examination and laboratory tests, with hydroxyphosphatasia, hyperparathyroidism, hypocalcaemia and secondary hyperparathyroidism.

Aim of study
We present a case of an almost 2-year-old boy with type 1 vitamin D-dependent rickets with normal levels of 1,25-dihydroxycholecalciferol (25(OH)D3) and vitamin D receptor (VDR) and recurrent episodes of bone pains.

Methodology
The patient was referred to our attention at age 6. He had presented postaxial hexadactyly of the hands and feet, surgically corrected when he was 2 years old. Other skeletal anomalies were short stature with short limbs, short terminal phalanges and mild retrognathia. Weight was above the 97th percentile. In addition, the patient had hypoplastic nails and dental anomalies (conical teeth, enamel hypoplasia, agenesis of permanent lower incisors). Electrocardiogram and echocardiography were normal. A previous array CGH was negative.

Clinical management
The phenotype was evocative of clinical spectrum of EVC-EVC2 mutations. Molecular analysis of EVC and EVC2 identified a heterozygous mutation in exon 22 of EVC2 gene (c.3805G>T). This mutation was not found in the child’s parents and it had been previously described in a single patient with WAD3. Therefore, the phenotype and the mutation were consistent with WAD. Regular endocrinological evaluations showed and confirmed altered bone quality (AD-SoS Z-score −3.99, BTT −2.80) and severe obesity with hyperinsulinism and insulin resistance.

Discussion
Our patient’s skeletal, nail and dental anomalies were typical of WAD. The mutation had been previously described in an Italian patient with WAD, whose clinical features were normal stature, postaxial polydactyly, hypoplastic nails, hypodontia, enamel hypoplasia, abnormally shaped teeth. Altered bone density and severe obesity are not typical features in WAD and had not been described in the child with the same mutation. Our current finding expands the WAD clinical spectrum. Further studies are needed to demonstrate an association of altered bone density and severe obesity with the specific mutation of our patient.

Disclosure
The authors declared no competing interests.

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P122 Bisphosphonate treatment initiated in the newborn period – our experience
Milan Bayer1 & Jana Jiriotkova1
1Department of Children and Adolescents, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 2Department of Pediatrics, Thomayer Hospital of Prague, Prague, Czech Republic.

Abstract withdrawn.
Background
Osteogenesis imperfecta (OI) is a clinically heterogeneous heritable connective tissue disorder with increased bone fragility. Intravenous bisphosphonate therapy is the most widely used medical approach. This treatment leads to an increase of the bone mineral density and reduces the fracture rate.

Presenting problem
We present our experience with five OI patients (one female, four males) between 2005 and 2016 who had bone fractures either in utero or in their first month of life. Targeted genetic testing identified causative mutation in 4/5 patients, three in COL1A2 and one in COL1A1 gene. Children have been treated with cyclic intravenous pamidronate, the mean age of treatment initiation was 6.6 weeks.

Clinical management
Children were clinically defined as OI type IV (patient 1), OI type I (patient 2), and OI type III (patients 3, 4, 5). Moderate side effects of pamidronate were observed only during the first infusion - temporary granulocytopenia was documented in patient 1; flu-like reaction with fever in patients 3 and 5. Treatment for 30.6 months on average reduced the rate of fractures and improved mobility of patients. Lumbar spine areal bone mineral density showed a rapid increase. Repeated intramedullary nailing surgery has been performed in patient 1 and 3. The patients 2 and 3 developed pectus excavatum. All children are short for age (<3.48 ± 1.78 s.d.). The patient 1 is able to walk short distances with aids, the patients 2, 3, 4, 5 are able to walk short distances independently. All patients show normal mental development.

Discussion
Mutations in genes COL1A1 and COL1A2 are mostly inherited through an autosomal dominant pattern. They could be associated with moderate to severe phenotype OI – type I, II, III, and IV. Bone fractures and deformities documented before or early after birth call for an urgent intervention. Early treatment with intravenous pamidronate seems to be safe and efficient. Only moderate side effects were observed. Most of the patients show very good mobility in spite of their severe perinatal status. Early pharmacotherapy, long-lasting rehabilitation, and corrective surgery significantly improve quality of life in children with a manifestation of multiple OI fractures immediately after birth.

Disclosure
The authors declared no competing interests.

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P123

Abstract withdrawn.

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P124

The treatment of Hyperphosphatemic Familial Tumoral Calcinosis
Karine Khatchadourian1, Lou Lawton2, Baxter Willis2 & Leanne Ward1
1Departments of Pediatrics, Children’s Hospital of Eastern Ontario, and The University of Ottawa, Ottawa, Canada; 2Department of Surgery, Children’s Hospital of Eastern Ontario, and The University of Ottawa, Ottawa, Canada.

Background
Hyperphosphatemic familial tumoral calcinosis (HFTC) is characterized by hyperphosphatemia and the formation of tumor-like extra-ossseous calcifications. Tumors often necessitate surgical management although medical treatment may limit the need for surgical intervention. We present two cases of HFTC successfully managed with combination acetazolamide (ACTZ) and sevelamer carbonate.

Case Report #1
An 11-year-old Arab girl with no mutation in FGF23, KL, GALNT3 and SAMD9 genes presented with TC at the left elbow which was gradually increasing in size over the last 5 years. Combination therapy with ACTZ and sevelamer was initiated at age 12. Biochemistry prior to therapy was as follows: serum phosphate 1.43 mmol/l (N:1.05–1.75) although patient was mildly hyperphosphataemic at presentation (phosphate 1.82 mmol/l), 1,25-dihydroxyvitamin D3 143 pmol/l (N:39–193), c-terminal FGF23 1435 RU/ml (N: < 230). After 2 years of therapy, the patient remained normophosphataemic with serum phosphate level at 1.27 mmol/l despite maintaining mild metabolic acidosis with serum bicarbonate 23 mmol/l. Clinically, the patient has shown mild regression of the tumor and normal activity. Despite mild hypercalciuria with treatment, there has been no nephrocalcinosis.

Conclusions
Combination ACTZ and sevelamer therapy resulted in significant tumor regression in patient 1, and mild tumor regression in patient 2. The agents were well tolerated and safe without evidence of nephrocalcinosis after 2 years of therapy.

Disclosure
The authors declared no competing interests.

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P125

Identification and characterization of a novel microRNA inhibiting osteoblast functions by suppressing actin polymerization
Ajaz Ahmad John, Ravi Prahak & Divya Singh
CSIR-Central Drug Research Institute, Lucknow, India.

MicroRNAs (miRNAs) are small non-coding RNAs that have emerged as critical post-transcriptional regulators of gene expression. There is increasing evidence that miRNAs play an important role in osteoblast commitment and differentiation. The main aim of this study was to identify and characterize novel miRNAs regulating osteoblast functions. We report the role of mmu-miR-1187 in osteoblast differentiation and the mode by which it regulates osteogenesis. MicroRNA profiling of calvarial osteoblasts revealed that mmu-miR-1187 was ~8.5 fold down regulated in response to Med treatment. This data was further validated by qRT-PCR in calvarial osteoblasts. Over-expression of mmu-miR-1187 inhibited osteoblast differentiation, whereas inhibition of mmu-miR-1187 function promoted osteoblast differentiation and mineralization. Target prediction analysis tools and experimental validation by luciferase 3’ UTR reporter assay identified BMPRII as a direct target of mmu-miR-1187. Over expression of mmu-miR-1187 in osteoblasts led to down regulation of BMP-2 induced and cdc42 mediated actin cytoskeletal organization. All these results were reversed on transfection with anti-miR-1187. Additionally, after visualizing actin with TRITC-conjugated phallolidin, it was revealed that over expression of anti-miR-1187 resulted in increased actin polymerization and cortical protrusions formation. Invivo experiments revealed that on injecting miR-1187 subcutaneously in 1–2 days old Balb/c pups inhibited osteoblast differentiation, whereas inhibition of mmu-miR-1187 function promoted osteoblast differentiation in Balb/c calvaria. Our data suggests that binding of mmu-miR-1187 represses BMPRII thus inhibiting BMP2 signalling pathway which is required to activate cdc42 and phosphorylate LIMK1. LIMK1 is not able to inactivate cofilin which is an actin depolymerizing factor. mmu-miR-1187 may thus be inhibiting osteoblast functions by suppressing actin polymerization. Our findings suggest that therapeutic approaches targeting mmu-miR-1187 for enhancing osteoblast functions may be useful.

Disclosure
The authors declared no competing interests.

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P126

A case of severe reaction following the use of Bisphosphonates in a patient with Osteogenesis Imperfecta
Julie Park1, Hussain AlSaffar1, Louise Apperley1, Nick Bishop2, Poonam Dharmaraj2 & Renuka Ramakrishnan3
1Alder Hey Children’s Hospital, Liverpool, UK; 2Sheffield Children’s Hospital, Sheffield, UK.

Background
We present a case of unusual delayed multi-systemic reaction, following treatment with Pamidronate. The reaction, resembling rhabdomyolysis, requiring intensive care support, has not been reported previously to our knowledge.

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Presenting problem
An 11 month old boy with severe osteogenesis imperfecta (OI) presented with hyperpyrexia and respiratory distress 10 days after his fifth cycle of Pamidronate. His respiratory distress was out of proportion to the chest radiograph changes. BiPAP was required for ventilatory support.

Clinical management
He was initially treated as presumed sepsis. However, there were no significant positive microbiology cultures and investigations for Haemophagocytic lymphophytosis were negative. He was managed with supportive treatment and gradually improved. He subsequently developed decreased head movement, stiffness of limbs, poor interaction with carers and an anxious look. He had a normal CT head and cervical spine raising the possibility of continuing muscle and bone pain. He showed an excellent response to morphine.

Discussion
Serious adverse reactions have been reported with bisphosphonate use including renal failure after several cycles, severe muscle and bone pain, dermatomyositis and rhabdomyolysis in adults. Our patient may have developed rhabdomyolysis following Pamidronate treatment, which has not been reported previously in children. He was extensively investigated to exclude other possibilities and made a full recovery with only supportive management.

Disclosure
The authors declared no competing interests.

References

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P127

Growth hormone treatment in two short peri-pubertal brothers with X-linked hypophosphatemic rickets
Rachel Bello Vitiria, Ariel Tenenbaum1,2, Liara Lazar1,2, Miriam Davidovitz2 & Yael Lebenthal1
1The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, Schneider Children’s Medical Center of Israel, Petah Tikva, Israel; 2Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 3Institute of Nephrology, Schneider Children’s Medical Center of Israel, Petah Tikva, Israel.

Background
X-linked hypophosphatemic rickets (XLH) is characterized by hypophosphatemia, bone deformities, and growth retardation. Conventional treatment of XLH with oral phosphate supplementation and high doses of vitamin D fails to normalize linear growth and adult stature remains disproportionately short. Few studies report on the use of recombinant human growth hormone (rhGH) therapy in pre-pubertal children with XLH.

Presenting problem
Two brothers were diagnosed clinically at infancy with XLH. Genetic analysis confirmed a frameshift mutation in the PHEX gene consistent with XLH. At endocrine referral extreme short stature (−3.7 SDS and −2.5 SDS) and an elevated upper segment/lower segment ratio were revealed.

Clinical management
Since treatment with alfacalcidol, phosphate and vitamin D failed to improve growth velocity, the GH axis was tested and GH reserve was normal. Both siblings underwent osteotomy for correction of their limb deformities (11.3 years and 10.8 years) with a beneficial effect on height-SDS (−3.7 to −2.4 and −2.5 to −1.9). Due to short stature at initiation of puberty and predicted compromised final height despite adherence to conventional therapy and osteotomy, rhGH therapy was initiated. Twelve-month rhGH treatment (0.05 mg/kg per d) initiated one year after surgical correction - improved height-SDS (−2.4 to −1.8 and −1.9 to −0.9). BMI centile remained stable 75%; U/L ratio (increased from 1.08 to 1.18 (within normal range) and remained stable 1.25 (increased)). puberetal stage advanced from Tanner 2 to 3 in both siblings and as expected IGF-1 levels increased (less than +2 SDS). During rhGH therapy metabolic markers of calcium phosphate metabolism remained largely unchanged; a transient increase in renal phosphate reabsorption and serum phosphate levels were observed and PTH levels slightly increased. No adverse effects were reported.

Discussion
Previous reports on rhGH treatment of short children with primary or metabolic bone diseases (XLH, skeletal dysplasias) have raised concern regarding disproportionate skeletal growth and questioned the benefit of this therapy in advanced age and pubertal stage. We report our preliminary clinical experience with short-term rhGH therapy in two peri-pubertal GH-sufficient short boys with XLH without progression of body disproportion. Long-term controlled studies are warranted to elucidate whether this adjuvant treatment can improve adult height without exacerbating the disproportionate body segments.

Disclosure
The authors declared no competing interests.

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P128

Growth and clinical outcome in a 16 year-old male with childhood hypophosphatasia after 1 year therapy with asfotase alfa
Sasigarn Bowden & Brent Adler
Nationwide Children’s Hospital, Columbus, Ohio, USA.

Background
Asfotase alfa therapy improves clinical outcome in young children with severe form of hypophosphatasia (HPP). Treatment outcome in older children (≥12 years) has not been reported.

Presenting problem
We report clinical outcome of a 16 year-old male with childhood HPP who started enzyme therapy at age 15 years.

Clinical management
The patient was diagnosed with HPP at age 2 years when he presented with premature loss of primary teeth and genu varum. He had a history of multiple fractures requiring 16 orthopaedic surgeries with rod and pin placement in his lower extremities. He had chronic skeletal pain and used cane to ambulate with great difficulty. He presented to Endocrine Clinic at age 15 years with height of 126.4 cm (Z score −4.7, height age 7.5 years), arm span 139 cm, weight 25.2 kg (Z = −5.78), Tanner stage 3 for pubertal development. He had severe scoliosis and deformity of both legs. Serum alkaline phosphatase level was <20 U/L with elevated pyridoxal 5-phosphate (836.8 nmol/l; normal 20–125). Bone age was delayed at 12.5 years with marked metaphyseal fraying and lucency in distal radius and ulna. He was started on asfotase alfa 2 mg/kg Q20 3 times/week. He had marked clinical improvement in growth and mobility with no report of pain after 3 months of treatment. At 6 month follow up, he walked without cane and became more sociable and liked to play outdoor with peers. Bone radiograph at 6 months showed striking improvement in previous lucency areas. At 9 months, height was 133.5 cm (growth velocity of 9.5 cm/year), while arm span increased to 148 cm (growth velocity of 12 cm/year). However, at 12 months, he was noted to have worsening scoliosis from 70 degrees before therapy to 110 degrees, with slightly decreased height at 129.5 cm, necessitating a scoliosis surgery. His lumbar bone density improved from baseline of 0.319 to 0.381 gram/cm² at 1 year (height-adjusted Z score decreased from −2.7 to −3.1).

Discussion
Treatment with asfotase alfa for 1 year significantly improved growth, physical function, pain, overall quality of life and skeletal radiographic findings in this patient.

Disclosure
The authors declared no competing interests.

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The case of severe osteoporosis in patient with recessive dystrophic Epidermolysis Bullosa
Nataliya Balatska & Vladyslav Povoroznyuk
D. F. Chebotarev Institute of Gerontology, NAMS of Ukraine, Kyiv, Ukraine.

Background
Epidermolysis bullosa (BE) is a group of inherited diseases that are characterized by skin and mucosal fragility and blister formation. The various complications such as malnutrition, anemia, growth retardation, esophageal stenosis and deformed teeth may develop. Low bone mass and fractures recognized as complications of generalized forms of EB.

Presenting problem
In the Ukrainian medical center of osteoporosis there were examined nine children with generalized recessive dystrophic form BE from 10 to 15 years old. Low bone mass was diagnosed in 36.4% cases after adjusting for height Z-score. Nobody had fractures. All children were prescribed the calcium (1000 mg) and vitamin D (800 IU) supplements. In seven months after examination one of the patients who had normal BMD (aBMD Z-score at total body – 1.7±1.6, and –1.8 at the level L1-L4) and reduced TBS (0.851) was diagnosed with multiple vertebral fractures. His 25(OH)D level was 15.8 ng/ml, calcium - 2.46 mmol/l, β-CTX - 1.05 ng/ml, alkaline phosphatase - 79.72 U/ml (norm 26–117). Patient complained of severe low back pain which reduced his mobility.

Clinical management
The diagnosed vertebral fractures due to osteoporosis are indication to bisphosphonate therapy. Patient was prescribed Pamidronate in doses 1 mg/kg infusion. Unfortunately the prescribed osteotropic treatment in combination with analgesics did not release the pain syndrome and did not improve mobility of the child during next 3 months.

Discussion
Patients with BE are suffering with chronic pain syndrome which have substantial effects on their quality of life while vertebral fractures due to osteoporosis may incredibly increase the pain syndrome. However, in children the low bone density without history of clinically significant fragility fracture is not indication to the specific osteotropic treatment. Vertebral fractures prevention for children with BE is extremely important.

Disclosure
The authors declared no competing interests.

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The impact of intravenous bisphosphonate on vertebral morphometry in children with secondary osteoporosis and vertebral fractures
JG Timmons1, R Morrice1, S Joseph1,2, S Shepherd3, A Mason1, SF Ahmed4 & SC Wong4
1Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK; 2Paediatric Neurosciences Research Group, Department of Paediatric Neurology, Royal Hospital for Children, Glasgow, UK.

Background
Intravenous (IV) bisphosphonate (BP) is used for treatment of painful vertebral fractures (VF) in children with underlying chronic conditions. BP effect on vertebral height reshaping in this population is however poorly studied.

Aims/Objectives
To evaluate the impact of IV BP on vertebral morphometry in children with VF and underlying chronic medical conditions with associated glucocorticoid (GC) therapy.

Methods
Retrospective study of eight children (6M) with VF treated with IV BP for 1–2 years: 5 Duchenne Muscular Dystrophy (DMD), 2 Crohn’s Disease (CD), 1 Juvenile Dermatomyositis (JDM). Vertebral height from lateral spine x-rays (T10-L5) were measured on two occasions by one single observer (JT). Repeatability co-efficient of vertebral height ratios were determined. Improvement and deterioration in vertebral height ratio was considered to be significant if changes exceeded the 99% confidence level of the repeatability co-efficient at a particular vertebral level and also changed Genant staging. Results presented as median (range).

Results
Median age at baseline was 12.2 years (5.6, 17.0). Height SDS at baseline was −2.4 (−3.4, −1.4), significantly lower than height SDS a year prior to commencement of therapy, −1.5 (−2.7, −0.8) (P=0.0009) but did not improve following BPs, −2.5 (−4.9, 0.2) (P=0.87). All eight were on GC at baseline but only 5/8 were on GC at last follow-up. Lumbar spine bone mineral content SDS for bone area at start was −0.9 (−2.8, 1.9) and did not change with BP, −0.4 (−1.7, 1.6) (P=0.08). Vertebral height ratio improved in 3/8 children (2 DMD, 1 CD) in 6 vertebrae (2 DMD, 1 CD). Vertebral height ratio decreased in 3/8 children (2 DMD, 1 CD) in 8 vertebrae. One boy with DMD who showed deterioration of vertebral height ratio also sustained fracture femur during BP therapy. Four out of the 8 (3 DMD, 1 JDM) had stabilization of vertebral height ratio with therapy.

Conclusion
Intravenous bisphosphonates in children with chronic disease and vertebral fracture led to stabilization in vertebral height in half of the cohort and improvement in vertebral height in some children but did not prevent the development of new VF.

Disclosure
The authors declared no competing interests.

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The treatment of Camurati-Engelmann disease with Losartan: a case report
Alex Moylan1, Emma L. Wakeling2, M. Zulf Mughal1, Richard Keen1, Matt Thornton1, Daniela Peeva3 & Benjamin Jacobs1
1Royal National Orthopaedic Hospital, Stanmore, UK; 2Northwick Park Hospital, London, UK; 3Royal Manchester Children’s Hospital, Manchester, UK.

Background
Camurati-Engelmann disease (CED) is a rare bone dysplasia characterised by hyperostosis and sclerosis of the diaphyses of the long bones and skull. It is caused by autosomal dominant gain-of function mutations within TGFBI1, which result in increased activity of transforming growth factor β1 (TGF-β1). It typically presents in mid-childhood with bone pain, myopathy and progressive immobility. Evidence for treatment is based on a number of case reports, most of which describe the response to glucocorticoids. Losartan, an angiotensin-II receptor antagonist, is known to reduce expression of TGF-β1 and there are reports of two children with CED who showed significant improvement in pain and mobility in response to this treatment.

Presenting problem
A 10 year old child with a clinical and radiological diagnosis of CED (Figures 1 and 2) was found to have a heterozygous TGFBI1 mutation (p.Y104H). He had significant leg pain and difficulty walking.

Clinical management
We commenced losartan treatment at a dose of 0.6 mg/kg daily. Response to treatment was assessed using the 6 minute walk test, Child Health Assessment Questionnaire (CHAQ) and formal assessment of gait, and bone health using biochemical markers of inflammation and bone turnover and radiological appearance including radiographs and densitometry. We monitored for side effects, including specific monitoring for hypotension and electrolyte abnormalities. He reported a significant decrease in pain and improvement in walking, his progress is shown in the table.

Discussion
The early response to treatment in our patient and the lack of side-effects supports the use of losartan as a first line treatment for CED.
**P132**

**Anti-RANKL treatment in a murine model of fibrous dysplasia**

Biagio Palmisano1, Rosella Labella1, Emanuela Spica2, Cristina Remoli2, Alessandro Corsi1, Pamela Robey3 & Mara Riminucci4

1Sapienza University of Rome, Rome, Italy; 2NIDCR, NIH, Bethesda, USA.

Fibrous dysplasia of bone (FD) is a crippling skeletal disease caused by activating mutations (R201C, R201H) of the Gsa gene. We recently generated Gsa<sup>201H</sup>transgenic mice that develop a FD skeletal phenotype. The analyses of these mice demonstrated that increased bone resorption is one of the main morbidity factors in FD and that RANKL is the major molecular mediator of osteolysis at affected skeletal sites.

**Objective**

The aim of this study is to investigate the effect of RANKL-inhibition on the development and evolution of skeletal lesions in our mouse model of FD.

**Methods**

Twenty-four mice (2 months of age) with radiographically detectable lesions in the tail vertebrae were selected for the study. The mice were treated with either anti-RANKL antibody, or isotype rat IgG2a as a control (300 μg/mouse) by intraperitoneal injection, twice a week for 14 weeks. In each experimental group, half of the animals were euthanized at the end of the treatment, whereas the remaining half underwent a 3-month follow-up. The mice were radiographically monitored during treatment and follow up, at the end of which histological analysis was performed.

**Results**

The anti-RANKL antibody induced a progressive increase in bone density with disappearance of lytic areas in all treated mice. In addition, it prevented the development of new bone lesions and deformities. In contrast, the radiographic phenotype steadily progressed in control mice. Histological analysis performed at the end of the treatment confirmed a higher amount of bone in the tail vertebrae of the mice treated with anti-RANKL antibody compared to controls. The newly formed bone obliterated the medullary cavity and showed, at least in part, a lamellar structure. In contrast, radiographically non-affected vertebrae remained a normal space. As expected, virtually no osteoclasts were identified by histological and cytochemical (TRAP) analysis. The discontinuation of the treatment was associated with a rebound of the disease. In both anti-RANKL-treated and control mice, radiographs after 3 months of follow up were similar. The presence of fibro-osseous tissue along with clusters of TRAP-positive osteoclasts confirmed the relapse of the disease at histological level.

**Conclusions**

This preliminary study indicates that treatment with the anti-RANKL antibody is effective in preventing the progression of the disease. However, once treatment is discontinued, rebound of the disease occurs. Design of alternative therapeutic strategies are in progress.

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**Disclosure**

The authors declared no competing interests.

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**P134**

**Improvement in spinal involvement with zoledronic acid in pediatric patients with chronic recurrent multifocal osteomyelitis: a case series**

Marie-Eve Robinson, Anne Marie Shroccoli & Rosie Scuccimarrri

McGill University, Montreal, Quebec, Canada.

**Background**

Chronic recurrent multifocal osteomyelitis (CRMO) is a rare inflammatory bone disease characterized by chronic, non-infectious osteomyelitis. Spinal involvement has been reported in up to 26% of patients (1). Three studies evaluated the effect of Pamidronate (PAM) on spinal lesions in pediatric patients with CRMO (1, 2, 3) and showed partial or complete resolution of vertebral hyperintensities on MRI (1). However, the effect of Zoledronic acid (ZOL) in pediatric patients with CRMO with spinal involvement has not been previously reported.

**Presenting problem**

We report a case series of 3 patients seen at a tertiary pediatric center between March 2014 and January 2016 with CRMO and vertebral spine involvement, treated with ZOL 0.025 mg/kg every 3 months. We performed full body MRI before and 6 to 12 months after ZOL. A 14-year-old girl had hyperintense vertebral lesions which completely resolved after 6 months, while a 10-year-old girl with a diagnosis of William syndrome also had hyperintense vertebral lesions which significantly improved within one year. Both patients reported back pain before initiating ZOL, which completed resolved within 3 months with concomitant use of NSAIDs. The third case, a 4½ year old girl, did not have hyperintense vertebral lesions or back pain, but rather a Genant grade 3 vertebral compression fracture. Her fracture did not progress further after 6 months of treatment. ZOL was well tolerated with no significant adverse effects in all patients.

**Discussion**

This case series is the first to document the effect of ZOL on spinal involvement in pediatric CRMO patients. The positive effect of ZOL in our patients is concordant with the reported effect of PAM in CRMO patients treated for spinal involvement and back pain. One of our patient had a severe vertebral compression fracture, possibly due to a previous and inactive spinal lesion from CRMO. Follow-up imaging in this patient only 6 months after treatment might explain the absence of improvement in her vertebral fracture. In conclusion, our data shows that ZOL can be useful in treating vertebral hyperintensities and back pain in pediatric CRMO. Further experience with the use of ZOL in this context is needed to confirm these findings.

**Disclosure**

The authors declared no competing interests.

References

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**P133**

**Long term treatment with intravenous pamidronate in two children with severe form of juvenile Paget’s disease**

Katja Verfürth1,3, Matthias Höffel1,3, Michael Schindeln1,3 & Corinna Graesemann1,3

1Sapienza University of Rome, Rome, Italy; 2NIDCR, NIH, Bethesda, USA.

Severe forms of juvenile Paget’s Syndrome (JPP) result in extreme bone turnover, necessitating long-term treatment with anti-resorptive drugs to control bone pain and modeling of bones.

**Objective**

To report clinical and biochemical effects of intravenous treatment with Pamidronate in two children with severe forms of JPP over a time period of 3 and 9 years, respectively. Treatment was commenced at 12 months and 3 years of age, respectively.

**Clinical management**

Over time, doses of Pamidronate and infusion intervals were adjusted according to the presence of bone pain, bone turnover markers and bodyweight. Yearly doses ranged from 5.5 mg/kg per year to 9 mg/kg per year and infusion intervals varied from 3 monthly to 5 weekly. Growth, motoric development (developmental scale), occurrence of fractures, bone turnover markers and bone pain were recorded.

**Conclusion**

Individualized intravenous treatment with Pamidronate resulted in sufficient control of bone pain and suppression of bone turnover markers, with few side effects. At present, both children are fully ambulatory. Signs of bone pain present about 6 weeks after each treatment in the patients. Motoric development was delayed in both children, but improved significantly with treatment.

**Disclosure**

The authors declared no competing interests.

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Continuous subcutaneous PTH infusion in autosomal dominant hypocalcaemia

Evelien Gevers1, Jacqui Buck2, Neil Ashman3, Rajesh Thakker4 & Jeremy Allgrove5
1Department of Paediatric Endocrinology, Queen Mary University London and Barts Health NHS Trust, London, UK; 2Department of Paediatrics, Ipswich Hospital, Ipswich, UK; 3Department of Nephrology, Barts Health NHS Trust, London, UK; 4Academic Endocrine Unit, University of Oxford, Oxford, UK; 5Department of Endocrinology, Great Ormond Street Hospital, London, UK.

Objectives
Autosomal Dominant Hypocalcaemia (ADH) is due to gain-of-function mutations of the CASR resulting in constitutive activation of the GPCR Calcium Sensing Receptor (CaSR) leading to hypercalcic hypercalciuria, hypoparathyroidism and occasionally Bartter syndrome type V. Patients usually present with hypocalcaemic seizures at young age. Conventional treatment is with Alfacalcidol and Calcium or PTH injections. We describe a series of five patients with ADH in whom stabilization of calcium concentrations could not be achieved with conventional treatment and in whom continuous subcutaneous PTH infusion (CSPI) using insulin pumps was started.

Methods and results
CaSR mutations were P.Thr828Asn, not previously described, and the previously described p.Ala843Glu, p.Tyr829Cys, p.Phe821Leu. Patients presented with hypocalcaemic seizures or tetany in the first few weeks of life. Additional features were bilateral cataracts, hypomagnesaemia, Bartter type V. One patient had nephrocalcinosis before CSPI. Age at start of CSPI was 3 weeks, 6 weeks, 6 months, 6 years and 20 years. Medtronic and Omnipod patch pumps were used to deliver diluted PTH(1-34). Treatment was started in an inpatient setting. Duration of treatment is currently 1–3 years. PTH requirement was 0.21, 0.13, 0.15, 0.5 and 3 μg/kg per day. Four patients required Magnesium supplementation. All patients received Cholecalciferol. Calcium concentration stabilised and patients continue to require weekly or bi-weekly blood tests. Number of admissions significantly reduced during CSPI. Seizures stopped in all patients on CSPI. Current calcium concentrations range from 1.75 to 2.15 mmol/l. Current urine Calcium/creat ratios range from 1.2 to 2.5 mol/mol. Nephrocalcinosis has remained stable. One patient stopped pump treatment temporarily due to instable calcium concentrations.

Conclusion
We describe continuous subcutaneous PTH infusion as a suitable treatment for ADH that cannot be controlled conventionally. We also describe a new CaSR mutation resulting in ADH and cataracts, which is also a feature of the mouse model for ADH. Cataracts have since been found in some patients with ADH. Longer follow up is required to assess whether continuous sc PTH treatment delays the progression of nephrocalcinosis.

Disclosure
The authors declared no competing interests.

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P136 Preliminary results for a ramping model of pamidronate administration
Alyssa K Givens1,2, Steven Bachrach1, H Theodore Harcke1 & Heidi H Keesekemeyer1
1Nemours/AI duPont Hospital for Children, Wilmington, Delaware, USA; 2University of Delaware, Newark, Delaware, USA.

Objectives
Examine the effects of a ramped dosage schedule of pamidronate on BMD, fracture rate and location compared to a uniform 5-course regimen. The ramping regimen is intended to alter the tendency for post-treatment fractures to occur at the juncture of pamidronate bands where stress-riser related fractures have been described.

Methods
Ten non-ambulatory children (seven females) with neuromuscular disabilities who received IV pamidronate with a tapering dose were identified (Group 1) and compared to a cohort of 25 patients who received a uniform 5-course regimen (Group 2). Periodic DXA evaluations were performed every 6 months. Fracture rate before and after treatment was calculated using the person-years method, with post-treatment observation starting with the first dose. Radiographs were examined for location of fracture.

Results
Over treatment, lumbar spine (LS) BMD increased 47.7% and lateral distal femur (LDF) BMD of all regions (R1, R2 & R3) increased 38.0%, 30.1%, and 22.9%, respectively. Group 2 BMD increases were 40.3% at LS and 68.3%, 15.6% and 10.3%, for LDF R1–R3. Mean post-treatment observation period was 4.6 years. Two of ten children in Group 1 sustained a fracture during treatment (right distal femur at 3 weeks, left foot at 10.5 months) but no fractures occurred after treatment ended. Pre and post-treatment fracture rates were 18.5% and 4.3%. Four of 25 in Group 2 sustained 5 fractures after treatment over the same time period; 80% occurred at pamidronate bands. Pre and post-treatment fracture rates in were 36% and 13% for Group 2.

Conclusion
Regardless of dosing schedule, BMD improved, post-treatment fracture rate decreased after treatment started, and fractures occurred during treatment. After treatment ended, no fractures occurred in those who received the ramped dosage, suggesting reduction in stress riser formation. A longer treatment period and greater total amount of drug administered with the ramped dose might be contributory. Further study of a ramped dosing schedule is warranted.

Disclosure
The authors declared no competing interests.

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P137 Growth, bone and muscle mass are adversely affected in bone marrow transplant recipients: a body composition analysis
Artemis Doulgeraki1, E Vlachopapadopoulou2, I Peristeri2, A Pasiou3, G Polizos1, K Kaisari1, I Monopolis2, G Vessalas1, S Michalacou1 & V Plastira2
1Department of Bone and Mineral Metabolism, Institute of Child Health, Athens, Greece; 2Department of Endocrinology, Growth and Development, “P.&A. Kyriakou” Children’s Hospital, Athens, Greece; 3Bone Marrow Transplant Unit, Oncology Unit M. V. Vardinogianni “ELPIDA”, “Agia Sophia” Children’s Hospital, Athens, Greece; 4Biostatistician, Athens, Greece.

Objectives
There are many factors leading to poor bone health and imbalanced body composition in bone marrow transplant (BMT) recipients. We aimed to report our patients’ profile and to correlate it with clinical parameters.

Methods
Cross-sectional study of paediatric BMT patients. Assessment of growth (height, weight, BMI) and dual-energy X-ray absorptiometry (DXA) for evaluation of bone mineral density (BMD) and geometry, muscle and fat mass. All results were converted to Z-scores. Also, lean tissue mass and fat mass indexes were calculated (LTMI and FMI, respectively) and BMD was corrected for height, where indicated. Comparisons were made with 57 Greek controls and between patient subgroups.

Results
34 patients, aged 14.6 ± 3 years were studied (of which 15 girls, 27 adolescents and 12 with a previous diagnosis of acute lymphoblastic leukaemia). Six patients (17%) sustained a total of 12 fractures (1 vertebral) and three had osteonecrosis. 85% were on vitamin D and calcium supplements and 41% were exercising regularly. 35% were on hormone replacement therapy for hypogonadism and 23% had low vitamin D. Compared to controls, our population had impaired growth, lower lumbar BMD Z-score (mean: −0.5 ± 1.3, P < 0.01) lighter and smaller bones, with lower strength and less muscle mass (LTMI Z-score: −1.7 ± 1.3, P < 0.01). Within-group analysis revealed that female sex, prepubertal status, hypogonadism and lack of regular exercise adversely affected both total body (less head) BMD and LTMI. History of graft-versus-host disease led to lower Z-score for bone strength (bone mineral content/lean tissue mass ratio), mean value 0.2 ± 1.1, P = 0.03). Of note, BMD and body composition were not affected by inadequate calcium intake, history of bone pain, radiotherapy or corticosteroid treatment (P > 0.05). Finally, strong and positive correlations were found between BMI, bone width and BMD at both sites of measurement, LTMI and FMI (P < 0.01).

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Conclusions
In our cohort, and despite proper dietary supplementation and hypogonadism treatment, growth, bone and muscle mass were adversely affected, whereas fat mass was comparable to controls. Optimizing BMI through lifestyle interventions and enhancing bone width through mechanical loading, may prove to be useful clinical targets, in order to improve body composition profile in these patients.

Disclosure
The authors declared no competing interests.

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P138
Fibrodysplasia ossificans progressiva: baseline characteristics of 101 subjects participating in a global, longitudinal, natural history study
Frederick S Kaplan1, Edward C Hisao2, Genevieve Bautaj3, Matthew A Brown4, Carmen De Cunto5, Jada Di Rocco6, Richard Keen7, Mona Al Makkad8, Donna R Grogan9 & Robert J Pignolo9

1Center for Research in FOP and Related Disorders, The University of Pennsylvania, Philadelphia, Pennsylvania, USA; 2Division of Endocrinology and Metabolism, University of California, San Francisco, California, USA; 3Hospital Necker-Enfants Malades Centre de Reference Maladies Ossesues Constitutionnelles Departement de Genetique, Paris, France; 4Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia; 5Department of Pediatrics, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; 6Unit of Rare Diseases, Department of Pediatrics, IRCCS Giannina Gaslini, Genoa, Italy; 7Royal National Orthopaedic Hospital, Stanmore, UK; 8Clementia Pharmaceuticals Inc., Boston, Massachusetts, USA; 9Division of Geriatric Medicine and Gerontology, Mayo Clinic College of Medicine, Rochester, Minnesota, USA.

Objectives
Progressive heterotopic ossification in fibrodysplasia ossificans progressiva (FOP; OMIM #135100) begins in childhood and leads to irreversible restriction of movement, functional impairment, and shortened life-span. Baseline data from an on-going, global, 3-year, natural history study (NHS) describe FOP disease characteristics, and retrospective flare-up history, causes/symptoms, and outcomes.

Methods
Data from 101 subjects (recruited from 23 countries/five continents) were analysed overall and by age (<8, 8 to <15, 15 to <25, and 25 to <65 years).

Results
The median age of this cohort was 14.0 years (range 4-56 years; 55% male; 74% Caucasian). By self-report, all but one subject (99%) had great toe malformations. Thumb malformations (51%) and tibial osteochondromas (37%) were also common. Lesional biopsy (18%, 26%, 52%, and 73% in the respective age groups) and misdiagnoses (24%, 46%, 67%, and 64%, respectively) occurred less often in younger versus older subjects. FOP-associated medical conditions included hearing loss (43%), restricted chest expansion (38%), fracture (32%), and ankylosed jaw (32%). Initial flare-ups (median onset 4.5 years) were reported in the cervical spine (20%), upper back/thoracic spine (20%), and head (19%); older subjects had more flare-ups in the hip (see table). Retrospective accounts of subjects’ last flare-up prior to enrolment are summarized in the table.

Conclusions
These baseline results are similar to a prior retrospective, international survey in 500 patients (Pignolo et al., 2015). It demonstrates that the NHS sample (~13% of the world’s known FOP population) is representative. The results also indicate that lesions biopsy and misdiagnoses are decreasing, suggesting improved diagnostic awareness among clinicians. Ultimately, the NHS will provide important prospective data on FOP disease progression. The authors would like to thank the International FOP Association for fostering patient participation in this study.

Disclosure
De Grogan is an employee of Clementia Pharmaceuticals Inc., which sponsored this study.

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P139
Relative impact of muscle strength and muscle mass on bone mineral density in Japanese adolescents: data from the Kitakata Kids Health Study
Katsuyasu Kouda1, Yuki Fujita1, Takahiro Tachik1, Akiko Yura2, Yuho Sato2, Jaakkhorol Myadagmaa3, Namiraa Dongmei3, Kumiko Ohara4, Harunobu Nakamura5 & Masayuki Ik1
1Department of Public Health, Kindai University Faculty of Medicine, Osaka-Sayama, Japan; 2Department of Health and Nutrition, Faculty of Human Life, Jin-ai University, Echizen, Japan; 3Department of Orthopedic Medicine, Second Affiliated Hospital of Inner Mongolia Medical University, Inner Mongolia, China; 4Department of Health Promotion and Education, Graduate School of Human Development and Environment, Kobe University, Kobe, Japan.

Objective
Little is known about the effects of muscle strength and muscle mass on bone health in children and adolescents. We examined the relative impact of muscle strength and muscle mass on bone mineral density in Japanese adolescents.

Methods
Subjects were 236 adolescents aged 15–18 years in August 2010 and August 2013 who were enrolled in the Kitakata Kids Health Study in Japan. Cross-sectional data including appendicular skeletal muscle mass (ASM) and areal bone mineral density (aBMD) were obtained. ASM and aBMD were measured using a dual-energy X-ray absorptiometry scanner. The ASM index (ASMI, kg/m²) was calculated as ASM (kg) divided by height (m) squared. Grip strength was measured as an indicator of muscle strength.

Results
Grip strength was significantly (P < 0.05) and positively associated with aBMD at several skeletal sites after adjusting for age and sex (standardized partial regression coefficient, β: lumbar spine, 0.61; total hip, 0.52; femoral neck, 0.56; whole body, 0.56). However, after additional adjustment for age, sex, and ASMI, grip strength was only associated with lumbar spine aBMD (β: 0.22). On the other hand, ASMI was significantly and positively associated with aBMD at all sites even after adjustment for age, sex, and grip strength (β: lumbar spine, 0.56; total hip, 0.79; femoral neck, 0.82; whole body, 0.72).

Conclusion
Muscle strength was positively associated with aBMD in a muscle mass-dependent manner in Japanese adolescents. This association may be partly mediated by the amount of muscle mass.

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Disclosure
The authors declared no competing interests.

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P140
Bone mineral accretion is increased during winter and is positively related to lean mass accretion in healthy children 2–8 years
Neil Brett, Catherine Vanstone & Hope Weiler
McGill University, Sainte-Anne-de-Bellevue, Québec, Canada.

In children, it is not well understood how bone mineral accretion is related to lean mass accretion and vitamin D status.

Objective
To explore over 12 mo how bone parameters relate to lean mass and vitamin D metabolites in children 2–8 years.

Methods
This was a secondary analysis of data from 2 trials (clinicaltrials.gov: NCT01902160, NCT02327878) in Montreal, Canada. Children consumed their normal diet without vitamin D supplements for 12 mo starting in Apr 2014 (n = 21) with 4 study visits (Apr and Oct 2014, Jan and Apr 2015). At all 4 time-points, vitamin D status (total serum 25(OH)D: Liaison, DiaSorin) was assessed. At 6, 9 and 12 mo, bone biomarkers were measured (Liaison, DiaSorin, IDS iSYS) followed by standardized anthropometry, demographics, activity and dietary questionnaires. Bone mineral content (BMC) and body composition were measured at baseline, 6 and 12 mo and using dual-energy X-ray absorptiometry (Hologic Discovery, APEX v13.3). Statistical analyses included linear regression and mixed model ANOVA.

Results
In Apr 2014, children were 5.0 ± 1.9 y, 52% (11/21) male, with BMI Z-score of 0.79 ± 0.90. Calcium and vitamin D intake were 1097 ± 396 mg/d and
P141

Unique correlation pattern between cortical trabecular bone qualities and standard dynamometer handgrip strength in girls with adolescent idiopathic scoliosis (AIS)...

Elisa M S Tam1, Ka-Yee Cheuk1, Vivian W Y Hung1, Fiona W P Yu1, Bobby K W Ng1, X Edward Guo1, Jack C Y Cheng2 & Tsz-Ping Lam1
1The Chinese University of Hong Kong, Hong Kong, Hong Kong; 2Columbia University, New York City, New York, USA.

Objective

Grip strength is a marker of muscle mass which can optimize bone strength during puberty. While previous studies have shown AIS girls had poor bone qualities and mechanical properties when compared with non-AIS girls, the correlation between bone qualities and handgrip strength in AIS remains undefined. This study aimed to investigate the correlation between handgrip strength and bone qualities including volumetric bone mineral density (vBMD), bone geometry, trabecular micro-architecture and bone mechanical properties in girls with adolescent idiopathic scoliosis (AIS) versus age- and gender-matched normal controls.

Methods

212 AIS girls and 247 controls aged 12 to 14 years old were recruited. Maximum handgrip strength was measured by dynamometer and bone qualities of non-dominant distal radius were measured by high-resolution peripheral quantitative computed tomography (HR-pQCT). Trabecular plate and rod structure was evaluated by Individual Trabecula Segmentation (ITS) and bone mechanical properties with Finite Element Analysis (FEA). Partial correlation was used to control confounding from age, height and weight.

Results

After adjusted for confounders, positive correlation between handgrip strength and bone geometry (including cortical area, trabecular area and cortical thickness) was detected in both AIS girls and controls (all P < 0.05). In contrast, positive correlations between handgrip strength and cortical trabecular vBMD, trabecular plate structure (including pBv/TV, pTv.N, P-P Junc. D. and P-R Junc. D.) by ITS were only seen in AIS girls (p ranged from 0.003 to 0.015) but not in controls. Stiffness and failure load by FEA were positively correlated with handgrip strength in both AIS and controls.

Conclusions

Handgrip assessment can be useful for predicting bone qualities in AIS. Unique correlation patterns between bone qualities and handgrip strength were seen in AIS when compared with controls suggesting the characteristic muscle-bone cross talk in AIS could play a role in the etiopathogenesis of AIS. Further longitudinal studies are warranted to investigate the relationship between muscle strength, bone qualities and curve severity and the therapeutic implications.

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Disclosure

The authors declared no competing interests.

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P142

Walking within 12 months of age is related to higher whole body lean mass and bone mineral density in children at 3 years of age

Hope Weiler & Catherine Vanstone
McGill University, Montreal, QC, Canada.

Gross motor development is positively associated with bone mineral density in teenagers and is thought to be mediated by lean mass. Age at walking is an accepted milestone in motor development, achieved by 50% of infants by 12 mo of age according to the WHO Motor Development Study.

Objective

To examine if walking within 12 mo of age is related to bone mineral density (BMD) and if this relationship is mediated by lean mass.

Methods

Participants (35 girls; 46 boys) of a randomized dose-response trial of vitamin D (NCT00381914) returned at 3 y of age for assessment of anthropometry, whole body composition, bone mineral content (BMC) and BMD using dual-energy x-ray absorptiometry (Hologic 4500 APEX v13.3.3). Children were term born, appropriate size for gestational age, born to healthy mothers and breastfed. Age at walking was parent-reported and categorized according to: < 12 mo or ≥ 12 mo.

Activity was surveyed using the Habitual Activity Estimation Scale. Mixed model ANOVA accounted for maternal education, gestational age at birth and age at follow-up.

Results

At follow-up, n = 37 walked on their own before or at 12 mo of age compared to n = 44 after 12 mo (mean ± s.d.: 10.8 ± 1.0 vs 14.6 ± 1.7 mo, P = 0.0001). No differences were observed in weight or gestational age at birth, maternal characteristics, or BMI-Z score or activity level at 3 y of age. After accounting for covariates, walking by 12 mo was associated with greater lean mass (10183 ± 1233 vs 9621 ± 1149 g, P = 0.019), BMC (610.22 ± 46.53 vs 591.85 ± 46.61 g, P = 0.023) and BMD (0.637 ± 0.040 vs 0.619 ± 0.036 g/cm², P = 0.023). No differences were observed in fat mass or percent body fat. The relationships between walking by 12 mo and BMC (P = 0.347) and BMD (P = 0.195) were eliminated by including lean mass in the model.

Conclusion

These data suggest that in healthy term born children, earlier attainment of walking relates to greater lean and bone mass by 3 y of age and that the relationships to bone are mediated by lean mass.

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Disclosure

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P143

Gender differences in bone health in a cohort of adolescents with developmental coordination disorder

Paola Chivers1, Timo Ranta-Laiminen1, Fleur McIntyre1,2,3, Beth Hands1, Benjamin Weeks4, Belinda Beck4, Nicholas Hart5 & Aris Siafarikas1,6
1Institute for Health Research, University of Notre Dame, Fremantle, Australia; 2Institute for Physical Activity and Nutrition, Deakin University, Melbourne, Australia; 3School of Health Sciences, University of Notre Dame, Fremantle, Australia; 4Menzies Health Institute Queensland, School of Allied Health Sciences, Griffith University, Gold Coast, Australia; 5Exercise Medicine Research Institute, Edith Cowan University, Joondalup, Australia; 6Department of Endocrinology and Diabetes, Princess Margaret Hospital, Subiaco, Australia.

Objective

Individuals with Developmental Coordination Disorder (DCD) have difficulty coordinating movements and are often unable to perform common, age-appropriate tasks. Approximately 5–6% of school-aged children are affected by DCD and the condition may persist throughout adolescence and into adulthood. Australian adolescents with DCD have poor bone health compared to European normative data. It can be hypothesized that this is due to a lack of loading resulting from decreased physical activity levels. This study examines whether these differences remain when compared with non-DCD Australian age-matched adolescents with a similar environmental opportunity for physical activity.

Methods

Analysis of peripheral Quantitative Computed Tomography (pQCT) data from Australian adolescents aged 12–18 years with (DCD, n = 39) and without movement difficulties (non-DCD, n = 147). Outcome measures were Stress Strain Index (SSI, mm²), Total Bone Area (TBA, mm²), Functional muscle bone unit (FMBU): (SSI/bone length) and Robustness (SSI/bone length²). A general linear
model was used to determine differences between groups controlling for gender, age and bone length. Specific group differences were examined using Mann-Whitney U Test.

**Results**

DCCD participants were younger (mean = 14.4 years s.d. = 1.3) than the non-DCCD group (15.3 years s.d. = 1.8) \( (P = 0.007) \). Gender was equally represented for radius (54.1% male and 53.2% male), although sample size differed for each bone site due to motion artefact (DCCD radius \( n = 26 \), tibia \( n = 39 \), non-DCCD radius \( n = 96 \), tibia \( n = 147 \)). DCCD participants had lower scores for tibial SSI, TBA and Robustness, and no gender-group interaction was observed. A radius Z test was performed to compare mean bone geometry in each bone site due to motion artefact (DCD radius \( Z = 6.5 \), tibia \( Z = 6.3 \), P = 0.004). In contrast tibial FMBU scores were not significantly different between female groups: 40.4 s.d. = 13.6 compared to 38.1 s.d. = 6.3, P = 0.542. No significant group differences were observed for radial bone measures.

**Conclusion**

Comparisons in bone measures between motor competence groups are similar to European results however gender differences were found in the present study. Australian male adolescents with DCD have weaker bones compared to Australian non-DCD peers, whereas there was no difference between female groups. These differences may be due to lower levels in habitual weight –bearing physical activity in DCD boys. It needs to be further explored whether male individuals are at higher risk of developing bone changes resulting from decreased activity levels than females.

**Disclosure**

The authors declared no competing interests.

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**P145 Longitudinal growth and bone development in glucocorticoid treated boys with Duchenne muscular dystrophy**

**S Joseph1,2, N Capaldi1, M DiMarco1, J Dunne2, I Horrocks3, I Horrocks2, S Shepherd1, S F Ahmed1 & S C Wong1**

1Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK; 2Paediatric Neurosciences Research Group, Department of Paediatric Neurology, Royal Hospital for Children, Glasgow, UK.

**Background**

There is still limited information on changes in growth especially segmental growth and bone mass of glucocorticoid (GC) treated boys with Duchenne Muscular Dystrophy (DMD).

**Objectives**

To evaluate changes in growth and bone mass in GC treated boys with DMD.

**Methods**

Retrospective study of 15 boys with DMD treated with GC, median age 7.6 years (4.1, 15.5) who had repeated DXA scan for clinical monitoring of bone health, median follow-up 1.6 years (1.0, 4.7). Height (Ht), sitting height (SH) and leg length (LL) were obtained from DXA images. Total body less health bone mineral content (TBBLH-BMC) and lumbar spine bone mineral apparent density (LS-BMAD) were converted into SDS based on recent published information in 3598 UK children and adolescents (1). Results reported as median (range).

**Results**

At baseline, median duration of GC therapy was 3.0 years (0.04,9.8). Nine out of 15 (60%) were on daily Dexamethasone, 3/15 (20%) daily Prednisolone, 1/15 (6.7%) pulsed Dexamethasone and 2/15 (13.3%) pulsed Prednisolone. GC regimen did not change during the follow-up period. At baseline, 13/15 (86.7%) were ambulant whereas this was 7/15 (46.7%) at follow-up. At baseline, median Ht-SDS was \(-1.4 (0.4, -4.5)\) and was significantly lower at follow-up: \(-3.6 (1.1, -7.2)\) (\(P = 0.001\)). At baseline, median SH-SDS and LL-SDS were \(-1.4 (0.2, -4.0)\) and \(-2.3 (0.5, -4.8)\) and both were significantly lower at follow-up respectively: \(-2.6 (1.4, -6.1)\) and \(-3.8 (0.2, -5.8)\) (\(P = 0.001\)). Despite profound growth failure, median TBBLH-BMC SDS at baseline and follow-up were not different: \(-3.5 (7.4, 0.2)\) and \(-2.4 (6.5, 0.4)\) (\(P = 0.18\)). Similarly, median LS-BMAD SDS at baseline and follow-up were not different: \(-1.3 (2.9, 1.9)\) and \(-1.4 (2.8, 1.8)\) (\(P = 0.68\)).

**Conclusion**

GC treated boys with DMD show profound growth failure with follow-up but this was not reflected in changes in DXA measured bone mass. Novel methods of assessment of bone strength and microenvironment require further exploration in these boys.

**Disclosure**

The authors declared no competing interests.

Reference


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**P146 Stature and longitudinal growth in glucocorticoid naïve boys with Duchenne muscular dystrophy**

**S Joseph1,2, G Edwards1, M DiMarco1, I Abu-Arefeh4, A Baxter5, I Horrocks2, K McWilliam3, K Naismith5, E Stephen3, S F Ahmed1 & S C Wong1**

1Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK; 2Paediatric Neurosciences Research Group, Department of Paediatric Neurology, Royal Hospital for Children, Glasgow, UK; 3Scottish Muscle Network, Queen Elizabeth University Hospital, Glasgow, UK; 4Department of Paediatrics, Forth Valley Royal Hospital, Stirling, UK; 5Department of Paediatric Neurology, Royal Hospital for Sick Children, Edinburgh, UK; 6Department of Paediatrics, Ninewells Hospital, Dundee, UK; 7Department of Paediatrics, Royal Aberdeen Children’s Hospital, Aberdeen, UK.

**Background**

Previous studies with small number of boys with Duchenne Muscular Dystrophy (DMD) suggest that growth failure occurs in glucocorticoid naïve (GC) boys.

**Objective**

To evaluate height and longitudinal growth in boys with DMD prior to GC.

**Method**

Retrospective evaluation in boys with DMD with height measurements obtained for clinical purposes. Out of the 91 boys currently managed in Scotland, 51 had at
least one height (Ht) measurement prior to GC, 36 had two height measurements prior to GC and 18 had three height measurements prior to GC. Ht and BMI were converted to SDS according to the 1990 UK growth standards. Results reported as median (range).

Results

At median age of 3.5 years (0.1, 7.0), median Ht SDS was $-1.0 (-2.8, 1.0)$ with 11/51 boys (22%) with Ht SDS $<-2.0$. Median BMI SDS was $+0.8 (-3.3, 3.4)$ with 2/51 (4%) boys with BMI SDS $<-2.0$. Ht SDS was not associated with age ($r=0.03$, $P=0.81$) and BMI SDS ($r=0.19$, $P=0.18$). For the 36 boys with two height measurements prior to GC, median Ht SDS at baseline was $-1.2 (-2.8, 1.0)$ at median age of 2.8 years (0.1, 6.6). Median Ht SDS at follow-up was $-1.1 (-3.4, 0.2)$ ($P=0.60$ vs baseline) at median age of 4.6 years (2.4, 8.9). For the 18 boys with three height measurements prior to GC, median Ht SDS at baseline was $-0.9 (-2.7, 1.0)$ at median age of 1.6 years (0.2, 6.6). Median Ht SDS at first follow-up was $-1.3 (-2.8, 0.7)$ ($P=0.09$ vs baseline) at median age of 3.8 years (1.3, 6.9). Median Ht SDS at second follow-up was $-1.0 (-3.2, 0.6)$ ($P=0.19$ vs baseline) at median age of 5.3 years (3.1, 8.6). Ht SDS $<-2.0$ was observed in 5/18 (28%), 6/18 (33%) and 6/18 (33%) at baseline, first and second follow-up. Height velocity at first and second follow-up were 7.9 cm/year (2.7, 11.2) and 6.6 cm/year (0, 10.1) ($P=0.15$).

Conclusion

Our results suggest that short stature occurs in GC naïve boys with DMD but severe growth failure is however not frequently encountered.

Disclosure

The authors declared no competing interests.

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P147

Bone health in boys with Duchenne muscular dystrophy (DMD): the dichotomy between bone density and fracture

Nicola Crabtree1, Wolfgang Hogler1,2, Helen Kooper3 & Nicholas Shaw1,2

1Birmingham Women’s and Children’s NHS Foundation Trust, Birmingham, UK; 2Institute of Metabolism and Systems Research, Birmingham, UK; 3Heart of England NHS Foundation Trust, Birmingham, UK.

Objectives

Current guidelines recommend annual assessments of bone densitometry in boys with Duchenne muscular dystrophy (DMD). However, this recommendation is based on the assumption that bone density is a predictor of fractures in this patient group. The aim of this study was to evaluate the relationships between long-term changes in bone density, corticosteroid exposure and mobility with vertebral and long bone fractures.

Methods

Twenty-four DMD boys (mean age 10.1 (s.d. 2.4) years) with at least six annual DXA assessments were included in the study; each boy had three measures whilst ambulant and three measures once ambulation had ceased. A repeated measures model was used to compare size adjusted lumbar spine BMD (BMAD), total body less head BMD (TBLH BMD), lean body mass (LBM) and corticosteroid (CS) cumulative exposure with fractures and mobility.

Results

Over 5 years, nine long bone fractures were reported in 8 boys and 41 vertebral fractures in 14 boys, of which 6 and 4 respectively, occurred after loss of ambulation; only 7 boys (29%) remained fracture free. At baseline, no differences were seen between the fracture and non-fracture groups for height, LBM and BMAD. Boys who developed vertebral fractures were heavier ($P=0.04$) and had a higher CS exposure ($P=0.02$) whilst those who developed long-bone fractures were lighter ($P=0.04$) and had lower TBLH BMD ($P=0.05$). BMAD, TBLH BMD, & LBM Z-scores declined consistently over the measurement time frame but the rate of decline was greatest once ambulation ceased ($P<0.001$). There was a significant positive interaction between CS exposure and vertebral fracture but this was not seen in those who developed long bone fractures.

Conclusion

The only distinguisher of long-bone fractures was low TBLH BMD whereas vertebral fractures were not associated with low BMAD, TBLH BMD or rate of loss of bone density. Cumulative CS exposure was associated with vertebral fractures but not long-bone fractures. Both fracture types were more likely after loss of mobility. This dichotomy between bone density as assessed by DXA and fractures may be potentially misleading when monitoring bone health in boys with DMD. Current guidelines should be revised to reflect these issues.

Disclosure

The authors declared no competing interests.

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P148

Muscle density measurement in muscular dystrophy

Susanne Bechtold, Astrid Blaschek, Wolfgang Müller-Felber, Julia Roeb, Claudia Weissenbacher, Carmen Sydlik & Heinrich Schmidt

LMU University Children’s Hospital, Munich, Germany.

Objective

Muscular dystrophy is characterized by lower skeletal muscle quality, lower muscle strength and physical performance. The aim of the study was to assess regional muscle density and its correlation with regional muscle area in Duchenne muscular dystrophy (DMD) subjects and able bodied controls.

Method

Skeletal muscle pQCT (peripheral quantitative computed tomography) scans at the non-dominant forearm were performed in patients with muscle dystrophy at different ages and compared with muscular healthy patients with familiar short stature or diabetes type 1.

Results

We included 45 children and adolescence with clinical and molecular diagnosis of MD (2 Becker-Kiener, 2f) and 105 controls (68 f). Mean age for MD was 9.73 ± 3.7 years and 14.77 ± 4.6 years for controls. Younger MD patients were ambulatory, the majority of them were treated with intermittent glucocorticoids. Muscle density was constant between 70 and 80 mg/m3 in the control population (mean 77.90 ± 2.16) irrespective of age and sex, whereas muscle density for MD was significantly reduced with 48.38 ± 12.8 mg/m3 and decreased with age ($r=-0.39$, $P=0.009$). There was no correlation between muscle density and muscle cross sectional area (MCSA) for each of the groups. With age MCSA increased in controls ($r=0.73$, $P<0.001$) but not in MD.

Conclusions

In healthy or able bodied controls muscle density is a constant parameter. Measurements of this parameter in MD seem to reflect the progressive loss of muscle fibers and might be an early marker at a stage where muscle CSA is still within normal range.

Disclosure

The authors declared no competing interests.

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P149

Abstract withdrawn.

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DXA based evaluation of the bone mass and body composition in a group of Romanian cystic fibrosis children

Carmen Gabriela Barbu1,2, Diana Lungu3, Valentina Daniela Comanici1,2 & Iustina Stan3,2
1Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; 2Alfred Russescu1 National Institute for Mother and Child Health, Bucharest, Romania; 3Academia Medical Center, Bucharest, Romania.

Background
The significant increase in the life expectancy of the patients with cystic fibrosis (CF) came with some costs, as new complications have emerged. Among endocrine disorders, CF-related bone disease (CFBD) is a leading complications reported in the adult patients.

Objectives
Our study present the first results of the bone mass and body composition evaluation by DXA in a small group of Romanian children with CF treated in the author’s departments.

Results
Seventeen children aged between 8 and 18 years were diagnosed with CF were evaluated through a whole body DXA scan (Prodigy-Lunar GE). We performed routine biochemical tests, respiratory function and endocrine diseases (growth, development, thyroid function and serum vitamin D). Medical history data were collected from medical records. Mean age of the children was 12.1±2.5 years, with a BMI of 20.5±2.3 kg/m² and a height centile (WHO reference) of 28.6±23. Mean values of measured parameters were 77.9±18.44 for the total body less head bone mineral density (TBLH-BMD) Z score, patients with more severe form of CF but seven patient had below -1 S.D. value.

Two patients had short stature, one was overweight and one underweight. Vitamin D deficiency was found in 14 patients, deficiency in 9 and only 3 out of 17 patients had normal serum 25HO vitamin D, in spite of vitamin D supplementation. TBLH BMD Z score below -2 SD was found in only two patients with more severe form of CF but seven patient had below -1 S.D. value. Two patients had prevalent fractures but with TBLH BMD above -2 S.D. only. Only three out of 17 patients had normal body fat percent, the other showing extremely low values. None of the patient had extreme values for LMI.

Conclusions
Specific treatment for CF and maintenance of a good lean mass seems to compensate overall the deleterious effects of the disease and corticotherapy on the bone; however, prevalent fractures seems not to be correlated only to BMD parameters, suggesting additional quality factors in the pathogeny of CFBD.

Acknowledgement
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Disclosure
The authors declared no competing interests.

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Characterisation of skeletal developmental in mouse models of Duchenne Muscular Dystrophy

Claire Wood1,3, Sze C Wong1, Volker Straub2, S Faisal Ahmed1 & Colin Farquharson1
1Department of Developmental Biology, Roslin Institute, Edinburgh University, UK; 2Developmental Endocrinology Research Group, University of Glasgow, UK; 3John Walton Muscular Dystrophy Research Centre, Newcastle University, UK.

Short stature and osteoporosis are common in DMD. Disease progression can be slowed by glucocorticoids but these are associated with further growth retardation and skeletal fragility. The defect in growth and skeletal development in children with DMD is probably multifactorial and not solely dependent on glucocorticoid exposure. The muscular dystrophy x-linked (mdx) mouse is the most commonly used animal model of DMD. However, its growth phenotype has not been studied in detail and the phenotype is relatively mild. Few medications that have shown therapeutic benefit in the mdx have also shown efficacy in DMD clinical trials. The utrophin heterozygous mdx mice might represent a more appropriate model but their growth and bone phenotype have not been investigated. We tested the hypothesis that: Mouse models of DMD (mdx and mdx:utr) have an intrinsic abnormality of linear growth and skeletal development. A cross-sectional study of 49 male mice sacrificed at 3, 5 and 7 weeks was performed. Mdx and mdx:utr mice were obtained from the Jackson laboratory, alongside C57BL/10 controls (WT). Animal growth was assessed twice weekly using digital weighing scales and ruler. Forelimb grip strength testing was performed according to the TREAT-NMD SOP. Creatine Kinase was measured using an Abnova assay kit, on blood taken at sacrifice. Histopathology was assessed using H+E sections of tibials anterior muscle. Left tibiae were scanned using SkyScan microtomography to assess cortical and trabecular bone structure. 3-point bending determined biomechanical properties.

Muscle
WT mice had the greatest normalised grip strength at all ages. Mdx:utr had higher mean grip strength at 7 weeks than mdx mice. Results indicated significantly higher serum values from mdx (P<0.002) and mdx:utr (P<0.002) mice, compared to WT. Muscle histology was consistent with these observations.

Bone
There was no significant difference in bodyweight gain between groups at any age and no difference in tail length by 7 weeks. Gain in body length was 0.3 mm less/day when comparing the mdx and mdx:utr to WT mice culled at 7 weeks, but Micro-CT of tibial length revealed no genotype difference.

There were no significant differences in trabecular bone parameters between groups at any age, except for structural model index (greater in 3-week WT mice, P<0.04). Cortical bone parameters and bone mechanical properties were similar at all ages. There are very limited strategies available to treat short stature and osteoporosis in DMD and the impaired osteoblast function described in DMD suggests that an anabolic treatment would be optimal. We have demonstrated that young mdx and mdx:utr mice exhibit muscle weakness, but do not show a bone or growth phenotype and therefore have clear limitations. Finding a more suitable pre-clinical mouse model is therefore essential.

Disclosure
The authors declared no competing interests.

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P153

Vitamin D intake and status in children 2–18 years: a meta-analysis
Neil Brett & Hope Weiler
McGill University, Sainte-Anne-de-Bellevue, QC, Canada.

Evidence is unclear on the effect of vitamin D intake on vitamin D status in children. Objective
In a meta-analysis, investigate the effect of vitamin D supplements and/or fortified foods on vitamin D status, using the biomarker 25-hydroxyvitamin D (25(OH)D) in children 2–18 years.

Methods
Eligible studies were randomized placebo-controlled trials, published in English, in children 2–18 years that compared vitamin D supplements or fortified foods. Using PRISMA guidelines, literature searches of Ovid MEDLINE, PubMed, CINAHL, Embase, and Cochrane Central Register of Controlled Trials were conducted up to December 2016. The Cochrane qualitative bias tool and the Jadad scale assessed evidence strength and I² assessed heterogeneity. Subgroups included age (2–8, 9–18 years), baseline 25(OH)D (<30, 30–49.9, ≥50 nmol/l), latitude (≥40°N or S) and daily supplements, fortified foods or high dose injections.

Results
We included 29 trials (4972 children) with interventions (10 using fortified foods, 17 using supplements, 2 using bolus injections) from 2.5 to 100 µg/d vitamin D equivalent over 4 weak to 2 years. Due to the variation in design, heterogeneity was high (I² = 73%). Once adjusted for dose, heterogeneity was low (I² = 0%). Study designs were qualitatively high and 97% had Jadad scores ≥4. The 25(OH)D weighted mean difference (20·5 nmol/l, 95% CI 22.8–30.2 nmol/l) was greater with mean baseline 25(OH)D <30 nmol/l, compared to higher status categories (P < 0.05). The 25(OH)D increase per µg/d of vitamin D (2.3 nmol/l, 95% CI 2.1–2.5 nmol/l) in trials using fortified food was greater than daily supplements (P = 0.02), but not bolus injections (P = 0.20). Interventions of < 10 µg/d had greater 25(OH)D increase per µg than those of ≥25 µg/d (P = 0.03), but not 10–24.9 µg/d (P = 0.08). Using a segmented-plateau quadratic regression, the 25(OH)D change per µg of vitamin D plateaued at 0.5 nmol/l when the dose reached 33 µg/d.

Conclusion
To the best of our knowledge, this is the first vitamin D intake and status meta-analysis specific to children. The 25(OH)D response to vitamin D intake appears to differ based on baseline status and delivery mode, but not age, sex or latitude. (Funding: Canada Research Chairs).

Disclosure
The authors declared no competing interests.

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P154

Maximal suppression of parathyroid hormone as a determinant of optimal vitamin D status in adolescents
Taryn Smith,1 Laura Tripkovic,1 Camilla Damsgaard2, Christian Molgaard2, Aine Hennessy, Kirsten Dowling3, Kevin Cashman,1 Mairead Kiely3, Susan Lanham-New1 & Kathryn Hart1
1 University of Surrey, Guildford, UK; 2University of Copenhagen, Copenhagen, Denmark; 3University College Cork, Cork, Ireland.

Suppression of parathyroid hormone (PTH) has been suggested as a potential biochemical outcome measure for determining the optimal serum 25-hydroxyvitamin D (25(OH)D) concentration for bone health in adults. However, in adolescents increases in PTH may not be driven by the same mechanisms and may not be detrimental to bone health. Adolescent studies have provided a wide range of estimates of the 25(OH)D concentration at which PTH is suppressed (the inflection point) in 14–18 year old white male and female adolescents in the UK. 25(OH)D and plasma PTH were measured in 102 adolescents (mean age 16.2 ± 1.4 years; 41% male) recruited onto a vitamin D dose-response randomised controlled trial. Regression models were used to estimate the 25(OH)D concentration at which PTH plateaued and a linear model was selected based on best fit. Mean 25(OH)D concentration was 49.3 ± 18.0 nmol/l and mean plasma PTH was 41.6 ± 15.6 pg/ml. Plasma PTH was significantly inversely associated with 25(OH)D (r = −0.315, P = 0.001) and serum corrected calcium concentrations (r = −0.214, P = 0.029), but was not associated with sex, age, Tanner stage or calcium intakes. There was no plateau in plasma PTH, suggesting that in this data there was no inflection point (Figure 1).

In conclusion, a point of inflection of plasma PTH could not be identified in 14–18 year old adolescents. This may be due to the narrow range of 25(OH)D <100 nmol/l within this sample of adolescents (13.8–86.3 nmol/l). Therefore, based on this data, maximal suppression of PTH is not an appropriate measure for determining optimal vitamin D status in healthy adolescents.

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Disclosure
The authors declared no competing interests.

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P155

Dietary protein is associated with bone adaptations and performance of pre-adolescents
Theodoros Stampoulis1, Diamanda Leontsini1, Alexandra Avloniti1, Dimitrios Draganidis2, Athanasios Chatzinikolaou1, Fotini Venetsanou2, Chariklia Deli1, Dimitris Vlachopoulos2, Luis Gracia-Marco3, Maria Michalopoulou1, Athanasios Jamurtas2, Ioannis Fatouros2 & Antonis Kambas1
1Democritus University of Thrace, Komotini, Greece; 2University of Thessaly, Trikala, Greece; 3University of Athens, Athens, Greece; 4University of Exeter, Exeter, UK; 5University of Zaragoza, Zaragoza, Spain.

Objectives
Nutrition in childhood is a major factor for healthy living during adulthood. Bone mass is influenced immensely by nutritional intake, especially protein intake which is very important for bone matrix and the integrity of skeletal structure. This study aimed to identify the effects of dietary protein intake on bone mineral density (BMD), bone mineral content (BMC) and performance of children aged 6–12 years old.

Methods
A repeated measures design with 114 pre-adolescent (9–12 years) children (boys: N = 61; girls: N = 53) was employed. Children were evaluated at baseline and following 12 months. Maturity was determined with Tanner stages of sexual maturity. Participants had their body mass, body height and tibia length measured. Dual energy X-ray absorptiometry (DEXA) was used to measure bone composition as well as bone mineral density (BMD) and content (BMC) at hip and lumbar spine. Daily physical activity was measured (once every 6 months of the study) using accelerometry (for 7 days). Power of lower limb muscles and cardiovascular endurance were determined using long jump and shuttle run testing, respectively. 7-day diet recalls (administered once every 6 months of the study) were used to measure daily protein intake. Analysis of variance was used to compare groups of low, normal and high daily protein intake. A partial correlation analysis (adjusted for BMI) was used to correlate BMD, BMC and performance indices with protein intake.

Results
Children were assigned to a low (<1.5 g/kg body weight), normal (1.5–2.0 g/kg body weight) and high (>2.0 g/kg body weight) protein intake groups based on their daily intake recorded using dietary recalls. Normal and high protein intake groups had higher BMD, BMC whereas no significant interaction was found with
**P156**

**Bone health status of underprivileged Indian adolescent girls**

Rubina Mandlik1, Veena Ekbote1, Neha Kajale1, Anjali Jaival1, Sahsh Chiplonkar1, Vannan Khadilkar1, Raja Padidela2, Zarif Mughal2 & Anuradha Khadilkar1

1Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India; 2Royal Manchester Children’s Hospital, Manchester, UK.

**Objectives**

Earlier studies performed using dual energy X-ray absorptiometry indicate that underprivileged Indian girls acquire low bone mass during adolescence. Therefore, aim was to assess bone geometry of underprivileged Indian adolescent girls (age 12–19 years) using peripheral quantitative computed tomography (pQCT).

**Methods**

Fifty-six adolescent girls were assessed for anthropometry (height, weight) (February 2016 to January 2017) and pQCT (STRATECXCT-2000). Socio-demographic information was recorded. Dietary intakes (3-non-consecutive days) of 24-hr diet recall and nutrient intakes were computed using (c-diet software). Biochemical parameters: serum 25OHD and intact parathyroid hormone were assessed using standard laboratory kits. Statistical analysis was performed using SPSS (version 21). Level of significance was set at P<0.05.

**Results**

Mean age was 15.4 ± 1.6 years (with mean age at menstruation 12.9 ± 0.9 years) (mean monthly family income 218 ± 140 EUR). Anthropometry and biochemical parameters were within reference range except for serum 25OHD deficiency (mean 23.8 ± 11.6 nmol/l; 95% below reference range of 50 nmol/l), mean PTH (5.47 ± 3.14 pmol/l; 33% above reference range of 7.05 pmol/l). Majority of the girls (59%) were exposed to sunlight for less than 30 minutes/day. 16% of girls reported history of fracture. pQCT measurements demonstrated that mean trabecular density was 160 ± 27 mg/cc, mean Z-score was −0.9 ± 1 and 11% girls had low bone mass (Z-score < −2) at radius 4%: mean total density was 279 ± 47 mg/cc, mean Z-score was −1.1 ± 0.9 and 22% girls had low bone mass. Mean cortical bone density at radius 66% site was 1082 ± 60 mg/cc, mean Z-score was −1.2 ± 2.0 (29% Z-score < −2) and mean Stress Strain Index (SSI) was 165.6 ± 38.1 mm² and mean Z-score was −1.4 ± 2.5; SSI was below −2 in 33% girls. All macro (protein = 29.0 ± 11.3 gm/day) and micronutrient (calcium = 302 ± 113 mg/d) intakes were below recommended dietary allowance except for dietary fat.

**Conclusion**

These underprivileged girls with poor sunshine exposure and hypovitaminosis D with poor habitual calcium intake had low bone mass as measured by pQCT and urgent attention needs to be focussed on bone health of these girls.

**Disclosure**

The authors declared no competing interests.

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**P157**

**Are there gender differences in abdominal fat distribution in healthy teenagers?**

François Duboeuf1, Stéphane Bouttry1, Tiphanie Ginhoux2, Jean-Paul Roux1, Roland Chapurlat1 & Justine Bacchetta1,3

1INSERM 1033, Lyon, France; 2EPICIME, Lyon, France; 3Pediatric Nephrology, Bron, France.

**Background**

While the relationship between visceral (VFAT) and subcutaneous (SFAT) fat mass with cardiometabolic risk has been demonstrated in adults, fat mass evolution during teen agehood remains poorly explored and usually assessed with irradiative (CT) or expensive (MRI) techniques. Our aim was to evaluate a novel technique derived from DXA to assess VFAT and SFAT in healthy teenagers. Subjects and methods

Healthy teenagers from the VITADOS study underwent whole body DXA scans for body composition analysis (Discovery A, HOLOGIC Inc; Bedford, MA).

Outcome parameters were considered on the sub-total body (total body without head) and included mass (M), fat mass (FM), lean mass (LM), bone mineral density (BMD), as well as the androind-gyroid ratio (And/Gyn). Abdominal fat mass, including VFAT and SFAT, was assessed on the same scan using APEX V4.0.2, on a 5 cm-wide region placed across the entire abdomen just above the iliac crest approximately at the level of the 4th lumbar vertebrae.

**Disclosure**

The authors declared no competing interests.

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**P158**

**Bone metabolism in adolescent girls with anorexia nervosa**

Lubica Ticha, Juraj Payer, Zdenko Killiong, Alibeta Lenesesova & Ludmila Podracka

Paediatrics Department of Faculty of Medicine Comenius University in Bratislava, SR, Bratislava, Slovakia.

**Introduction**

Anorexia nervosa usually has its onset during adolescence, the critical time when peak bone mass is accrued. Inadequate nutrition abnormalities and endocrine changes during starvation have a negative effect on bone health. The aim of this work was to investigate the effects of hormonal and autological parameters on markers of bone metabolism, bone mineral density in girls with anorexia. Patients and methods

In groups of 45 girls with anorexia nervosa (x = 16.15 ± 2.67 years) at different stages of the disease, we examined deficit of weight, disease duration, duration of amenorrhea and laboratory parameters of bone metabolism (vitamin D, osteocalcin CTx, estradiol, IGFI). Dual X-ray absorptiometry (DXA) was used to assess the bone mineral density (BMD). The results were evaluated as DXA Z-scores for the age - matched controls and sex pubertal development.

**Results**

BMD Z-score – lumbar spine (LS) was −0.8 ± 1.22, proximal femur −1.1 ± 1.22. 11 patients (24%) had bone density less than −2 s.d. for the age. Anorectic
girls with reduced density had a longer duration of disease (22 months versus 12.85 months), significantly lower vitamin D (vs 19.48, 27.50 ng/ml, P ≤ 0.05), estradiol (19.48 vs 27.50 pg/ml, P ≤ 0.05) and IGF1 (174.11 vs 261.89 ng/ml) as anorectic girls with normal BMD. Low BMD was associated with lower concentrations of osteocalcin CTX. BMD of proximal femur correlated with estradiol (r = 0.48). An inverse relationship between vitamin D and PTH (r = -0.55) points (proves) to maintaining control of calcium metabolism even during the critical weight loss.

Conclusion
Severe nutritional deficiency and adaptive hormonal changes in anorexia nervosa could lead to a reduction of bone mass as well as to increase fracture risk later in life.

Disclosure
The authors declared no competing interests.

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**P159**

Maternal calcium supplementation in a rural Gambian population associated with reduced blood pressure among adolescent female, but not male, offspring

Simon Schoenbuchner1, Sophie Moore2,3, Landing Jarjou1, Kate Ward1,4 & Ann Prentice1,3

1MRC Elsie Widdowson Laboratory, Cambridge, UK; 2Division of Women’s Health, King’s College London, London, UK; 3MRC Unit The Gambia, Banjul, Gambia; 4MRC Lifecourse Epidemiology Unit, Southampton, UK.

We have previously observed sex-specific effects of maternal calcium supplementation on offspring childhood growth, in a rural Gambian population with habitually low calcium intake (~300 mg daily). In this study, we aim to investigate longer-term effects of maternal calcium supplementation on adolescent growth in same cohort.

We aimed to investigate effects on offspring blood pressure (BP) in the same cohort. We recruited 205 female, 182 male from a randomised, placebo-controlled trial of calcium supplementation during pregnancy and included in these analyses the 150 participants (98 female, 52 male) who had complete information on height, weight, and BP at these follow-up occasions, at mean(S.D.) ages 9.2(0.9), 13.8(1.2) and 16.3(1.3) years.

We have previously reported sex-specific effects of maternal calcium supplementation on offsprings childhood growth,1,2 in a rural Gambian population with habitually low calcium intake (~300 mg daily). In this study, we aim to investigate longer-term effects of maternal calcium supplementation on adolescent growth in same cohort. We recruited 230 female, 217 male born following a randomised, placebo-controlled trial of calcium supplementation during pregnancy (1500 mg daily from gestational week 20 until delivery, ISRCTN96502494). Height and weight were measured on three occasions, at mean(S.D.) ages 9.2(0.9), 13.8(1.2) and 16.3(1.3) years.

The authors declared no competing interests.

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**P160**

Maternal calcium supplementation in a rural Gambian population associated with reduced height and weight among adolescent female, but not male, offspring

Simon Schoenbuchner1, Sophie Moore2,3, Landing Jarjou1, Ann Prentice1,3 & Kate Ward4

1MRC Elsie Widdowson Laboratory, Cambridge, UK; 2Division of Women’s Health, King’s College London, London, UK; 3MRC Unit The Gambia, Banjul, Gambia; 4MRC Lifecourse Epidemiology Unit, Southampton, UK.

We have previously reported sex-specific effects of prepubertal calcium supplementation on the timing of adolescent growth,1,2 as well as sex-specific effects of maternal calcium supplementation on offspring childhood growth,2,3 in a rural Gambian population with habitually low calcium intake (~300 mg daily). In this study, we aim to investigate longer-term effects of maternal calcium supplementation on adolescent growth in same cohort. We recruited 230 female, 217 male born following a randomised, placebo-controlled trial of calcium supplementation during pregnancy (1500 mg daily from gestational week 20 until delivery, ISRCTN96502494). Height and weight were measured on three occasions, at mean(S.D.) ages 9.2(0.9), 13.8(1.2) and 16.3(1.3) years.

The authors declared no competing interests.

Disclosure
The authors declared no competing interests.

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(1) Prentice et al. AJCN 2012 96 1042–50.
(2) Ward et al. JBMR 2015 30 (S1) OP1096.

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**P161**

Early-life vitamin D status and bone mass at five years in a prospective birth cohort study

Carol ni Chaoimh1,2, Deirdre Murray3,4, Louise Kenney2,4, Alan Irvine5,6, Jonathan Hourihane7,3 & Mairead Kiely1,2

1Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences, University College Cork, Cork, Ireland; 2The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Cork, Ireland; 3Department of Paediatrics and Child Health, University College Cork, Cork, Ireland; 4Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland; 5Department of Clinical Medicine, Trinity College, Dublin, Ireland; 6Department of Paediatric Dermatology, Our Lady’s Children’s Hospital, Dublin, Ireland; 7National Children’s Research Centre, Our Lady’s Children’s Hospital, Dublin, Ireland.

Objective
We aimed to investigate associations between early-life vitamin D status, mode of infant milk-feeding and bone outcomes at five years.

We have previously reported sex-specific effects of prepubertal calcium supplementation on the timing of adolescent growth,1,2 as well as sex-specific effects of maternal calcium supplementation on offspring childhood growth,2,3 in a rural Gambian population with habitually low calcium intake (~300 mg daily). In this study, we aim to investigate longer-term effects of maternal calcium supplementation on adolescent growth in same cohort. We recruited 230 female, 217 male born following a randomised, placebo-controlled trial of calcium supplementation during pregnancy (1500 mg daily from gestational week 20 until delivery, ISRCTN96502494). Height and weight were measured on three occasions, at mean(S.D.) ages 9.2(0.9), 13.8(1.2) and 16.3(1.3) years.

The authors declared no competing interests.

References
(1) Prentice et al. AJCN 2012 96 1042–50.
(2) Ward et al. JBMR 2015 30 (S1) OP1096.

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Methods: Participants were from the prospective mother-infant SCOPE-BASELINE Birth Cohort Study. Serum 25 hydroxyvitamin D (25(OH)D) concentrations were quantified at 15 weeks gestation, in umbilical cord sera and at two and five years using a gold-standard CDC-accredited LCMS method. Whole-body bone mineral content (BMC), bone area (BA) and areal bone mineral density (aBMD) were assessed in 596 children at five years by dual-energy x-ray absorptiometry (DXA). To adjust for body size, estimated volumetric bone mineral density (vBMD) was calculated by adjusting BMC for BA, weight, and height.

Results: The prevalence of maternal vitamin D deficiency (25(OH)D <30 nmol/l) was 12%, and 41% of mothers were <50 nmol/l at 15 weeks gestation. Forty-three percent of neonates were <30 nmol/l, decreasing to 6 and 2% at 2 and 5 years, respectively. Maternal and cord 25(OH)D concentrations were positively correlated (Spearman’s r = 0.345, P < 0.001). There were no differences in bone outcomes at 5 years across categories of maternal or cord 25(OH)D concentrations (<30, 30–49, and ≥50 nmol/l). By 6 months, 85% of children were receiving infant formula, 95% were receiving complementary foods and 60% were using a vitamin D supplement. Four children (<1%) were exclusively breastfed without supplementation at 6 months. Bone outcomes at 5 years did not differ significantly by type of milk feeding at 6 months. However, among children born to mothers <50 nmol/l, those who were receiving breast milk as their predominant milk source at 6 months (n = 27) had lower BMC than children who were mixed- or formula-fed (median (IQR): 406 (375, 441) vs 436 (396, 481) g, P = 0.040). These children also had lower aBMD, but differences did not persist for estimated vBMD (P = 0.389).

Conclusion: We report a high prevalence of maternal and neonatal vitamin D deficiency that did not track into childhood. Lower maternal vitamin D status, followed by breast milk as the predominant milk source at 6 months was associated with lower BMC, but not size-adjusted BMC at 5 years.

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Disclosure: The authors declared no competing interests.


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Nutritional rickets presenting to secondary care in children (<16 years) – A UK surveillance study

Prisca Jules1, Karina Pull2, Richard Lynn2, Alistair Calder3, Zulf Mughal3, Nicholas Shaw4, Ciara McDonnell5, Helen McDevitt5 & Mitchell Blair1

1Royal Free Hospital, London, UK; 2British Paediatric Surveillance Unit, London, UK; 3Royal Manchester Children’s Hospital, Manchester, UK; 4Birmingham Children’s Hospital, Birmingham, UK; 5Royal Hospital for Sick Children Glasgow, Glasgow, UK; 6Children’s University Hospital, Dublin, Ireland; 7Northwick Park Hospital, London, UK; 8Great Ormond Street Hospital, London, UK.

Objectives: Rickets is a disease of growing children with potentially serious short and long-term complications. The United Kingdom (UK) national incidence of Nutritional Rickets(NR) is unknown and thought to be increasing. This study aims to describe the incidence, presentation and clinical management of children with NR in the UK and Republic of Ireland.

Methods: Data is being collected prospectively monthly between March 2015 and March 2017 from 3500 paediatrics using British Paediatric Surveillance Unit reporting methodology.

Results:

During 22 months of surveillance, 89 cases met the case definition. Table 1 shows demographic and clinical findings. There was little difference by sex. Most were young children, of African and South Asian ethnicity and on solids with dairy. At the time of diagnosis 84% of children were not receiving vitamin D supplements. Cows milk protein allergy and/or multiple food allergies (10%; 9/89) and iron deficiency (7%; 6/89) were the commonest associated conditions. Bony (wrist swelling, bowed legs) and radiological abnormalities were the commonest presentation. Eight children (9.2%) had associated fractures. All confirmed radiological cases had either high parathyroid hormone and/or low phosphate. One child died of dilated cardiomyopathy. There is huge variability in management practices of Vitamin D deficiency amongst clinicians.

Conclusions: Interim findings are that NR continues to affect children in the UK with serious sequela. Uptake of vitamin D supplementation remains low and constitutes a failure of current public health guidance and policy. We recommend performing both radiological and biochemical tests for accurate case ascertainment. This surveillance of NR will provide robust and current data to inform UK national policy on management of this preventable condition.

Supported by a grant from the European Commission (ODIN grant 613977).

Disclosure: The authors declared no competing interests.

Table 1 Demographics and clinical characteristic.

<table>
<thead>
<tr>
<th>Sex (n=89)</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Male</td>
<td>46</td>
<td>52</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
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<td>Ethnicity (n=89)</td>
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<td>Other Asian background</td>
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<td>6</td>
</tr>
<tr>
<td>Not known</td>
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<tr>
<td>Age at Presentation (n=89)</td>
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<tr>
<td>&lt;1 year</td>
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<td>5–15 years</td>
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<td>18</td>
</tr>
<tr>
<td>Exclusively formula fed</td>
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<tr>
<td>Mixed</td>
<td>8</td>
<td>10</td>
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<tr>
<td>Solids (with dairy n=50)</td>
<td>60</td>
<td>71</td>
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<td>Clinical Presentation (n=89)</td>
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<tr>
<td>Bony Sign (in 8, the only abnormality)</td>
<td>69</td>
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<td>Radiological Abnormalities</td>
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<td>Incidental Blood test or X-ray</td>
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P163

Vitamin D insufficiency and inadequate bone mineral status in newcomer immigrant and refugee children in Canada

Hassan Vatamparast & Ginny Lane

University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Nutrition and physical activity are two main important factors influencing bone mineral mass accumulation during childhood and adolescence. Newcomer immigrant/refugee children are at a high risk of poor nutritional status. Vitamin D deficiency, in particular, and its related diseases is a major concern due to minimal sun exposure in countries in high latitude and limited dietary sources. Using Healthy Immigrant Children (HIC) polite data (n=72), we previously reported vitamin D and bone mineral status of sample of newcomer children. No large scale study is available in Canada to evaluate the relationship between nutrition, physical activity and bone mineral status on newcomer immigrant and refugee children.

Objective:

To evaluate the association between nutrition, physical activity and bone mineral status in immigrant and refugee newcomer children to Canada.

Methods:

In a cross-sectional design, we recruited 299 immigrant (n=133) and refugee (n=166) children aged 3–13 years who had been living in Saskatchewan and Regina, Canada for no more than five years. Measurements included serum 25OHD using LC-MS/MS method, total body bone mineral content (TBBMC) using DXA, dietary assessment using 24-h recalls, physical activity and USDA food security questionnaires.

Results:

The mean age of children was 8.0±2.8 years. The rate of childhood food insecurity was 18.8% and 32.3% in immigrants and refugees respectively. Most children (83.7%) were meeting the recommended level of physical activity (>60 min/day). Over 40% of children had the TBBMC lower than the predicted optimal values for their age, sex and ethnicity. We found 63.7% of participants had inadequate levels of serum vitamin D (<50 nmol/l) for bone health. Prevalence of inadequacy in vitamin D intake was 92%. In stepwise regression...
analyses, after controlling for all potential covariates; height and serum vitamin D status were found to be determinants of TBBMC ($R^2 = 0.82$, $P < 0.001$). Children who were taller and had significantly greater serum vitamin D also had greater TBBMC with $\beta = 0.93$ for height and $\beta = 0.12$ for serum vitamin D. In accordance with HIC polite data, a considerably high rate of vitamin D deficiency and insufficiency in newcomer immigrant and refugee children and its association with bone mineral mass during this important stage of life requires immediate preventive interventions to minimize the risk of serious vitamin D related diseases.

Disclosure
The authors declared no competing interests.

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P164

Abstract withdrawn.

DOI: 10.1530/boneabs.6.P164

P165

Abstract withdrawn.

DOI: 10.1530/boneabs.6.P165

P166

Abstract withdrawn.

DOI: 10.1530/boneabs.6.P166

P167

Stable and functional osteosynthesis with intramedullary growing rods: results of surgical correction in eleven patients with systemic skeletal disease
Andrii Zyma1, Iurii Guk1, Stepan Martynak1, David Stewart2, Andrii Cheverda1, Tamara Kincha-Polishchuk1 & Yurii Denyyan1
1Institute of Orthopedics and Traumatology, Kiev, Ukraine; 2Childrens Bone and Spine Surgery, Las Vegas, Nevada, USA.

Goal
The use of intramedullary telescopic constructs for osteosynthesis in surgical correction of bone deformities in children with systemic skeletal disease can be complicated by delayed bony union, and the structural and functional pathology of bone in patients with these disorders do not always make it possible to avoid displacement of bone fragments and effectively correct the deformity.

Methods
Analysis of treatment of 11 patients of femoral and tibia deformity in patients with the skeletal system diseases (osteogenesis imperfecta – 2 patients with type I by Sillence; fibrous dysplasia - 3, vitamin D-resistant rickets - 4, vitamin D-dependent rickets - 1, Camurati-Engelmann syndrome - 1). Patients underwent corrective osteotomy of the femur and tibia with osteosynthesis using an advanced intramedullary locking rod with a T-shaped telescopic part. There were 19 surgical interventions: the hips - 7, tibia - 12.

Results
Average age was 9.8 years (range 8–11). The intramedullary construct consisted of a rod with proximal holes for locking screws, distal holes for locking screws in two planes, and a T-shaped telescopic part with holes of the same diameter and distance between them as in the rod. In the first stage, a corrective osteotomy was performed and stabilized by the intramedullary construct with distal locking of the rod and its T-shaped telescopic component. In the second stage, distal locking screws were removed after consolidation of the osteotomy, dynamizing the construct to growth mode. Correction of the deformity and bony union were achieved in all cases with no recurrence of the deformity or implant failure over five years follow-up.

Conclusion
The efficacy of the application of the improved intramedullary telescopic construct for the surgical correction of bone deformities in children with systemic skeletal disease which is based on the principles of locking intramedullary osteosynthesis and telescoping intramedullary rod that grows.

Disclosure
The authors declared no competing interests.

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P168

Cortico-cancellous bone allografting in treatment of children with orthopedic diseases
Mikhail Mikhovich, Lianid Hlazkin & Viktoryja Kazlova
Mogilev Regional Children’s Hospital, Mogilev, Belarus.

Objectives
Allografting is often used in the surgical treatment of skeletal deformities in children’s orthopedics. The aim of our study was to investigate the response of child bone tissue to the cortical and cortico-cancellous allografting, the dynamics of remodelling in various areas of the skeleton.

Methods
Bone grafting was applied in 166 children over 3 years in the department of orthopedics and traumatology. 93 had foot deformities, 33 – benign skeletal tumors and dysplastic processes, 17 – bone fractures with delayed union, 13 – dysplasia and other disorders of the hip, 10 – congenital and acquired deformities of the long bones including bone shortening. Allografts were used most frequently during: heel bone procedures – 54 children, metatarsals – 42, femur – 18. Analysis of graft reconstruction and recovery of bone was performed by examining radiographs at 1, 3 months, then every 6 months until complete resorption.

Results
The results were observed in 140 children in the period from 1 month to 3 years. Good early results observed in 137 children. Complications occurred in 4 children: 1 – nonunion, 1 – allograft migration, 1 – chronic ostemyelitis, which required removal of the graft, and long-term treating. X-ray observation showed that significant changes in the structure of cortical grafts did not occur within the first 6 months and they provided effective mechanical correction in osteotomy or resection zone. The first symptoms of partial resorption appeared after 6 months. Complete resorption with replacement of graft with recipient bone occurred in most cases after 2.5–3 years.

Conclusion
Cortical and cortico-cancellous allografts did not render pathological effects on the bone regeneration in the area of use. We found no significant difference in terms of consolidation of the fragments after surgery using cortico-cancellous and cortical allografts. Allografts provided effective mechanical correction in zone of osteotomy or bone resection within 6 months, however, required a minimal fixation. Perforation of the cortical plate is recommended, if acceleration of resorption is necessary, however, there is the possibility of loss of strength. We consider that the further use of bone allograft is appropriate in treating children when the need to fill bone defects or to fixate the fragments present.

Disclosure
The authors declared no competing interests.

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P169

Orthopaedic management of leg length discrepancy in Proteus syndrome: a case series
Molly Crenshaw1, Cara Goerlich2, Lauren Ivey1, Julie Sapp1, Kiri Keppler-Noreuil1, Allison Scott1, Leslie Biesecker1 & Laura Tosi2
1National Institutes of Health/National Human Genome Research Institute, Bethesda, Maryland, USA; 2Children’s National Health System, Washington, District of Columbia, USA.

Background
Proteus syndrome (PS) is a rare mosaic disorder comprising asymmetric bony and soft tissue overgrowth leading to significant morbidity. Placement of guided

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growth hardware with subsequent epiphyseal arrest improves leg length and angular deformities in pediatric patients without PS.

Presenting problem
The purpose of this study was to review the surgical approach and present outcomes, complications, and recommendations in eight patients with PS and leg length discrepancy (LLD). Although children with PS typically appear normal at birth, disproportionate growth of bone and soft tissue is usually identified by 6–18 months of age. PS is caused by a somatic activating mutation in the oncogene AKT1. The overgrowth in patients with PS is asymmetric, disturbing, and relentless with a rate and severity that vary greatly among patients.

Clinical management
We conducted a retrospective chart review of eight patients with PS whose primary reason for surgery was LLD. Patients were eligible if they met clinical diagnostic criteria for PS and if the NIH team performed at least one of their surgical interventions between 2005 and 2015. Surgical techniques included guided growth, with tension band plates, applied one or more times, and epiphyseal arrest. Eight patients, followed for an average of 4.6 years (range 1.0–7.1 years) after the index procedure, were included in this analysis. Average age at first LLD surgery was 9.4 years (range 6.1–13.6 years); the average LLD was 3.6 cm (range 0.4–8.9 cm) at presentation, and 5.0 cm (range 1.8–10.0 cm) at the time of the first LLD surgery. Participants underwent 23 total surgeries (range 1–5 per patient) and seven patients have completed surgical intervention. For six patients, the average LLD correction at last follow-up was 3.2 cm (range 0.8–6.6 cm). We encountered three complications: one patient developed a fracture of the fibula and ankle varus following development of a distal lateral tibia exostosis, and two patients developed mild knee valgus, which responded to standard guided growth techniques.

Discussion
This case series suggests that guided growth and epiphyseal arrest in children with PS can reduce leg length discrepancy with few complications. Careful monitoring, rapid mobilization, DVT prophylaxis, and sequential compression devices were also integral elements of our surgical protocol.

Level of Evidence: Level IV

This research was conducted under the National Human Genome Research Institute IRB-approved protocol 94-HG-0132: The Phenotype and Etiology of Proteus Syndrome, and was supported by research funding from the Intramural Research Program of the National Human Genome Research Institute (1 ZIA HG020388).

Disclosure
The authors declared no competing interests.

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P170

Physical activity is negatively correlated with circulating sclerostin in 6–12 year-old children
Theodoros Stampoulis1, Diamanda Leontsina1, Alexandra Avloniti2, Dimitrios Draganidis2, Konstantinos Papanikolaou2, Athanasios Chatzinikolaou1, Maria Michalopoulou1, Dimitris Vlachopoulos3, Luis Gracia-Marcos4, Konstantinos Makris3, Symeon Tournis3, Athanasios Jamurtas2, Ioannis Fatouros2 & Antonis Kambas1
1Democritus University of Thrace, Komotini, Greece; 2University of Thessaly, Trikala, Greece; 3KAT General Hospital, Athens, Greece; 4University of Athens, Athens, Greece; 5University of Exeter, Exeter, UK; 6University of Zaragoza, Zaragoza, Spain.

Objectives
Bone mass development through childhood is very important for osteoporosis prevention during adulthood. Physical activity (PA) and/or exercise can influence positively bone matrix and its contents in pre-adolescents Sclerostin, a glycoprotein produced by osteocytes, promotes osteoclastic activity and it is associated with reduced bone formation. The purpose of this study was to describe the relationship between PA and sclerostin levels in pre-adolescent boys and girls aged 6–12 years.

Methods
A cross-sectional design with 206 children (6–8 years: n = 105) was employed. The sample included both boys (n = 108) and girls (n = 98). Maturity was examined with Tanner stages of sexual maturity. Participants had their body mass, body height, tibia length and hip and waist circumference measured. Dual energy X-ray absorptiometry (DEXA) was used to measure body composition as well as body mineral density (BMD) and content (BMC) at hip and lumbar spine. Daily physical activity was measured using accelerometry (for 7 days). Power of lower limb muscles and cardiovascular endurance were determined using long jump and shuttle run testing, respectively.

Blood samples were collected at rest to measure serum sclerostin. Comparisons between PA (low, moderate and vigorous), sex (boys and girls) and age (6–8 vs 9–12 years) groups were performed using analysis of variance while a partial correlation analysis (adjusted for BMI) was used to correlate PA and serum sclerostin concentration.

Results
Serum sclerostin was lower in children with moderate and vigorous higher daily PA compared to those with low PA independent of age. Boys had higher sclerostin levels than girls in the low but not in moderate and high PA groups. Older children (9–12 years) demonstrated higher sclerostin levels than younger children (6–8 years) independent of PA level and sex. Sclerostin was negatively correlated with PA (r = −0.58, P < 0.05), muscle power (r = −0.61, P < 0.05) and endurance (r = −0.52, P < 0.05).

Conclusion
The results of this study indicate that serum sclerostin are affected by PA level, performance, age and sex during childhood suggesting that increased PA may promote osteogenic activity probably through a down regulation of sclerostin.

Disclosure
The authors declared no competing interests.

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P171

Review of lower limb range of movement following intramedullary fixation in children with Osteogenesis Imperfecta
Caroline Marr
Sheffield Children’s NHS Foundation Trust, Sheffield, UK.

Osteogenesis Imperfecta (OI) is a genetic condition which alters collagen biosynthesis[1]. Prevalence is estimated at 1 in every 15,000 births. It is a disorder with a wide spectrum of severity, with cases ranging from the extremely mild to those of perinatal mortality. Typical features include bone fragility; short stature; long bone deformity and persistent blue sclera[2]. Although currently there is no cure for OI, with the input of a multidisciplinary team those with the condition can be supported to live a full and independent live. Intramedullary fixation is a common orthopaedic intervention in children with osteogenesis imperfecta. It is associated with reduced fracture incidence and improved ambulation[3-4]. Complications such as rod migration are documented in the literature[5-6]. However a reduction in knee extension, a common clinical finding is not reflected in the current evidence base. As such a retrospective review of knee range of movement in children aged three to eighteen years diagnosis with OI who underwent intramedullary fixation at Sheffield Children’s Hospital (SCH) between 2007 and 2014 was completed. This service evaluation gained approval from the clinical governance department at SCH. Thirty children and 35 limbs were reviewed. Of the 30 children who had received orthopaedic surgery 26 had rodding of their femurs; 8 of their tibia’s and 1 of both femur and tibia jointly. Type of OI varied as did the type of intramedullary rod and post-operative care. Of the 15 limbs reviewed 15 lost knee extension following the surgery. Of these 7 resolved fully, 4 had an unknown outcome and 5 remained restricted. There is evidence to suggest that knee range of movement can be restricted post intramedullary rodding, with the suggestion that this is more likely following rodding of the tibia. This piece of work had many limitations and as such there is a need to examination the issue further with more robust techniques.

Disclosure
The authors declared no competing interests.

Reference

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Bone health at 11–12 years, physical activity and sedentariness: a cross-sectional Australian population-based study

William Osborn1,2, Peter Simm1,2, Tim Olds4,5, Kate Lycott1,2, Fiona Mensah1,2, Josh Mulles1,2, François Fraysse4, Najmi Ismail1,2, Jennifer Vlok1,2 & Melissa Wake2,3
1University of Melbourne, Parkville, Victoria, Australia; 2Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 3Department of Endocrinology and Diabetes, The Royal Children’s Hospital, Parkville, Victoria, Australia; 4Alliance for Research in Exercise, Nutrition and Activity (ARENA), University of South Australia, Parkville, Victoria, Australia; 5Centre for Community Child Health, The Royal Children’s Hospital, Parkville, Victoria, Australia.

Objectives: Activity duration and the daily patterns of activity during childhood and adolescence could contribute to long-term bone health. We examined cross-sectional associations between 11 and 12 year old children’s bone health and (1) durations, (2) patterns, and (3) combined durations and patterns of moderate-vigorous physical activity (MVPA) and sedentary behaviour.

Methods: Design: Population-based cross-sectional study nested within the Longitudinal Study of Australian Children. Participants: 11–12 year olds attending the Child Health CheckPoint physical module. Exposures: MVPA and sedentary behaviour (7-day wrist-worn accelerometry) yielding: (1) daily average durations (hours/day), and (2) patterns (power law alpha, representing the relationship between frequency and length of bouts of activity). Outcomes: Peripheral quantitative computerised tomography, yielding tibial bone density (cortical and trabecular), geometry (endosteal and perosteal circumference) and strength (polar stress-strain index (SSI)). Analysis: Multivariable regression models adjusting for sex, age, height, puberty, neighbourhood disadvantage, body fat percentage and muscle cross-sectional area (Aims 1–2), and mutually for durations and patterns (Aim 3). Interaction tests also assessed the effect of child sex.

Results: Of the 3,764 eligible children, 866 (23%) had both bone and accelerometry data available (mean age 11.14 years; 50% 50% boys). On average, children accumulated 0.6 (S.D.: 0.5) hours/day) of MVPA and 11.1 (S.D.: 1.2) hours/day of sedentary behaviour. Each additional daily hour of MVPA was associated with small bone health benefits, including larger periosteal and endosteal circumference (standardised effect sizes 0.26 (95% CI 0.10, 0.43) and 0.22 (95% CI 0.02, 0.41), respectively) and greater bone strength as evidenced by higher SSI (0.29 (95% CI 0.15, 0.42)). Duration of sedentary behaviour showed little association with bone health. In mutual models, bone health was slightly better with patterns of longer continuous MVPA and shorter fragmented sedentary behaviour, but these largely attenuated after adjusting for duration. There were no interactions for sex.

Conclusions: In early adolescence, more time spent in MVPA is associated with better bone health. While small, these associations are of population level importance. Activity guidelines to optimise adolescent bone health may need to focus explicitly on increasing daily duration of MVPA, rather than on its pattern or on sedentary behaviour.

Conflicts of interest: The authors declare no potential conflicts of interest.

Funding: This work has been supported to date by the National Health and Medical Research Council (NHMRC) of Australia (Project Grants 1041352, 1109355), The Royal Children’s Hospital Foundation (2014-241), Murdoch Childrens Research Institute and The University of Melbourne. Research at the Murdoch Childrens Research Institute is supported by the Victorian Government’s Operational Infrastructure Program.

Disclosure: The authors declared no competing interests.

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Management of Gorham disease in the cervicothoracic spine with mobile gravity traction and Sirolimus

Paul Foster1, M. Zulf Mughal2, Julian Leong2 & Benjamin Jacobs1
1Royal National Orthopaedic Hospital, Stanmore, UK; 2Royal Manchester Childrens Hospital, Manchester, UK.

Background: Gorham Disease is a rare condition characterised by massive osteolysis. The pathophysiology is related to angio/lymphatic proliferation within bone. No genetic transmission has been identified and onset occurs in patients of all ages.

Results: A clinical case of Maffucci Syndrome and highlight its clinical and orthopedic features and differences from the Ollier disease. Methods: Risk of secondary chondrosarcoma higher at Maffuccici syndrome – 46%. Almost 100% extraskeletal malignant transformation compared to Ollier disease which, according to various estimates, is 5–43% of all cases. Maffucci syndrome is a rare ‘(orphan)’ disease, and therefore not well known by scientists and practicing orthopedics. In world literature about 200 cases of this syndrome are described (Dyshondroplasia/multiple hondromatosis) – as a result of sporadic (post-zygote) mutations, there is an infringement of regulation, proliferation and residual differentiation of chondrocytes. As a consequence, there is a presence of abnormal ‘embryonic’ tissue inclusions in short and long tubular bones and the pelvis. The above-mentioned areas are located in close proximity to areas of bone growth, causing deformation, shortening of the affected segments, pathological fractures, and in some cases transformation into chondrosarcoma 5–43% (low or medium malignancy degree). Thereby, clinical understanding of features and differences between Ollier disease and Maffucci Syndrome (with almost 100% of likelihood of cancer complications) causes necessity of careful monitoring of patients with multiple enchondromatosys, early diagnostics, timely surgical treatment and prevention of malignant complications. Significance: Early diagnostics, timely surgical treatment and prevention of malignant complications – basis for patients with Maffuchici Syndrome.

Disclosure: The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P174
Surgical fixation of the spine may be unsuccessful due to progressive osteolysis of bone surrounding the metalwork, or of the bone graft.

Presenting problem
An 11 year-old boy presented with a 2 year history of back and shoulder pain. He then suffered weakness and paraesthesia in both legs for a few minutes after performing a forward roll. He was noted to have scoliosis, but was otherwise well. Blood bone and inflammatory markers were normal. Imaging demonstrated a severe kyphosis of the thoracic spine with extensive marrow hyperintensity and fatty signal within the bone. Biopsy of the spine was consistent with Gorham disease; transiliac bone biopsy was normal.

Clinical management
A Halo traction system was applied and a customised wheelchair constructed, connecting the Halo to weights via a pulley system. This provided continuous traction to the spine, but allowed him to mobilise in a chair. At night he was transferred to bed traction. Traction weight was gradually increased to a maximum of 1/3 body weight over 7 weeks. Pharmacological therapy with intravenous Zoledronate and Srornimus was initiated to attempt to improve bone quality ahead of spinal surgery.

Discussion
Mechanical traction and pharmacological treatment were used to prepare this patient's spine for surgery. Pre-operative traction has provided a minor improvement in the kyphotic deformity and it is hoped that this combination will improve the post-operative outcome.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P175

P176
System epidermal nevus with hyperkeratosis and violations of bone tissue metabolism – therapy of drug of pamidronic acid and surgical orthopedic treatment. Case from practice

Andrii Cheverda, Yuriy Guk, Andrii Zyma, Tamara Kincha Polishchuk, Yuriy Demyan, Yuriy Shkurko, Inna Molnar & Andrii Zotya
Institute of Orthopedics and Traumatology, Kiev, Ukraine.

Introduction
System epidermal nevus with hyperkeratosis (SENHK) – congenital epidermal formation characterized by hyperkeratosis, papillomatosis and acanthosis with elongation of intrapapillary epithelial strands. The main manifestations of metabolic bone disorders are system osteoporosis (SO) and violation of vitamin D metabolism. The urgency message is caused by a combination of the two above mentioned diseases in one patient.

Methods
We present 18-year-old patient with a complex problem: SENHK, SO and VDD. The diagnosis is established on the basis of clinical, radiological, biochemical and genetic testing.

Result
Clinically: muscle weakness, bone deformation of the limbs, pathological mobility in the diaphysis of the left femur, the inability for independent movement. Radiological findings: false joint of the left femur, expand growth areas of all long bones with intact areas of enchondral ossification, bone deformities of meta-epiphysial regions. Biochemical investigations found P1NP 758.3 ng/ml, β-CTx 2.78 ng/ml, osteocalcin 129.6 ng/ml, PTG 40.88 pg/ml, vitamin D 10 nmol/l. X-ray densitometric: decrease of bone density (z-score L1-L4 – 3.7, z-score femur neck left – 5.1, right – 5.4). Conservative treatment: pamidronate intravenously three times at intervals of 3 months (one cycle – 1 mg/kg per day for 2 days under the control of calcium serum), then – bivalos (1 pack a day), alpha-D3-Teva (alfacalcidol) – 1 mg/kg once a day, 10 drops of vitamin D a day – for 3 years. After 4 years seen significant increase muscle strength but deformation of the upper and lower limbs preserved. X-ray densitometric: z-score L1-L4 – 0.7, z-score femur neck left – 3.4, right – 2.6; biochemical P1NP 177.5 ng/ml, β-CTx 2.38 ng/ml, osteocalcin 91.01 ng/ml, PTG 54.75 pg/ml, vitamin D 13.05 nmol/l. Surgical treatment: resection of the false joint of the left femur, osteosynthesis with intramedullary locking rod.

Discussion
In the literature there are single reports of potential orthopedic manifestations in patients with SENHK. However, we did not encounter information of bone metabolism with the development of system osteoporosis (SO) on the background of the low levels of vitamin D in this disease. We do not exclude the possibility that the combination SENHK and SO are sporadic cases and has no genetic predisposition.

Disclosure
The authors declared no competing interests.

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P177
The elbow in type V osteogenesis imperfecta: is early functional loss related to radiographic findings?

Claire Hill1, Amaka Offiah1,2, Nick Bishop1,2 & Paul Arundel1
1Sheffield Children’s NHS Foundation Trust, Sheffield, UK; 2University of Sheffield, Sheffield, UK.

Objectives
Type V osteogenesis imperfecta (OI) results in abnormal modelling of the ulna, dislocation of the radial head and interosseous membrane calcification (ROM). Individuals develop reduced functional ability as a consequence of reduced range of movement (ROM) including elbow flexion and/or supination, which may be intrinsic or secondary to the radiographic findings. We describe the evolution of radiographic and functional parameters in a cohort seen in our centre.

Method
We performed a retrospective review of all type V OI cases seen in our institution. ROM data included earliest loss of elbow flexion (≤ 120°) and supination (≤ 60°). Radiographic images were double reported, consensus reached in cases of discrepancy. Earliest age of onset was determined for radiographic features: ROM; subluxation (SUBL) or complete dislocation (DISL) of radial head; abnormal modelling of proximal ulna (ULNMd); and ulna bowing ≥15° (UBOW15).

Results
Thirteen cases were reviewed (6 male/7 female; mean age 6.5 years (0.3–16.8 years)). ROM and radiographic data were available in 12/13 and 10/13, respectively; both available in 9/13. Loss of flexion and supination occurred in 12 elbows (8/12 children) and 14 elbows (8/12 children), respectively. Mean ages of loss of flexion and supination were 2.9 years and 4.6 years, respectively. Of those >2.9 years, 4/7 had flexion loss. Of those >4.6 years 6/7 had supination loss. Evolution of supination loss was variable over time. In contrast, flexion loss progressed steadily over time. In none of those with flexion ≤120°, was there recovery to normal. The pattern of evolution of both flexion and supination was remarkably symmetrical within individuals. Mean age (proportion of cases; range) at which radiographic features appeared were: IOM: 4.1 years (7/10;0.1–12.4 years); SUBL: 3.6 years (4/10;1.8–5.2 years); DISL: 5.5 years (4/10;3.2–6.9 years); ULNMd: 4.2 years (7/10;0.7–12.5 years); UBOW15: 2.4 years (3/10;1.5–2.9 years). Development of radiographic changes was not symmetrical within individuals.

Conclusion
We present data detailing the natural history of ROM and radiographic changes in the forearm in type V OI. We did not find a close relationship between loss of ROM and radiographic changes in the forearm and elbow. The symmetry of changes in ROM within individuals with type V OI suggests that these are partially due to intrinsic/systemic factors but not radiographic findings.

Disclosure
The authors declared no competing interests.

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Abstract withdrawn.

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Skeletal health of young patients with perinatal HIV infection: Experience from a reference centre
A. Doulgeraki1, E. Botsa2, A. Lourida2,3, G. Polizois1, I. Monopolis4 & V. Spoulou4
1Department of Bone and Mineral Metabolism, Institute of Child Health, Athens, Greece; 2Department of Paediatrics, University of Athens Medical School, Athens, Greece; 3Collaborative Center for Clinical Epidemiology and Outcomes Research (CLEO), The ‘Stavros Niarchos’ Foundation, Athens, Greece; 4Biostatistician, Athens, Greece.

Objectives
There are conflicting data on the skeletal health of patients with perinatal HIV. We aimed to evaluate the bone profile of a paediatric population followed in a reference centre for perinatal HIV.

Methods
The following data were recorded: dietary calcium intake, extra-curricular exercise, fracture history, medications and comorbidities. All patients were assessed for growth and skeletal deformities. They underwent laboratory tests: CD4 count, CD4/CD8, HIV viral load and basic bone profile, including %OH(D) and PTH. Finally, they had a DXA scan (GE Lunar Prodigy, paediatric software) for evaluation of bone mineral density (BMD) of L1-L4 and total body less head (TBLH), bone dimensions and strength, as well as fat mass (FM) and lean tissue mass (LTM). For calculation of Z-scores and statistical comparisons, 57 age- and sex-matched controls were used.

Results
Fourteen patients were studied, aged 9.9 ± 4.2 years (6 boys, 8 girls). They were on lopinavir/ritonavir, zidovudine and lamivudine. 50% of the patients had regular exercise. Only one post-traumatic fracture was reported; one patient had mild scoliosis and three patients complained of bone pain. Z-scores for height, weight, BMI, FM and LTM, as well as BMD, bone dimensions and strength were all comparable to controls. Their laboratory tests were also unremarkable, although 50% of the patients reported inadequate calcium intake. BMI Z-score was strongly and positively correlated to Z-scores for muscle and fat mass (r = 0.578 and 0.566, respectively, P = 0.03). Finally, only two patients had very low CD4 (< 500/ml); their BMD at both sites and bone strength (bone mineral content/LTM ratio) were lower (P = 0.02), compared to the other patients.

Conclusion
In our cohort, who were promptly diagnosed, treated and carefully followed through the years, growth, skeletal health and body composition were not compromised. CD4 count may have a prognostic value in detecting those patients in need of a more comprehensive bone health evaluation.

Disclosure
The authors declared no competing interests.

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Generalized arterial calcification of infancy: a case of a new mutation with central nervous system involvement and good response to bisphosphonates
Artemis Doulgeraki1, A. Nikas2, M. Vakaki3, G. Grigoriadou4, G. Servos4, H. Athanasopoulou1, K. Katsieris7 & I. Kapetanakis1
1Department of Bone and Mineral Metabolism, Institute of Child Health, Athens, Greece; 2Neonatal Intensive Care Unit, P.&A. Kyriakou Children’s Hospital, Athens, Greece; 3Department of Radiology, P.&A. Kyriakou Children’s Hospital, Athens, Greece; 4Department of Paediatric Cardiology, P.&A. Kyriakou Children’s Hospital, Athens, Greece.

Background
Mutations in the ENPP1 gene have been identified in individuals with generalized arterial calcification of infancy (GACI), a life-threatening disorder characterized by arterial calcification in the blood vessels, because of reduced availability of pyrophosphate. We describe a case of GACI due to a novel ENPP1 mutation.

Presenting problem
The patient, born at term to non-consanguineous parents, was referred to us at birth with weak femoral pulses for exclusion of aortic coarctation. Echocardiography showed left ventricular hypertrophy and low contractility, but normal aortic arch dimensions. Doppler studies revealed increased echogenicity of the wall of various arteries (common hepatic and splenic arteries, renal, iliac, femoral, carotid arteries and abdominal aorta), which within a 2-week interval progressed to frank calcifications. His cranial ultrasound revealed calcifications of the anterior cerebral and right lenticulostriate artery, as well as dilatation of the 3rd and 4th ventricle. His growth was within normal limits and he had no dysmorphic features.

Clinical management
Upon Doppler imaging and clinical suspicion of GACI he was started on IV infusions of pamidronate (0.1 mg/kg/week for 4 weeks), followed by oral risdrodonate (1 mg/kg/week), with close monitoring of growth, bone metabolism and progress of his calcification. Sequence analysis of ENPP1 gene was performed; the patient was found homozygous for the novel ENPP1 mutation c.825C>G in exon 8, which results in the substitution of an isoleucine by a methionine (ENPP1: p.Ile275Met). Both parents were heterozygous for the same mutation. The patient developed resistant hypertension requiring three antihypertensive medications. He also had a V-P shunt inserted and adequate calcium and vitamin D supplementation. He is now two years old and still on the aforementioned treatment. His calcifications have nearly regressed and his growth and neurodevelopment are satisfactory. However, his hypertension is still very hard to control and left ventricular hypertrophy remains unchanged.

Discussion
We identified a novel ENPP1 mutation causing GACI in our index patient. To our knowledge, this is the first time that hydrocephalus and calcifications of cerebral arteries are described in this disorder. More importantly, unexplained, drug-resistant hypertension requires a comprehensive Doppler study of all arteries to exclude rarities like this.

Disclosure
The authors declared no competing interests.

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Osteonecrosis results in significant long term morbidity in patients with acute lymphoblastic leukaemia
Nadia Amin1,2, Richard Feltbower2, Sally Kinsey1,2, Ajay Vora3, Talat Mushtaq1 & Beki James1
1Leeds Children’s Hospital, Leeds, UK; 2University of Leeds, Leeds, UK; 3Sheffield Children’s Hospital, Sheffield, UK.

Objectives
To determine the national prevalence, management and long term outcomes of patients who develop osteonecrosis after initiation of treatment for acute lymphoblastic leukaemia (ALL).

Methods
The central trials unit for the leukaemia trial UKALL2003 identified patients with reported bone toxicity out of the 3126 patients recruited into the study. Questionnaires were sent to each relevant treatment centre requesting information about each patient, covering demographics, diagnosis, scan results, management and outcomes. Details regarding previously unidentified patients was also requested.

Results
There was a 90% response rate for the 292 patients identified by the central trials unit. Of these 263 patients, 170 patients had radiographically confirmed
osteonecrosis, giving a prevalence of 5.4% of patients. Median duration of follow up was 70.5 months, and median time for development of symptoms of osteonecrosis after diagnosis of ALL was 16 months. Age was the most significant risk factor for development of osteonecrosis, with relative risk 17.72 (95% CI 11.28–27.82) and 19.97 (95% CI 12.19–32.71) for those aged 10–15 years and 16–25 years respectively at diagnosis of ALL, compared to those age < 10 years. 85% of patients had multifocal osteonecrosis, with hips and knees most commonly affected. There was significant variation in patient management with regards to cessation of steroids. Bisphosphonate therapy was given to 43 patients (25%), and use was centre specific. Surgery was required in 38% of all patients with osteonecrosis, with 99 surgical procedures reported in 65 patients. 33 patients with osteonecrosis required at least one hip replacement, and 16 patients required more than one joint to be replaced as a result of osteonecrosis. 3.6% of all patients over the age of 10 years at diagnosis of ALL required at least one joint replacement.

Conclusion
This is the largest study of symptomatic osteonecrosis with long term follow up data of childhood ALL in the UK. The considerable morbidity from this condition is clear, with important implications for quality of life. The greatest impact is on those over 10 years of age at diagnosis of ALL, with a surgical intervention required in a large percentage of patients.

Source of funding
Candlelighters charity.

Disclosure
The authors declared no competing interests.

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P183
The treatment of severe pain in melorheostosis with daily walking program only; a case report
Aysehan Akinci, Ismail Dundar & Ahmet Sigirci
Inonu University Medical Faculty, Malatya, Turkey.

Background
Melorheostosis is a rare, non-familial and progressive disorder characterized by hyperostosis, of the cortical bone. Typical clinical symptoms include chronic pain, limitation of joint movement, and soft tissue ossification and hand or foot deformity. Radiographic findings are helpful in the diagnosis, these consist of irregular hyperostosis extending along the length of one side of the long bone, resembling flowing candle wax. Medications including non-steroid anti-inflammatory drugs, nifedipine and high dose bisphosphonates are usually used for pain control. Here, we reported a case with melorheostosis whose pain control was abated temporarily by several drugs including local anesthetics but fully stopped by daily walking only.

Presenting Problem and Clinical management
A 16 years-old female had chronic hard pain around her feets and hands, and had been hurt in shoe gear. The physical exam revealed a severely ‘C’ shaped right and left feets that was nonreducible, and erythematous change, callosity formation were seen around the contact area between the skin and the shoes. Biochemical findings were within the normal range. Radiological examination showed that distal part of the ulna and metacarpal and carpal phalanges 3–4 and metatarsal phalanges were thickened and sclerosed. She was treated with high dose sodium pamidronate (1 mg/kg per day, 3 days each 3 months) for 9 months. At the end of this period, swelling in her hands and foots decreased and the pain abated, but when bisphospanate treatment was stopped, severe bone pain on her legs and arms began again. She was taking local anesthetics from time to time. After then, we recommended her to walk 45 minutes twice in a day only. After she began daily walking program, her pain disappeared in 2 months. She has been going on daily walking for one year, so she has no pain and restriction of extremities.

Conclusion
Treatment options are limited in melorheostosis. Non steroid antiinflamatuvar drugs, nifedipine and even sympathetic blockers have been prescribed to alleviate pain. Here, we observed that severe pain can be treated by daily walking program without taking any drugs in melorheostosis.

Disclosure
The authors declared no competing interests.

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P184
Physical activity and health-related quality of life in patients with chronic non-bacterial osteomyelitis – pilot and model project in a rare inflammatory bone disease
Julia Nentwich1, Annette Heilig-Wieden1, Henner Morbach1, Hermann Josef Girschick2, Katharina Ruf1, Helge Hebestrati1 & Christine Hofmann1
1Pediatric Rheumatology and Osteology, University Children’s Hospital, Wuerzburg, Germany; 2Children’s Hospital, Vivantes Hospital im Friedrichshain, Berlin, Germany; 2Pediatric Pulmonology and Sports Medicine, University Children’s Hospital, Wuerzburg, Germany.

Objectives
Chronic non-bacterial osteomyelitis (CNO) is an inflammatory, non-bacterial disorder of the skeletal system of yet unknown etiology (ORPHA 324964). CNO predominantly affects the metaphyses of long bones, but lesions can occur at any sites of the skeleton. Patients present with local bone pain and inflammation and - to our experience - often suffer from functional impairment with significant disabilities of daily life. The objective of this study was to assess physical activity, fitness and health-related quality of life (HRQOL) in patients with established diagnosis of CNO versus age and gender matched healthy controls (HC) (age 13–18 years) using established questionnaires, accelerometry and cycle ergometry.

Methods
Fifteen patients with CNO and 15 HC completed questionnaires (Pediatric Quality of Life Inventory PedsQL3.0 and 4.0, Child Health Assessment Questionaire CHAQ, Lipid Research Clinics LRC, in-house established activity questionnaire with visual analogue scales VAS, questionnaire to assess depression, anxiety and stress DASS-G), performed an incremental exercise test with gas exchange measures (Godfrey protocol) up to voluntary fatigue and wore an accelerometer (Actigraph GT3X) over 7 days at home to assess physical activity behavior.

Results
At the time of assessment 10 (66%) CNO patients were in clinical remission and 7 (47%) did not receive any therapy (median time after making the diagnosis/start ing treatment 3.7 years). The results of the exercise test (Wpeak, peak heart rate, VO2 peak and RQpeak) and of the accelerometry (time spend in moderate/vigorous/moderate and vigorous activity) did not show any significant difference between patients with CNO and HC. However, reported sports participation was lower in patients with CNO and PedsQL3.0 and 4.0 showed significant lower values in most of the scores indicating reduced HRQOL.

Conclusion
Although most of our CNO patients showed a favorable course of disease without any relevant differences in objective measurements of physical activity and fitness versus HC at the time of assessment, questionnaires (PedsQL3.0 and 4.0, LRC, and CHAQ) revealed self-reported limitations. Further studies are needed to measure HLQOL and to validate questionnaires in patients with CNO against objective measures including more participants with a higher level of disease activity.

Disclosure
The authors declared no competing interests.

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P185
Effects of vitamin D with calcium supplementation or zinc supplementation on the incidence of infections in school children: a randomized controlled trial
Rubina Mandalik1, Veena Ekbo1, Vivek Patwardhan1, Shashi Chipulkar1, Vaman Khadilkar1, Raja Padidela1, Zulf Mughal1 & Anuradha Khadilkar1
1Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India; 2Royal Manchester Children’s Hospital, Manchester, UK.

Objectives
Randomised controlled trials (RCTs) of vitamin D to reduce the incidence of infections among children have yielded variable results (Holland, 2012; Camargo, 2012). We performed a RCT of supplementation with vitamin D3 (cholecalciferol) along with calcium or supplementation with zinc in order to assess their effect on the incidence of infections in rural Indian school children.

Methods
A double-blind, placebo-controlled RCT was conducted on 465 children (248 boys), aged 6–12 years from Western India (18%). Fifty-four children dropped out of the study and supplements were administered to the remaining children as follows daily for 6 months: vitamin D (1000 IU) and calcium (500 mg) supplementation was administered to 124 children, zinc (10 mg) to 142 children and a placebo was given to 145 children. Detailed anthropometry, dietary and environmental data were collected at baseline and endline. Information on
Supplementation with vitamin D or zinc in school-children did not help to reduce infections were noted at baseline or endline among the three groups using morbidity status viz respiratory infections, gastrointestinal infections, or skin infections were noted at baseline or endline among the three groups using Kruskal–Wallis test.

The mean serum vitamin D concentration at baseline was 58.7 ± 10.7 nmol/l with no significant difference among the three groups. At 6 months there was significant increase (P < 0.05) in serum vitamin D levels in vitamin D and calcium supplemented group (82.2 ± 27.8 nmol/l vs 61.7 ± 16.4 in placebo and 56.3 ± 15.5 nmol/l in the zinc group). There was no significant difference noted in the serum zinc levels among the three groups at endline. No significant differences in morbidity status viz respiratory infections, gastrointestinal infections, or skin infections were noted at baseline or endline among the three groups using Kruskal–Wallis test.

Conclusion
Supplementation with vitamin D or zinc in school-children did not help to reduce the incidence of infections.

Disclosure
The authors declared no competing interests.

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Is cherubism a systemic disease? Prospective study about 9 patients
Aline Joly1, Gérard Maruani1, Valérie Cormier Daire1, Brigitte Faurox1, Ariane Beral1, Arnaud Picard1 & Amélie Coudert1
1Hopital Necker-Enfants Malades, Paris, France; 2Laboratoire physiopathologie orale moléculaire INSERM 1138, Paris, France.

Introduction
Cherubism is a rare pediatric disease with a maxillofacial localization caused by mutations of the SH3BP2 gene. Pathogenesis is well described in the SH3BP2 KI mouse model that presents a systemic inflammatory and bone phenotypes maintained by TNFα and due to the presence of hypersensitive myeloid precursors. In human, the disease is usually described as a maxillofacial exclusive disease. The aim of our study was to explore the systemic phenotype of cherubism patients in order to determine if cherubism is not only a maxillofacial disease but also a systemic disease.

Methods
Nine cherubism patients from 9 to 21 years-old and of various cherubism grade had been included. Clinical evaluation sought for systemic tissue infiltration, bone loss phenotype, biological bone remodeling markers, and biological systemic inflammation markers.

Results
From clinical evaluation, two patients presented systemic tissue infiltration (spleen, liver), one patient presented osteoporosis (Z-Score = −4). Osteoblastic biological markers tested were elevated in three patients, osteoclastic biological markers in 6 patients and inflammatory cytokines (IL1β, IL6, TNFα) were increased in five patients.

Conclusion
Our study suggest that cherubism is not an exclusive maxillofacial disease. For the first time, the potential systemic phenotype of cherubism was analyzed and variations of biological and radiological parameters in cherubism patients were demonstrated.

Disclosure
The authors declared no competing interests.

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Acroosteolysis presenting as nail resorption
Antonette Natino & Rashida Farhad Vasanwala
KK Women’s & Children’s Hospital, Singapore, Singapore.

Background
Acroosteolysis is a term used to describe bone resorption of the hands and toes. Typically involving distal phalanges, its causes may be hereditary, inflammatory, traumatic, toxin-mediated or idiopathic non-familial.

Presenting problem
An 11-year old Chinese girl presented to the dermatology clinic with nail resorption of the left index finger for 1 year. She was previously well with no history of connective tissue disorders and no history of frequent trauma to fingers or chemical exposure.

Clinical management
She was referred to the endocrine clinic to exclude metabolic bone disease. Hand X-rays revealed resorption of distal phalanges of the index and middle fingers of both hands (Figure 1). Bone biochemistry was normal (PTH: 3.6 pmol/l, calcium: 2.54 mmol/l, phosphate: 1.4 mmol/l), and inflammatory, autoimmune markers were not elevated. Her father had abnormal toe nails since adolescence and his foot X-ray showed resorption of second and fourth terminal phalanges of the right foot and his lesions seemed to be nonprogressive. With the given history our patient possibly has autosomal dominant hereditary phalangeal acroosteolysis. Reassurance was provided to the family.

Discussion
Acroosteolysis is a well-known consequence of chronic occupational exposure to vinyl chloride. Other causes include frequent trauma as in the case of guitar players and peripheral ischemia caused by frostbite and infectious diseases such as leprosy, meningococcemia, and syphilis. Hyperparathyroidism has been associated with resorption of terminal tufts of phalanges. Acroosteolysis is also linked with several connective tissue diseases. Idiopathic non-familial variant is of unknown etiology and usually affects fingers and is progressive. Hereditary causes of acroosteolysis follow an autosomal dominant or recessive pattern of inheritance and distal phalanges are primarily affected. Dystrophic nails may represent cutaneous manifestation of underlying bone involvement. Consider X-ray imaging in patients with disorders of the nail apparatus.

Disclosure
The authors declared no competing interests.

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Paradoxical response of serum parathyroid hormone concentration in response to vitamin D and calcium supplementation in undernourished Indian children
Rubina Mandlik1, Veena Ekbote1, Vivek Patwardhan1, Neha Kajale1, Vaman Khadilkar1, Raja Padidela1, Anuradha Khadilkar2 & Zulf Mughal2
1Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India; 2Royal Manchester Children’s Hospital, Manchester, UK.

Objectives
We have previously described biochemical evidence of resistance to PTH in Indian toddlers, which reversed on calcium supplementation. We performed a post-hoc analysis on data from an RCT of vitamin D and calcium supplementation, which was designed to assess if supplementation would reduce infection rate in undernourished school children with adequate sun exposure. Specifically we investigated the effect of oral vitamin D and calcium supplementation on biochemical parameters viz serum intact PTH, calcium, phosphorous and alkaline phosphatase (ALK-P) in children with habitually low calcium intakes.

Methods
A randomized, double-blind, placebo-controlled trial was conducted on 465 children (6–12 years) from Western India (18°N). The trial included 179 children: 79 received vitamin D-calcium (100IU/500mg) supplementation, 99 received placebo daily for 6 months. Anthropometric, dietary data and blood samples were collected at baseline, six months (end of supplementation) and 1 year post-supplementation.
Results
All anthropometric data at all three time points were below mean for age. Mean dietary calcium to phosphorus ratio was 0.4-1. Baseline mean serum 25OHD concentration was 58.2±10.9 nmol/l with no significant difference between the two groups. At 6 months, 25OHD concentration improved significantly (P<0.05) in supplemented group (83.9±30.1 nmol/l vs 58.3±15.7 nmol/l in placebo group). However, supplemented group also had significantly (P<0.05) higher PTH levels compared to non-supplemented group (6.7±3.6 pmol/l vs 5.5±3.2 pmol/l); positive correlation between serum 25OHD and PTH was noted (vs negative correlation in non-supplemented group). At 6 months mean levels of serum bone profile parameters were as follows: calcium (2.2±0.1 nmol/l), phosphorus (1.7±0.2 nmol/l) and ALK-P (178.7±40.7 IU/l). Neither at 6 months nor at 1 year post-supplementation was there significant difference between the groups in serum calcium, phosphorus and alkaline phosphatase levels. A year post-supplementation, PTH concentrations continued to remain high (but not significantly different from levels at six months); with low normal serum calcium, high normal phosphorus and normal ALK-P in supplemented group.

Conclusion
In nutritionally-deprived but vitamin D sufficient children, vitamin D and calcium supplementation paradoxically increased serum PTH concentration with no apparent effect on other bone biochemistry. The mechanism for this phenomenon is unknown. However, we speculate that chronic low dietary calcium to phosphorus ratio might be responsible for this paradoxical response.

Disclosure
The authors declared no competing interests.
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P189
A deep phenotyping of autosomal dominant osteopetrosis type 2 (ADO2) mouse model revealed multiorgan dysfunctions
Antonio Maurizi, Mattia Capulli, Rajvi Patel, Nadia Rucci & Anna Teti
Department of Biotechnological and Applied Clinical Sciences, University of L’Aquila, L’Aquila, Italy.

Objectives
ADO2 is a genetic bone disease induced by dominant negative mutations of the proton/chloride antiporter ClC7 encoded by the Clcn7 gene. In osteoclasts, ClC7 is crucial for lysosome function and resorption lacuna acidification. However, Clcn7 is expressed in several cell types in various organs, including brain, lungs, kidneys and spleen. Therefore, we asked whether Clcn7 mutations could affect other tissues beyond the bone.

Methods
A mouse model of ADO2, carrying the heterozygous Clcn7G213R mutation, was subjected to in-depth phenotyping.

Results
ADO2 mice exhibited 1.4 fold increased anxiety (P<0.05) and depression (P<0.01) and their related enzymes Gliol and Gad1 were more expressed in ADO2 brains (+1.77 and +1.23-fold respectively; P<0.05). Increased β-amyloid accumulation was found in hippocampus (+3.64-fold), thalamus (+4.6-fold) and amygdala (+2.28-fold; P<0.05). Cryosections of ADO2 hippocampus, as well as cultured ADO2 neurons, showed enlarged γ-adaptin-positive areas (+2.5-fold; P<0.02), suggesting alterations of Golgi-related clathrin-coated vesicular trafficking. Immunohistochemistry showed ClC7 expression in kidney tubular cells, lung bronchiolar epithelium and alveolar and spleen macrophages. Masson’s trichrome staining revealed pervascular fibrosis in ADO2 kidneys (+4.4-fold; P<0.0001). Moderate pervascular fibrosis was also observed in lungs (+1.5-fold; P<0.001), which in homozygous Clcn7G213R mice was more pronounced and associated with severe atelectasis and airway closure. Interestingly, pervascular fibrosis was confirmed in muscle, a tissue that does not express ClC7 but that is often damaged in ADO2 patients. ADO2 spleens did not show fibrosis but had elevated number of megakaryocytes (+1.4-fold; P<0.05), sign of an enhanced ectopic hematopoiesis.

Conclusion
Our study demonstrates that ADO2 is not only a bone disease, but it affects several organs at multiple cellular levels. It exemplifies the complexity of this pathology and the need to develop a targeted therapy with a systemic effect. In fact, the pervascular fibrosis was repeated, along with the bone phenotype, by systemic administration of an effective Clcn7G213R-specific siRNA.

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P190
Development of an in vitro model of cancer stem cells from a rare human telangiectatic osteosarcoma
Gaia Palmini1, Roberto Zonfrati1, Cecilia Romagnoli1, Alessandra Aldinucci2, Gianna Galli2, Carmelo Mavilia1, Gigliola Leoncini1, Antonella Simoni1, Alessandro Franchi1, Domenico Andreu Campanacci1, Rodolfo Capanna2 & Maria Luisa Brandi1
1Department of Surgery and Translational Medicine, University of Florence, Florence, Italy; 2Department of Neurofarba, University of Florence, Florence, Italy; 3SOD Ortopedia Oncologica e Ristrotruttiva, AOU Careggi, Florence, Italy; 4Clinica universitaria Ortopedia e Traumatologia, Azienda Ospedaliera, Pisa, Italy.

Objective
Even though recent studies have proved the presence of cancer stem cells (CSCs) in osteosarcoma (OSA), with this study, for the first time, the existence of CSCs in a rare high grade type of OSA, the telangiectatic osteogenic sarcoma (TOS), is showed.

Methods
TOS sample was collected at the ‘Ortopedia Oncologica e Ristrotruttiva Unit’, AOU Careggi, Florence, with informed consent approved by the local Ethical Committee. First of all, the primary human cancer cell culture of TOS has been established. After that, the subpopulation of CSCs has been isolated from this by the sarkoscape formation assay. Consequently, several cellular assays/stainings and molecular analyses have been performed to assess the presence of markers and properties, which are unique signature of the cancer stem cells phenotype.

Results
We have set up a primary cell line of a high grade TOS, from which we have isolated the CSCs and we have obtained a TOS-CSCs line, called TOS1-CSCs. The cancer stem cells phenotype of TOS1-CSC line was confirmed by observing the capacity of the TOS1-CSCs to differentiate into osteoblasts and into adipocytes and by showing the positive presence of the mesenchymal stem cells (MSCs) markers (by immunofluorescence assays and by the flow cytometry, too). The TOS1-CSCs line has showed a good rate as clonogenic capacity and a good rate as tumorigenic capacity, which has been evaluated in vitro by the agar soft assay. We have also studied, and confirmed, their embryonic phenotype by verifying the presence of the expression of the embryonic stem cells (ESCs) marker genes together with CD133 other genes involved in the pluripotency of CSCs and in the metastatic process.

Conclusions
In conclusion we have established and completely characterized, for the first time, a TOS-CSCs line at cellular and molecular level, setting up an in vitro model to study/find new targets to permit the development of molecular therapy against this high grade type of OSA.

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Disclosure
The authors declared no competing interests.
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P191
Juvenile hypophosphatasia presenting with short stature: a case report
Bertil Ersoy & Ozen Atik
School of Medicine, Celal Bayar University, Manisa, Turkey.

Background
Hypophosphatasia (HPP) is a heterogeneous disease; it can reveal itself at any age, through a wide range of symptoms. Findings of childhood (juvenile) HPP
Development of an osteogenesis imperfecta specific quality of life measure
Claire Hill¹, Wendy Baird² & Stephen Walters²
¹Sheffield Children’s NHS Trust, Sheffield, UK; ²University of Sheffield, Sheffield, UK.

Objectives
Osteogenesis Imperfecta (OI) is a hereditary disorder affecting approximately 1 in 20,000 births. Symptoms include; low bone mass, recurrent fractures, varying degrees of short stature and deformity. There is currently no disease specific quality of life (QoL) measure for children with OI. This study used a mixed methods approach to develop a QoL measure for the paediatric OI population. Patient reported outcome measure development is an iterative process, moving back and forth between concept elicitation, questionnaire development, pre-testing and psychometric analysis.

Methods
In order to encourage a balance between good content validity, alongside promoting a robust, reliable and responsive measure, the methods chosen involved several stages:
- Literature review to ensure no suitable QoL measure already existed and to begin eliciting themes.
- Interview and focus groups with the target population to uncover relevant concepts, develop a conceptual framework and subsequently validate themes.
- Questionnaire development; transforming themes into items, using the children’s’ language to ensure high content validity and acceptability.
- Pre-testing the instrument alongside a sample of the OI population, making revisions as required.
- Psychometric evaluation to assess validity, reliability and responsiveness of the questionnaire, informing potential item elimination and revision of the measure.

Results
Interviews and focus groups with the target population uncovered six main themes when describing QoL in children with OI; being safe and careful, reduced function, pain, fear, independence and isolation. These themes and related sub themes informed the development of the conceptual framework, which alongside the children’s own thematic based quotes, was used to develop the OIQoL. Pre-testing of the OIQoL highlighted logistical issues and understanding, which lead to revisions of the initial version.

Conclusion
The final version underwent field testing, Cronbach’s alpha for the 39-item questionnaire was 0.86. Concernts surrounding construct validity and internal consistency reliability highlighted the need to re-word some items and eliminate others, resulting in a 33-item questionnaire. Future research is proposed, involving multiple specialist centres, to include a larger patient cohort, which would further promote improved validity, reliability and responsiveness of the OIQoL, alongside the development of a short form.

Disclosure
The authors declared no competing interests.
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**P194**

Variable learning disability and behavioural difficulties in children with familial hypocalciuric hypercalcaemia type 3

Amish Chinyo, Mars Skae, Jacqueline Nicholson, Zulf Mughal & Raja Paddidela

Royal Manchester Children’s Hospital, Manchester, UK.

Background

Familial hypocalciuric hypercalcaemia type 3 (FHH3) is a genetically heterogeneous autosomal dominant disorder caused by loss-of-function mutations in the AP2S1 gene. This gene encodes the alpha-2 subunit of the adaptor protein-2 complex, which facilitates endocytosis of plasma membrane constituents such as G-protein coupled receptors.

Objective

It has been suggested that FHH3 may be associated with cognitive deficits (1). We assessed our cohort of 5 children with genetically confirmed FHH3 for evidence of learning and behavioural issues, using formal (such as the Vineland Adaptive Behaviour Scales Second Edition) and informal assessments.

Results

Table 1 summarises the patient characteristics of our cohort of children with FHH3, as well as extent of learning disabilities (LD) and behavioural difficulties.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>AP2S1 mutation</th>
<th>Learning disabilities</th>
<th>Behavioural issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>Female</td>
<td>Arg15His</td>
<td>Severe*</td>
<td>Possible evolving ASD</td>
</tr>
<tr>
<td>2</td>
<td>11.5</td>
<td>Male</td>
<td>Arg15Leu</td>
<td>Mild†</td>
<td>ADHD, aggression</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>Female</td>
<td>Arg15Leu</td>
<td>Mild†</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Male</td>
<td>Arg15Cys</td>
<td>Severe</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>Male</td>
<td>Arg15Leu</td>
<td>Mild†</td>
<td>ASD</td>
</tr>
</tbody>
</table>

Legend: *, formally assessed; †, requiring additional educational support but average scores on Vineland II assessment; ASD, autistic spectrum disorder; ADHD, attention deficit hyperactivity disorder.

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**P195**

Concentrated growth factor for the treatment of infrabony defects in chronic periodontitis in adolescents

Recep Orbak & Zerrin Orbak

1Department of Periodontology, Dental Faculty, Ataturk University, Erzurum, Turkey; 2Department of Pediatric Endocrinology, Medical Faculty, Ataturk University, Erzurum, Turkey.

Periodontitis is a health condition that involves inflammation of the periodontium, the supporting tissues, bones and ligaments around the teeth. In severe cases, the alveolar bone in the jaw area can become degraded and, without treatment, eventually lead to the loss of teeth. Periodontitis is the primary cause of tooth loss in adults. Regeneration of these tissues has become the most vital aim of periodontal surgery. For this situation, bone greft, syntetic materials and growth factors have been researching for years. Recently, there is a new material found, called 'Concentrated Growth Factor' (CGF). The current study was designed to evaluate the efficacy of CGF, with open flap debridement (OFD), in treatment of infrabony defects in chronic periodontitis (CP) in adolescents. Twenty patients with single defects were categorized into two equal treatments groups: group I: OFD alone, group II: OFD with CGF. Clinical parameters like site Plaque Index (PI), Gingival Index (GI), Gingival Bleeding Index (GBI), Probing Pocket Depth (PPD), Relative Attachment Levels (RAL) were recorded at baseline, before surgery and 6 months post-operatative. Percentage radiographic intra-bony defect depth reduction was evaluated using computer-aided software at baseline and 6th month. OFD with CGF group showed significantly greater percentage radiographic defect depth reduction (48.11±0.019%) as compared to Group I (11.21±0.082%) at sixth month. Adolescence are critical periods for the development of bones. Periodontal treatment is an important step in preventing alveolar bone loss. OFD+CGF group showed greater improvement in clinical parameters with greater percentage radiographic defect depth reduction as compared to OFD alone group in treatment of infrabony defect in chronic periodontitis in adolescents.

Disclosure

The authors declared no competing interests.

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**P196**

A qualitative enquiry examining the lived experience of mothers who have children with osteogenesis imperfecta

Caroline Mars & Zerrin Orbak

1The University of Sheffield, Sheffield, UK; 2Sheffield Children’s NHS Foundation Trust, Sheffield, UK.

Osteogenesis Imperfecta (OI) is a skeletal dysplasia which affects collagen biosynthesis. As with other chronic conditions it is recognised that the heterogeneity of perspectives between parents and health care professionals (HCP) can alter the course of a child’s health outcomes irrespective of the child’s disease. This qualitative study was employed to explore a mother’s lived experiences of having a child with OI.

Objectives

To gain improved understanding through qualitative semi-structured interviews a mother’s lived experience of having a child with OI. This includes a mother’s expectations for her child, both now and in the future, the relationships the mother has both within and outside the family unit; and a mothers’ own experience and perceptions of OI. To use template analysis to identify and explore factors that influence a mothers’ expectations for her child. Contrasting these expectations depending on the mother’s personal experience of the condition.

Method

A qualitative methodology was employed. Eight mothers were purposefully sampled. The sample size was derived from previous qualitative research in the area of interest. Each mother completed a semi-structured interview, which was digitally recorded and transcibe verbatim. The transcripts were analysed using template analysis. Ethical approval was obtained from the School of Health and Related Research Ethics Committee at the University of Sheffield.

Results

The analysis reviled four higher level themes: the multi-faceted role of mothers; a mother’s comprehension of OI; a mother’s relationship’s and a mother’s contemplation of the future. These four higher level themes were all permeated by the integrated theme of balance.

Conclusion

The findings echoed research conducted in other chronic conditions. However the mothers desire to decrease fracture risk seems to be unique to OI. The research suggests that HCP’s should recognise how a mother’s own perception of OI, established from the relationships she constructs and her own experiences and understanding of the condition, impacts upon her expectations for her child. This study is trustworthy and creditable but lacks some transferability. Future studies should include a larger cohort and review of the phenomenon family’s perspective.

Disclosure

The authors declared no competing interests.

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**P197**

Financial burden in families of children with osteogenesis imperfecta (OI)

Anne Murphy, Andrew Howard, Etienne Sochett & Jennifer Harrington

The Hospital for Sick Children, Toronto, Ontario, Canada.

Background

Families of children with Osteogenesis Imperfecta (OI) make costly modifications to their home, lifestyle and employment and incur costs of rehabilitative,
Hearing the patient’s voice: a focus group listening to the child and parent experiences of living with rare bone diseases

Evelina London Children’s Hospital, London, UK.

Objectives
- To establish the child and family experience of attending multi-disciplinary clinics within the rare bone disease service at Evelina London Children’s Hospital.
- To gain an understanding of the daily challenges the children, young people and families face.
- To understand how the tertiary multi-disciplinary team may support the child, young person and family.

Methods
Participants were recruited from Evelina London Children’s Hospital’s rare bone disease service. Twelve parents participated in the focus group along with seven unaffected siblings. The children participated in an activity-based group. The parents’ focus group ran in parallel. The discussion was voice recorded, transcribed and themes elicited. The themes from the children’s group were derived from written notes taken by the facilitators and written excerpts produced by the children.

Results
Thematic analysis revealed the following primary parental themes. i) The tertiay approach with professionals who cared about the wellbeing of their child and family is valued. ii) Continuity offered by local teams remains important iii) Practical suggestions for service improvement were offered iv) Transitions in childhood pose difficulties v) Daily life can present challenges for the child and their family. The children and young people’s themes encompassed the following: i) Ways to improve their clinic experiences ii) The challenges of daily life and iii) Which professionals may help to support them.

Conclusion
There is limited literature to date, which considers the patient’s voice in children with rare bone diseases. This articulate group have contributed valuable insight for clinicians working with this population. Results of this research will enable us to develop best practice in a tertiary setting. We highly recommend this family-centred approach, listening to the patient’s voice should be considered by other services internationally to drive care forward.

Disclosure
The authors declared no competing interests.

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Validation of questionnaire for measurement of sunlight exposure in children from Pune, India

Rubina Mandlik1, Veena Ekbote1, Neha Kajale1, Vivek Patwardhan1, Smruti Vishpute1, Utkarshni Kirtikar1, Anjali Jaiswal1, Dipali Ladkat1, Sonal Palande1, Pranati Deshpande1, Natasha Michael1, Varsha Deshpande1, Varsha Vartik1, Shashi Chiplonkar1, Vaman Khadilkar1, Raja Padidela1, Zulf Mughal2 & Amuradha Khadilkar1
1Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India; 2Royal Manchester Children’s Hospital, Manchester, UK.

Objective
Although there is adequate sunlight throughout the year, low serum 25OHD concentrations are being increasingly reported among Indian children (Gupta, 2014). Thus, quantifying individual sunlight exposure may be an important step in understanding hypovitaminosis D in sun-rich geographies. The objectives of our study were to quantify the sunlight exposure of school-children using a questionnaire and to use polysulphone film badges to validate the questionnaire administered.

Methods
The data reported is a part of an ongoing multi-centre, cross-sectional study to assess vitamin D status among Indian children. Sunlight exposure of a sub-set of 78 children aged (9.0–15.9 years) from a school in Pune city (18°N), Maharashtra was assessed during the month of July (the beginning of the monsoon season) using a questionnaire. Additionally, polysulphone (PSU dosimeter) film badges were given to all the children. These badges were mounted dorsally facing, in leather watch straps which were given to children to wear for 24 h on a typical school day. When all exposed badges were returned by participants they were sent to the laboratory at the University of Manchester for analysis and the resulting individual badge doses were reported in Standard Erythema Dose units (1 SED = 100 J/m²).

Analysis of the sunlight exposure questionnaire revealed a median sunlight exposure of 15 min (25th percentile, 75th percentile ~ 7.5 min, 30 min). Three children lost their badges and data obtained from three other children was found to be erroneous. Thus, 72 badges were analyzed and the mean standard erythemal dose (SED) was 0.57 ± 0.27 SED. The erythemal dose increased with the increase in sunlight exposure as assessed by questionnaire (0.53, 0.61 and 0.65 SED in groups with up to 7.5 mins, 15 mins and 30 mins or more of sunlight exposure respectively).

Conclusion
We present a questionnaire which was validated using PSU badges which may be used for assessment of sunlight exposure in Indian children. Lower duration of exposure to sunlight may be a major contributing factor to the low levels of serum 25OH D generally estimated among Indian children.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.P199
Methods
A multidisciplinary team (a communication officer, a bio-informatician, a geneticist, clinical research associates, an administrative manager and a project manager) helps and coordinates actions with the members of the platform.

Results
To improve the visibility, diagnosis and research studies of the reference centers of Paris-Sud several actions were undertaken by the platform team. The communication officer developed a unique website (http://maladiesrares-paris-sud.aphp.fr), produced videos showing the main clinical and research activities of the Paris-Sud reference centers (playlist available at https://www.youtube.com/playlist?list=PLRfa8NoVoR3EUIMqbfalu1yyWYylqD3), as well as many other communication tools. The bio-informatician, in collaboration with the team working on the on-site NGS platform, developed data analysis workflows specific for a rare disease or a group of rare diseases, accelerating diagnosis and reducing analysis costs. The geneticist, in collaboration with the INSERM UMR 788, identified 3 new genes linked to rare diseases by performing whole-exome sequencing. Clinical research associates accompanied the reference centers for clinical research projects by developing patient databases and collaborating with a team developing a ‘French database for rare diseases’ to allow local patient databases to be included in the national registry. Finally, the project manager helped to prepare grant proposals such as European Reference Networks and the national certification of reference centers for rare diseases.

Conclusions
The project of the platform of expertise for rare diseases Paris-Sud demonstrates that multidisciplinary and improved interaction between reference centers for rare diseases, research centers and patient associations permit translational research on rare diseases to advance. We strongly believe that this model can be used and implemented in the future in different medical structures in France and abroad.

Disclosure
The authors declared no competing interests.

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P201
Posture cushions impact on spinal alignment in children with osteogenesis imperfecta – true or false?
Alison Seasham
Sheffield Children’s Hospital, Sheffield, UK.

Background
Osteogenesis imperfecta (OI) is a rare skeletal disorder characterised by bone fragility usually due to inherited mutations in the genes for type 1 collagen. Children with OI present with numerous physical manifestations due to ligamentous laxity and bone fragility (1).

Presenting problem
Poor posture and associated back pain is a common problem for many children with mild to moderate OI. Hypermobility can result in muscle imbalance which may lead to excessive lengthening/weakening of the abdominal muscles and tightening of the iliopsoas and spinal erector muscle. Consequently is an increase in the natural lordosis of the lumbar spine which can result in spondylolysis/spondylolisthesis (2). This poster describes the case of a 14 year old with a diagnosis of OI type 1. In 2014 she presented with a lumbar sacral spondylolisthesis which deteriorated gradually over a 2 year period with resultant back pain and potential surgical intervention.

Clinical management
Good posture, dependent on the balance of the skeleton and optimal symmetrical alignment, is an active and dynamic process which underpins functional activities (3, 4). A posterior tilt wedge cushion was provided, used in conjunction with a standard classroom chair and foot rest. Positioning was checked for optimal alignment by the occupational therapist and monitored daily by teaching support staff.

Goals of provision
• Maintain skeletal alignment in sitting
• Provide a stable base of support to promote function
• Increase tolerance of optimal sitting posture
• Decrease pain and fatigue
• Correct skeletal deformity in sitting

Outcome
Six months following initial provision the patient attended a follow up outpatient appointment. Back pain had resolved and X-ray showed an improvement in the spondylolisthesis. No further spinal follow up was required.

Discussion
Evidence on the effectiveness of posture cushions in the OI population is scant. This case study suggests the use of a posterior wedge cushion to improve a lordotic posture and consequent spondylolisthesis can also reduce the incidence and severity of back pain in children mildly to moderately affected by OI. The need for surgical intervention may also be avoided.

Disclosure
The authors declared no competing interests.

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P202
Osteosarcoma-derived Extracellular Vesicles induce tumoral-like phenotypes in normal cells
Enrica Ursicoli1,2 & Barbara Peruzzi2
1University of Rome – Sapienza, Rome, Italy; 2Bambino Gesù Children Hospital – Multifactorial Diseases Unit, Rome, Italy.

Objectives
Osteosarcoma is the most common primary bone cancer and most frequent cause of cancer-related deaths in children and adolescents. Osteosarcoma cells are able to establish a crosstalk with resident bone cells leading to the formation of a deleterious vicious cycle. We hypothesized that osteosarcoma cells can release in the bone microenvironment transforming Extracellular Vesicles (EVs) involved in regulating the bone cell proliferation and differentiation, thereby promoting the tumour growth. So, our aims are to assess the EV production by osteosarcoma cells and to investigate the role of EVs in the communication between osteosarcoma cancer cells and normal recipient cells.

Methods
We used human osteosarcoma cell lines to set protocols aimed at isolating, visualizing and quantifying EVs. Once characterized, osteosarcoma-derived EVs were used to treat murine fibroblast cell line, NIH3T3. We studied the effects of tumoral EVs on normal NIH3T3 by cell count, cell cycle and apoptosis analyses. In order to verify tumoral-like phenotypes, we analyzed EV-treated NIH3T3 by wound healing, to test migration and soft agar assays, to assess anchorage-independent growth. Moreover, by exploiting the usage of human EVs and mouse recipient cells, we studied the presence of human osteoblastic and tumorigenic mRNAs in EV-treated NIH3T3 by using PCR assay.

Results
Our results showed that osteosarcoma cell lines are able to produce EVs that, in turn, induce tumour-like phenotype in recipient murine fibroblasts. In detail, EV-treated NIH3T3 showed an enhanced survival capability under low-serum conditions, high levels of activated survival pathways, an increased migration and the acquired capability to grow in an anchorage-independent manner. Moreover, in EV-treated NIH3T3 we found a de novo expression of specific mouse markers involved in osteoblastic differentiation and tumorigenesis, such as murine ALkaline Phosphatase (ALP) and Matrix MetalloProteinase (MMP)-9. Surprisingly, we also found the expression of human markers, as ALP and TNF-α, in EV-treated NIH3T3.

Conclusions
Our results demonstrate the ability of osteosarcoma-derived EV to transfer mRNAs and to induce tumoral-like phenotypes in normal recipient cells. Taken together, these findings highlight a crucial role of EVs in mediating tumoral transformation of normal cells.

Disclosure
The authors declared no competing interests.

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P203
Neurofibromatosis type 1 (NF1) associated congenital pseudarthrosis of the Tibia and Fibula misdianagnosed as non-accidental injury (NAI)
M Ziel Mughal1, Farhan Ali1, Rui Santos1, Grace Vassallo1, Siobhan West1, Susan Howard2, Judith Eelloo3, Eileen Hupton2, Elizabeth Rowles2 & Susan M Huson1
1Royal Manchester Children’s Hospital, Manchester, UK; 2St Mary’s Hospital, Manchester, UK.

Background
Congenital tibial pseudarthrosis (CTP) presents with anterolateral bowing of the lower leg in infancy, which often progresses to fracture and non-union (pseudarthrosis). CTP occurs in 2–3% of children with NF1. The distal end of the fibula and other long bones can also be affected.

Objective
We describe three children in whom NF1 related congenital tibial or fibular pseudarthrosis was initially misdiagnosed as NAI.

Presentation problem
The table summarises patient characteristics, presenting problem, safeguarding assessment and action taken by social services. Café-au-lait lesions were present

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in all 3 patients. The diagnosis of NF1 was confirmed by genetic testing in all patients.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age and gender</th>
<th>Presentation</th>
<th>Radiographic findings</th>
<th>Safeguarding referrals</th>
<th>Safeguarding investigations</th>
<th>Removed from parents</th>
<th>Family history of NF1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39M; F</td>
<td>Hard lump above the right lateral epidural</td>
<td>Fracture of the distal right tibia with pseudoarthrosis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>7M; F</td>
<td>Anterolateral bowing of the right lower leg</td>
<td>Anterolateral bowing of the right distal tibia with fracture and pseudoarthrosis</td>
<td>Yes</td>
<td>Under-taken</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>3M; F</td>
<td>Bilateral bowing of the lower legs from birth Pain when left leg moved</td>
<td>Fracture of the left distal tibia with pseudoarthrosis Anterolateral bowing of the right tibia &amp; fibula, i.e. bilateral CTP</td>
<td>Yes</td>
<td>Under-taken</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Legend: M, male; F, female; Safeguarding referrals: referral to social services and paediatric assessment; Safeguarding investigations: full radiological skeletal survey, CT head scan and fundoscopy; removed from parents, temporarily removed from parents into foster care or placed with a relative.

Discussion

Carefully taken family history, thorough clinical examination and classical radiological features of CTP could have avoided unnecessary safeguarding assessment/investigations in these patients, and their temporary removal from parents (Cases 3 and 4).

Conclusion

Clinical and radiological features of congenital tibial & fibular pseudoarthrosis, associated with NF1, may be confused with NAI.

Disclosure

The authors declared no competing interests.

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P204

Is a modified version of the Childhood Health Assessment Questionnaire (CHAQ) a useful tool to identify the level of disability in children with osteogenesis imperfecta?

Suzanne Ball1, Marie Roberts1, Vrinda Saraff1, Sophia Sakka1, Nick Shaw2 & Wolfgang Höglé1

1Department Endocrinology and Diabetes, Birmingham Children’s Hospital, Birmingham, UK; 2Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK.

Objectives

Different versions of the Childhood Health Questionnaire (CHAQ) have been used in Paediatric Rheumatology since 1994 to establish levels of functional disability. To date, use of the CHAQ has not been reported in Osteogenesis Imperfecta (OI). The aim of this study was to establish if disability scores generated from a modified CHAQ (MCHAQ) correlate with OI severity.

Methods

The MCHAQ was developed to reflect the specific needs of children with OI. All main features of the original CHAQ remain, but with a total of 32 questions. A category format was used and disability was graded 0–4 (0=no disability, 4=severe disability). Each patient was clinically categorized as having mild (Type I), moderate (Type IV) or severe OI (Type III), independent of genotype.

Results

The MCHAQ was completed by 100 patients with OI (median age 9.9 years (range 3.1–19.8)), with no age difference between clinical severity groups. MCHAQ scores were significantly higher in severe (2.06 (0.69–3.58); n=12) compared to moderate (0.59 (0–2.38), P=0.002; n=19) and mild OI (0.22 (0–1.61), P<0.001; n=69), and moderate OI tended to have higher scores than mild OI (P=0.051). MCHAQ scores (ρ=−0.291, P=0.003) and the percentage of tasks classified as ‘unable to do’ (ρ=−0.210, P=0.036) and ‘not-applicable’ (ρ=−0.617, P<0.001) were negatively associated with age, suggesting a learning effect. However, across age, children were consistently unable to perform certain skills such as riding a bike or tricycle (19.4% of children), cutting fingernails (14.3%), participating fully in physical education at school (14.3%) and reach up and get down a heavy object (such as a large or book) from above his/her head (14.1%).

Conclusion

The MCHAQ differentiated the functional level of disability in patients with OI based on clinical severity category and identified specific functional difficulties that can guide therapy intervention. MCHAQ allows monitoring of individual change but the score is also age dependent. Therefore, comparison with healthy children will be required to test the hypothesis whether children with OI acquire skills later than their normal peers.

Disclosure

The authors declared no competing interests.

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P205

Osteopathologies and endocrine late effects in a cohort of 102 juvenile survivors of brain tumors

Michael Schündeln1, Sebastian Fritzemeier2, Pia Hauffa1, Jens Bauer2, Berthold Hauffa2 & Corinna Graesemann2

1Department of Pediatric Hematology and Oncology, Kinderklinik III, Universitätsklinikum-Essen and the University of Duisburg-Essen, Essen, Germany; 2Department of Pediatric Endocrinology and Diabetology, Kinderklinik II, Universitätsklinikum-Essen and the University of Duisburg-Essen, Essen, Germany.

Objectives

Endocrine late effects, including osteopathologies, following diagnosis and treatment of childhood malignancies are studied in adult survivors with alarming results. However, in pediatric patients with brain tumors the risk to develop endocrine late effects is high even during childhood and adolescence.

Aim and design

To investigate osteopathologies and endocrine function in juvenile survivors of pediatric brain tumors we conducted a cross-sectional analysis in the hematology and oncology outpatient clinic at the University Children’s Hospital Essen, Essen, Germany. The study was approved by the local ethics committee (DRKS: 00003636). After informed consent was obtained, a thorough clinical and biochemical assessment of endocrine function (hypothalamic-pituitary-gonadal/thyroidal/adrenal axes) and signs of osteopathologies, (biochemical, radiographic and anamnestic parameters) was performed in 102 patients (42 female).

Results

50% of the patients displayed impaired function of at least one of the investigated endocrine axis. 20% of the patients experienced fractures after chemotherapy, but only 3 patients reported frequent fractures. 12% of patients reported bone pain after physical activity: 25 OH-vitamin D3 levels ≤20 ng/ml were observed in 77% of the patients. 38% presented with 25 OH-vitamin D3 levels ≤10 ng/ml and 11% with secondary hyperparathyroidism. Using an expert rating, 28% of patients were diagnosed with osteopathologies. Osteopathologies were more frequent in children with endocrine impairments (not significant). A positive association of cumulative vincristine dose, but not of methotrexate, and the presence of osteopathy was observed.

Discussion

Impaired endocrine function and bone health are present in about 50% of juvenile patients after treatment for childhood brain tumors. Our results point towards a possible dosage effect of vincristine for the development of osteopathologies, however we could not confirm previous observations of negative effects of methotrexate on bone health.

Conclusion

Impaired bone health is a frequent finding in young survivors of brain tumors. Identification of children at risk is difficult and requires continuous assessment of clinical, biochemical and radiological measures. Adequate supplementation of vitamin D is recommended to avoid secondary hyperparathyroidism.

Conflict of interest

None.

Disclosure

The authors declared no competing interests.

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P206

Lysinuric protein intolerance associated with vertebral fractures and IGF-1 deficiency

Emily Cottrell & Talat Mushtaq

Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Background

Lysinuric Protein Intolerance (LPI) is a rare autosomal recessive metabolic disorder affecting amino acid transport. The condition typically presents at weaning, with recurrent diarrhoea and vomiting especially following protein rich meals. It may have a multisystem clinical presentation including growth and haematological abnormalities and rarely osteoporosis. The diagnosis is based on biochemical findings, including increased urine and reduced plasma concentrations of lysine, arginine and ornithine.

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P207
Bone turnover in the obese children is related to gender, body composition and lepton level
Paweł Matsuki,1 Magdalena Olszanecka-Glinianowicz 2, Jerzy Chudek2, Edyta Matusik3 & Ewa Malecka-Tendera1
1Department of Pediatrics and Pediatric Endocrinology, Medical University of Silesia, Katowice, Poland; 2Department of Pathophysiology, Medical University of Silesia, Katowice, Poland; 3Department of Medical Rehabilitation, Medical University of Silesia, Katowice, Poland.

Introduction
Recently published data revealed that bone turnover is related to the body composition in pubertal children and may be impaired in obese adolescents. Bone turnover ratio (calculated as OC/NTx) was significantly lower in obese girls only (P<0.05 respectively). Bone turnover ratio (calculated as OCN/NTx) was significantly lower in obese girls only (P<0.01 respectively). Significant negative correlation was found between the OC level and BMI Z-score in the whole studied population of children. OC and OCN/NTx correlated significantly with all anthropometrical parameters only in girls. There was also a significant positive correlation between NTx and lepton in the entire group, being significantly higher in females (P<0.05 and P<0.0001 respectively).

Conclusions
Bone turnover is related to the amount of fat mass and its hormonal activity. We can suspect that, in obese children, particularly in obese adolescent girls, impairment of bone turnover may be a risk factor for the lower bone mass and higher fracture risk in the future life.

Disclosure
The authors declared no competing interests.

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P208
Social barriers and needs of children with osteogenesis imperfecta (OI): a qualitative descriptive interview-based study
Jerzy Konstantynowicz & Pawel Abramowicz
Medical University of Bialystok, Department of Pediatrics, Rheumatology, Immunology and Metabolic Bone Diseases, Bialystok, Poland.

Objective
Osteogenesis imperfecta (OI) which is a heterogenic group of diseases presenting with bone fragility, skeletal deformations and limited mobility may confer a risk of several non-skeletal health issues and is associated with adaptational social problems. The aim of the qualitative descriptive study was to determine the most important areas (problems?) related to social functioning, familial environment, hospital amenities and social needs in children with OI.

Methods
Nineteen children (10 boys, 9 girls) aged 5-17 years with different clinical forms of OI (type I, n=8; III, n=7; IV, n=3) and their caregivers/parents completed a questionnaire including 26 items, and were interviewed, using a semi-structured design and open questions, on fracture rate/prevalence, limitations in social functioning, access to facilities, locomotor abilities, medical care, housing and education, self-reliance, manual abilities, leisure time and habits, socializing with peers, essential difficulties in the familial and school environment, barriers in social integration, and needs for ameliorative interventions addressed to improve these domains.

Results
The severity of functional disability was strictly related to clinical type of the disease. The majority of patients (12) were able to walk independently, three were able to walk on crutches, two using four used wheelchair. Fine-motor skills (drawing, lacing up, puzzling, operating utensils) were not restricted by the disease. Only five respondents reported spending leisure time actively or outdoor with their peers. The interviews revealed emerging themes of which the most essential were limited daily activity resulting in coerced stay at home, unadjustedness of school and public institutions to locomotor disability, school absence and decreased academic performance, limited socializing, non-acceptance by age-mates. According to participants’ and parents’ views, the remedial and corrective actions to be undertaken included: elimination of architectural barriers, customization of housing according to individual needs, facilitation to physiotherapy access. Caregivers of three patients were neither able to express their expectations nor map out the needs.

Conclusions
Functional limitations, social integration neglects among children with OI appear as important as physical disability. The participants do not focus on their physical restrictions caused by the disease. Isolation from the school environment and contemporaries considerably disturb potential social roles in OI. Personalized care plan in OI should include not only physical health or rehabilitation, but should substantially address facilitation in social integration, breaking up barriers in local environment, and should implement an individualized age-adjusted corrective schedule regarding socializing.

Disclosure
The authors declared no competing interests.

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P209
Intensive therapy – a week of multi-disciplinary intervention at Sheffield Children’s Hospital: An example of goal setting and positive outcomes
Elizabeth Knowles
Sheffield Children’s Hospital, Sheffield, UK.

Patients with moderate to severe osteogenesis imperfecta commonly have functional difficulties following fractures or surgery, which generally result in long periods of immobilization. Patients quickly lose range of movement, muscle strength and conditioning, which impacts on daily living activities, mobility, self-confidence and motivation. It is natural for patients to become more dependent on their carers, some not returning to their previous level of independence, nor achieving what they had aspired to. Barriers include fear of pain and injury, as well as potential failure in aiming higher. At Sheffield Children’s Hospital Metabolic Bone Disease Service, we aim to facilitate independence in the areas that matter to our patients, and positively influence other aspects of their life. Patients who are considered to have reached plateau, are offered the opportunity to attend for a week of intensive therapy, involving a minimum of 8 two-hour sessions over 5 days. In order to meet the criteria for this intervention, the patient rather than the family
must be prepared to actively engage, and to identify goals prior to therapy. Both a physiotherapist and occupational therapist are present at each session, for multi-faceted intervention, with a combination of problem solving and activity analysis alongside graded exercise programmes that are designed to build the skills to achieve the goals. A variety of gross and fine motor activities are included throughout the week, dependent on patient’s specific interests and needs. Opportunities to develop and practice daily living skills are available. Parents/carers are encouraged to observe each day for reinforcement and continuity, as evening exercises are expected. At the end of the week, a closing assessment is performed, to identify goals that have been met, and to progress onto new goals. A patient evaluation form is completed, to inform better future outcomes. Patients are seen for follow-up review after a six-week period, to establish progress and compliance with the home programme. Many patients have made significant progress from the first episode, often opting to attend a further intensive week; suggestive that when patients are motivated to improve their functional outcomes, intensive therapy provides a good baseline to start from.

Disclosure
The authors declared no competing interests.
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Late Breaking Abstracts
L1

**Treatment with a Novel activin receptor IIB ligand trap improves muscle mass and bone geometry in a mouse model of severe Osteogenesis Imperfecta**

Josephine T. Tauer & Frank Rauch
Shriners Hospital for Children and McGill University, Montreal, Canada.

**Objective**
Osteogenesis imperfecta (OI) is primarily characterized by bone fragility but is also associated with lower muscle mass and function. As muscle mass and bone mass are closely linked, an intervention that increases muscle mass should also increase bone mass. Here we investigated the effect of a novel activin receptor IIB ligand trap, ACE-2494 (Acceleron Pharma), on skeletal muscle mass and bone properties in a mouse model of severe dominant OI, the Col1atm2+/+ mouse.

**Methods**
ACE-2494 (3 mg or 10 mg per kg body mass) or vehicle was injected subcutaneously twice per week for 4 weeks into male OI and wild-type (WT) mice, starting at 8 weeks of age.

**Results**
At baseline, OI mice had 20% lower body mass than control littersmates. This difference persisted during the intervention as OI and WT exhibited a similar dose-dependent increase in body mass during ACE-2494 treatment. ACE-2494 injections led to a dose-dependent gain in muscle mass in OI and WT cohorts (Figure 1). In WT, ACE-2494 treatment also increased soleus weights (by 16 and 34%) and EDL weights (by 20 and 65%) in a dose-dependent manner. In OI mice, ACE-2494 increased soleus and EDL mass to a similar extent in both dose groups (by 65 and 75%, respectively). ACE-2494 had no effect on heart muscle mass or liver mass. There was also no effect on either femoral length or trabecular bone volume in the distal femoral metaphysis. However, ACE-2494 treatment resulted in an increased mid-diaphyseal periosteal diameter in OI mice only, leading to an improved polar moment of inertia.

**Conclusion**
ACE-2494 increases muscle mass and seems to improve diaphyseal bone geometry in a model of severe OI.

**Disclosure**
The authors declared no competing interests.

Figure 1 Increase in muscle mass (weight/initial body mass (mg/g)) by ACE-2494 treatment. Data are shown as mean±SEM; α ≤ 0.001 vs vehicle-treated (Veh).

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L2

**The regulation of Smpd3 expression in skeletal tissues and its role in fracture healing**

Gartniga Manickam1,2, Jingjing Li1,2, Hironori Hojo3, Amjad Javed4 & Monaiz Marzouk1,2
1McGill University, Montreal, Quebec, Canada; 2Shriners Hospital for Children, Montreal, Quebec, Canada; 3The University of Tokyo, Bunkyo-ku, Tokyo, Japan; 4University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

Bone fractures can be a serious and frequent problem for patients suffering from osteoporosis, metastatic bone cancer and congenital bone disorders. The promotion of new bone formation and mineralization can facilitate healing and strengthen union of fractured bones. Our laboratory has identified important developmental roles of sphingomyelin phosphodiesterase 3 (SMPD3), which include the promotion of apoptosis of hypertrophic chondrocytes and mineralization of cartilage and bone extracellular matrix. However, the transcriptional regulation of this gene and its role during fracture healing is still unknown.

**Objectives**
1. To elucidate the transcriptional regulation of Smpd3 in skeletal cells
2. To investigate the role of SMPD3 in fracture healing

**Methods**
1. ATDC5 and MC3T3 cell lines were used to investigate the transcriptional regulation of Smpd3 in chondrocytes and osteoblasts, respectively. These cells were transfected with pGKpcX or pGKox expression vectors. For Smpd3 promoter studies, a 1.9-kb mouse Smpd3 proximal promoter was cloned into the pGL4.10 (Luc) vector. This construct was then co-transfected with or without pGLpcX or pGLox. 2. To investigate the role of SMPD3 in fracture healing, we generated a conditional knockout mouse, Smpd3flox/flox;Ox-Cre, which lacks Smpd3 in both chondrocytes and osteoblasts. Rodedd immobilized fracture surgeries were performed in the tibia of these mice. The bones were then analyzed at 1 and 4 weeks post-surgery by X-ray, micro-CT, histology and histomorphometry.

**Results**
1. A significant upregulation of Smpd3 was seen in the presence of pGKpcX and pGLox in ATDC5 and MC3T3 cells, respectively. Furthermore, Smpd3 promoter activity was significantly upregulated in the presence of pGLpcX and pGLox. 2. Histological analyses showed a prominent callus at the site of fracture in Smpd3flox/flox;Ox-Cre mice, whereas the fractures induced in the WT mice healed well. Histomorphometric analysis showed a significant increase in osteoid volume in Smpd3flox/flox;Ox-Cre bones compared to WT bones.

**Conclusion**
Our data provides compelling evidence that Smpd3 activity, regulated by Sox9 and Ossx, is critical for bone fracture healing. To test SMPD3’s potential as a therapeutic agent to improve fracture healing, studies have been initiated to encapsulate Smpd3 and inject it into the fracture sites in WT and Smpd3-deficient mice.

**Disclosure**
The authors declared no competing interests.

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L3

**Mediating effect of muscle on the relationship of physical activity trajectories and bone outcomes: The Iowa Bone Development Study**

Vera Zymbal1, Fatima Baptista1, Elena M. Letuchy2 & Kathleen F. Janz3
1Department of Sport and Health, CIPER, Faculdade de Motricidade Humana, Universidade de Lisboa, Lisboa, Portugal; 2Department of Epidemiology, University of Iowa, Iowa City, IA, USA; 3Department of Health and Human Physiology, University of Iowa, Iowa City, IA, USA.

**Objectives**
This study analysed prospective associations between two distinct developmental trajectories of objectively-measured physical activity and late adolescent bone parameters (age 17 yr) by exploring the mediating effects of lean soft tissue (LST), a surrogate of muscle mass.

**Methods**
In approximately 349 participants (191 girls) of the Iowa Bone Development Study, physical activity was measured by accelerometry starting at age 5 and continuing at 8, 11, 13, 15 and 17 years. Gender-specific group-based trajectory modelling was used to construct developmental trajectories of moderate-and-vigorous intensity physical activity (MVPA) from childhood to late adolescence. Bone parameters were assessed by dual X-ray energy absorptiometry and included bone mineral density (bMD), bMD distribution, and specific geometric measures of the proximal femur.

**Results**
A significant portion of the total effect of MVPA from age 5 to 17 yr on bone parameters at age 17 was explained by changes occurring in leg LST in both genders. These indirect effects were observed on all regional bMDs (neck, trochanter, intertrochanter, femoral and suprateral neck), on the ratio between the inferomedial and suprateral neck bMD, and on the hip axis length (HAL). The effects of MVPA mediated by leg LST were 43–49% on regional bMDs in girls (P < 0.01) and 27–32% in boys (P < 0.05). On the ratio between the inferomedial and suprateral neck bMD the effect of MVPA mediated by leg LST was 30% in girls (C = −0.011, bootstrap 95% CI: −0.027; −0.001) and 41% in boys (C = −0.013, bootstrap 95% CI: −0.029; −0.002). Regarding HAL, the effect of MVPA mediated by leg LST was 34% in boys (C = 0.083, bootstrap 95% CI: 0.016; 0.172) but inconsistent in girls (the sign of the coefficient of the mediated effect differed from that of the direct effect). Direct effects of MVPA were identified only in boys on all regional bMDs of the proximal femur.

**Conclusion**
To improve proximal femur bone parameters, physical activity interventions during childhood and adolescence should also focus on increasing muscle mass, particularly in girls.

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**Disclosure**
The authors declared no competing interests.

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**LB4**

Serious adverse effects of denosumab in adolescents treated for giant cell tumour of the bone: osteonecrosis of the jaw and rebound hypercalcaemia with acute kidney injury

Suma Uday1, Louie Gaston2, Robert Grimer3, Jonathan Joffe4 & Wolfgang Hoe elicier1,2

1 Birmingham Children’s Hospital, Birmingham, UK; 2 Institute of Metabolic and Systems Research, Birmingham, UK; 3 Royal Orthopaedic Hospital, Birmingham, UK; 4 Calderdale and Huddersfield NHS Trust, Calderdale, UK.

Introduction

Giant cell tumour of the bone (GCTB) is a benign, locally aggressive tumour whose neoplastic stromal cells express receptor activator of nuclear factor kappa-B ligand (RANKL) and activate its receptor RANK on osteoclast-like giant cells. Denosumab (RANKL inhibitor) is an FDA/EMA approved treatment for GCTB in adults and ‘skeletally mature’ adolescents. Safety concerns include over-suppression of bone remodelling, with risk of osteonecrosis of the jaw (ONJ) and atypical femur fractures during treatment, and rebound hypercalcaemia after treatment cessation. To date, ONJ has never been reported in children or adolescents.

Case descriptions

Two adolescents with sacral GCTB received denosumab as per trial protocol (Table 1). Following 4 years of therapy (age 19 years), P1 developed ONJ after a dental extraction necessitating surgical debridement and sequestration of exposed jaw bone. P2 completed GCTB treatment without complications. Both patients presented unwell with hypercalcaemia and acute kidney injury 6–7 months after denosumab cessation. Other causes of hypercalcaemia were excluded. Since hypercalcaemia was unresponsive to hyperhydration, P1 received repeated doses of calcitonin. P2 received low dose pamidronate and despite prophylactic oral calcium developed symptomatic hypercalcaemia requiring intravenous calcium. Both patients received treatment for vitamin D deficieny.

Conclusion

Here, we report the first case of ONJ in an adolescent. Both adolescents were naïve to chemotherapy, radiotherapy, bisphosphonates, corticosteroids and metastases free; hence, denosumab therapy was confirmed as the cause of P1’s ONJ, and both patients’ rebound hypercalcaemia. Over-suppression of bone remodelling due to this potent, high-dose antiresorptive drug has to be weighed up against its effect on tumour shrinkage. These cases call for close monitoring for side-effects during and after therapy, for safety data to be collected in adolescents and consideration on weight-based dosing.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics, treatment indication, duration, dosing and information and hypercalcaemia management.</th>
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<tbody>
<tr>
<td>Patient 1 (P1)</td>
<td>Patient 2 (P2)</td>
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<tr>
<td>Age at diagnosis</td>
<td>14 years 9 months</td>
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<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.5</td>
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<td>Location of GCTB</td>
<td>Sacrum</td>
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<tr>
<td>Denosumab indication</td>
<td>Tumour recurrence</td>
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<td>Denosumab regimen</td>
<td>following surgery and embolization</td>
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<td>ClinicalTrials.gov Identifier</td>
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<td>Individual dose (mg/kg)</td>
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<td>Total number of doses</td>
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<td>Cumulative dose over treatment duration (mg/kg)</td>
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<tr>
<td>Total treatment duration (months)</td>
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<tr>
<td>Reason for treatment cessation</td>
<td>Osteonecrosis of the jaw (ONJ)</td>
</tr>
<tr>
<td>Rebound hypercalcaemia</td>
<td>Yes, requiring repeat calcitonin</td>
</tr>
</tbody>
</table>

**LB5**

Role of type III sodium/phosphate co-transporters in the responsiveness of osteoblasts to extracellular inorganic phosphate

Toshimi Michigami1, Miwa Yamazaki1, Masanobu Kawai1 & Keiichi Ozono2

1 Osaka Women’s and Children’s Hospital, Izumi, Japan; 2 Osaka University Graduate School of Medicine, Suita, Japan.

Objectives

As osteoblasts mature, they acquire the expression of multiple molecules involved in phosphate metabolism, including dentin matrix protein 1 (DMP1) and fibroblast growth factor 23 (FGF23). This suggests that osteoblasts and osteocytes may sense and respond to alterations in the phosphate availability in their microenvironment. We previously reported that increased extracellular inorganic phosphate (Pi) triggered signal transduction in various cell types to alter gene expression and demonstrated the involvement of a type III sodium/phosphate (Na+/Pi) co-transporter Pit1 and FGF receptor. Pit2, another type III Na+/Pi co-transporter, is ubiquitously expressed similarly to Pit1, and loss-of-function mutations in Pit2 cause familial idiopathic basal ganglia calcification. Here we aimed to investigate the role of Pit2 in responsiveness of osteoblasts to extracellular Pi.

Methods

We applied CRISPR/Cas9 to an osteoblastic cell line MC3T3-E1 (subclone #4) to generate Pit2-knockout (KO) cells. Reduced Pi uptake in Pit2-KO cells was confirmed using 31P-orthophosphate. Then, Pit2-KO and control cells were cultured for 8 weeks in the medium containing 3 mM of Pi and 50 (μg/ml) of ascorbic acid, and mineralization was evaluated by alizarin red staining. Temporal change in gene expression was analyzed real-time PCR. Acute effects of increased Pi were also examined by incubating cells in the presence of 1, 4, or 7 mM Pi for 48 hours.

Results

After 8 weeks of culture in the presence of 3 mM Pi, both Pit2-KO and control cells were mineralized. However, the expression of Pit1, osteopontin and Fgf23 was increased in control cells but not in Pit2-KO cells during the culture. As to the acute effects of Pi, 48-hour treatment with 7 mM Pi increased the expression of Dmp1 and Fgf2 and reduced that of alkaline phosphatase (Alp) in both Pit2-KO and controls cells.

Discussion

Impaired induction of the expression of Pit1, osteopontin and Fgf23 in Pit2-KO cells cultured in the presence of 3 mM Pi for 8 weeks suggests that the effects of chronic elevation of Pi may be attenuated by reduced Pi uptake. On the other hand, the responsiveness to acute elevation of Pi was retained in Pit2-KO cells, implying the dispensability of Pit2 for Pi sensing.

Disclosure

The authors declared no competing interests.

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**LB6**

Altered bone metabolism in Fanconi anemia results from defective mesenchymal stem cell differentiation

Méloody Mazoit1,2, Jacinthe Julien2, Roth-Visal Ung1, Sylvain Picard2, Sarah-Kim Bisson1,2, Fabrice Mac-Way1,2 & Madeleine Carreau1,2

1 Laval University, Québec, Québec, Canada; 2 Research Center Chat de Québec-Université Laval, Québec, Québec, Canada.

Fanconi anemia (FA) is a rare genetic disease associated with a progressive decline in hematopoietic stem cells leading to bone marrow failure. FA is also characterized by various developmental defects including short stature and skeletal malformations of the upper and lower limbs. Indeed, more than half of children affected with FA have radiol-ray abnormalities with a tendency to early osteoporosis and osteosclerosis. However, the underlying mechanisms leading to bone defects in FA remains elusive.

Objective

We aimed to determine the mechanism leading to altered bone development and metabolism in FA.

Methods

Bone structure, mass and mineral content were evaluated using μCT-scan analyses of tibias from Fanc−/− and wild-type mice. Bone’s resorption activity...
was determined with tartrate-resistant acid phosphatase staining. To evaluate skeletal maturation, alizarin red and Alcian blue double staining were performed on mouse embryos (E15.5 to 19.5 dpc). To assess mesenchymal stem cell differentiation ability, in-vitro cultures and qPCR analysis of bone marrow stromal cells were performed.

Results
Our results show that FuncC−/− mice present a 15% decrease in bone mineral content, reduced cortical thickness and diameter combined with a 15% reduction of the bone marrow area. FuncC−/− mice also present elevated tartrate-resistant acid phosphatase staining as compared to wild-type littermates. In addition, FuncC−/− embryos show abnormal skeletal development indicated by decreased bone length and mineralization. Using in vitro studies, we found that FuncC−/− mesenchymal stem cells (MSC) have reduced osteoblastic differentiation capabilities and engraftment potential in favor of adipogenesis. Accordingly, FA-defective MSC present altered gene expression profiles of differentiation markers.

Conclusion
Together, our results suggest that defective bone metabolism in FA occurs in utero and results from altered MSCs function. These results provide, for the first time, valuable insights into the mechanism involved in FA developmental defects.

Disclosure
The authors declared no competing interests.

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**LB7**

Musculoskeletal system in adolescents with type 1 diabetes

Klara Maratova1, Ondrej Soucek1, Jana Matyskova1, Zdenek Hlayka2, Lenka Petruzalkova1, Barbara Obermannova1, Stepanka Prahova1, Stanislava Kolousova1 & Zdenek Sumnik1

1Department of Pediatrics, 2Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic; 3Department of Probability and Mathematical Statistics, Faculty of Mathematics and Physics, Charles University in Prague, Prague, Czech Republic.

Background
Sarcopenia and osteoporosis are among the late complications of type 1 diabetes (T1D) in adults. Whether and to what extent musculoskeletal impairment is present in childhood and adolescence has yet to be determined. The aim of this study was to assess volumetric bone mineral density (BMD) and dynamic muscle function in adolescents with T1D and to assess the clinical and biochemical predictors of their musculoskeletal system.

Methods
Ninety-five children and adolescents (59 boys and 36 girls, mean age 16.2±1.2 years) with T1D were included in this cross-sectional study. Study participants were divided into two groups according to the duration of the disease (less than 6 years and more than 9 years, respectively). Volumetric BMD of the non-dominant tibia was assessed using peripheral quantitative computed tomography. Dynamic muscle function was evaluated using jumping mechanography. Gender- and height-specific Z-scores were calculated using published reference data. HbA1c was calculated with an ion exchange resin method (Tosoh, Japan). Results

Results
Relative muscle power (Pmax/mass) and force (Fmax/body weight) were significantly decreased in T1D subjects (mean Z-scores −0.4±1.0; P<0.001, and −0.3±1.1; P<0.001, respectively). The duration of T1D negatively affected Pmax/mass (P<0.01) but not Fmax/body weight (P=0.54). Trabecular BMD and the Strength-Strain Index were significantly lower in subjects with T1D (mean Z-scores −0.78±1.3 and −0.49±0.84, respectively, both P<0.001). Cortical BMD was significantly increased when compared to controls (Z-scores 1.2±0.90, P<0.001). No association was observed between the HbA1c and 25-hydroxyvitamin D levels, bone or muscle parameters.

Conclusion
T1D influences the musculoskeletal system in adolescence. Decreased muscle function could contribute to the osteoporosis reported in adult diabetic patients.

Disclosure
The authors declared no competing interests.

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**LB8**

Breech presentation is associated with neonatal and early childhood deficits in bone mass and size

Alex Ireland1, Sarah Crozier2, Alexander Heuzel3, Kate Ward2, Keith Godfrey2,4, Hazel Insick3, S. Cyrus Cooper5,6 & Nicholas Harvey2

1School of Healthcare Science, Manchester Metropolitan University, Manchester, UK; 2MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK; 3Division of Developmental Biology and Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK; 4NIHR Southampton Nutrition Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK; 5National Institute for Health Research (NIHR) Biomedical Research Centre, University of Oxford, Oxford, UK.

Animal studies suggest that fetal movements are key to healthy skeletal development, but evidence in humans is limited. Breech presentation occurs in 3% of term births and is associated with reduced fetal movement and higher incidence of hip dysplasias, but more general effects on bone development have not been explored. Offspring whole body bone outcomes were measured using dual-energy X-ray absorptiometry (DXA) at mean(SD) 6.5 years after birth in 993 individuals (513 male) from the longitudinal Southampton Women’s Survey. Of these, 41 children (20 male) had a breech presentation at birth. To examine whether group differences were evident in later childhood, total body, hip and spine bone outcomes were examined by DXA at 4.1(2.06) years of age in 1015 individuals (524 male) of whom 39 (20 male) had been breech presentation.

Adjusting for maternal parity, social class, smoking, ethnicity, and offspring age at time of scan, infants with breech presentation had 11.0g lower neonatal total body bone mineral content (BMC; 95% CI −16.0g to −5.9g, P<0.001), 18.3 cm2 lower bone area (BA; 95% CI −26.9 cm2 to −10.2 cm2, P<0.001) and 6.7 g/cm2 lower bone mineral density (BMD; 95% CI −10.1 g/cm2 to −3.3 g/cm2, P=0.041). At four years, in similarly adjusted models breech presentation was associated with lower hip BMC (−5.3g, 95% CI −10.3g to −0.4g, P=0.036) and BA (−0.78 cm2, 95%CI −1.40 cm2 to −0.15 cm2, P=0.015) but not BMD (P=0.466); there were no associations between breech presentation and total body or spine bone outcomes. Additional adjustment for gestational age partially attenuated associations between breech presentation and bone outcomes e.g. neonatal BA (−6.7 cm2, 95%CI −14.2 cm2 to 0.3 cm2, P=0.083) and four year hip BA (−0.61 cm2, 95%CI −1.17 cm2 to −0.04 cm2, P=0.035).

These results suggest that breech presentation is associated with lower neonatal whole body BMC, BA and BMD, and with lower hip BMC and BA at 4 years. These associations are in part explained by differences in gestational age. The associations with hip bone parameters at age 4 years correspond to the location of dysplasias found commonly in breech presentation.

Disclosure
The authors declared no competing interests.

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**LB9**

The cellular immune response in children with inflammatory bowel disease may mediate their low bone mineral density: a pilot study

Gareth Pennan1,2 & David Camp2

1University of Sheffield, Sheffield, South Yorkshire, UK; 2Sheffield Children’s Hospital, Sheffield, South Yorkshire, UK.

Background
Children with inflammatory bowel disease (IBD) have reduced bone mineral density (BMD). The aim of this study was to investigate whether changes in patient’s cellular immune response correlate with reductions in BMD.

Methods
Children undergoing lower gastrointestinal endoscopy disease were approached with an aim of recruiting 15 patients newly diagnosed with Crohn’s Disease (CD) and 15 healthy controls. Lymphocytes were isolated from blood and mucosal biopsies, and analysed by flow cytometry including identification of gut primed lymphocytes expressing CD161. The bone turnover markers osteocalcin, Type 1 procollagen amino-terminal propeptide (P1NP) and N-telopeptide of type 1 collagen (NTX) were measured in all participants. In CD patients lumbar BMD was measured by DXA.

Results
About 14 cases were recruited, of which 10 were newly diagnosed with CD. In cases there was a reduction in the percentage of white blood cells that were lymphocytes (P=0.022), with an increase in expression of CD25 by circulating
CD4⁺ lymphocytes (P = 0.019), and αβ⁺ CD4⁺ lymphocytes (P = 0.005). Cases also had a significant reduction in their vBMD (0.29 vs 0.26 g/cm², P = 0.002), significant reductions in P1NP (P = 0.08) and non-significant reductions in both osteocalcin and NTX. There was a positive correlation between numbers of CD4⁺ and αβ⁺ CD4⁺ lymphocytes and vBMD, whilst those cells expression of CD25 was negatively correlated with vBMD.

Conclusion
This is the first study demonstrating reductions in BMD alongside alterations in the cellular immune response in children with IDA, with uncoupling of bone metabolism. The small patient cohort potentially explains why correlations between the cellular immune response and BMD were not statistically significant.

However, that the numbers of circulating lymphocytes and their activation status have potentially opposing influences makes further investigation of osteoimmune interactions in paediatric IDA warranted.

Disclosure
The authors declared no competing interests.

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**LB10**

Effects of long-term sedentary behaviour on the cortical bone mass and distribution during growth: THE HAPPY bone study

Rachel L. Duckham¹, Timo Rantalainen¹, Christine Rodda², Anna Timperio¹, Nicola Howley¹ & Kylie Hesketh¹

¹Institute of Physical Activity and Nutrition, Deakin University, Geelong, Australia; ²Institute for Musculoskeletal Sciences (AIMSS), University of Melbourne and Western Hospital, St Albans, Australia; ³Department of Chronic Disease Epidemiology, Yale School of Public Health, Yale University, New Haven, CT, USA.

Introduction
Whilst it is well-established that sedentary behaviour may increase the risk of paediatric obesity, and potentially result in greater bone turnover among the more active children, this may be greater following long-term sedentary behaviour, this may be due to increased activity resulting in greater bone turnover among the more active children.

Purpose
To determine if long-term sedentary behaviour affects accrual of bone mass, structure and strength during childhood.

Methods
A total of 99 (girls = 45) children (mean age 12.2 ± 0.8 years) were categorized into sustained high (n = 43) and low (n = 56) levels of sedentary behaviour (< 100 counts/min) based on at least two objective (Actigraph accelerometer worn for ≥ 4 days) sedentary behaviour measurements assessed at three year intervals from pre-school age to thirteen years. Peripheral quantitative computed tomography (pQCT) was used to assess the total bone area (ToA), cortical density (CoD), cortical area (CoA), marrow density (MaD), polyarticular strain index (SSIP), and total cortical and regional (endo-, mid- and peri-cortical) vBMD at the mid (66%) and distal (21%) radial site. Bone outcomes were compared across groups adjusting for maturity offset and gender using analysis of covariance followed by post hoc pairwise comparisons with Bonferroni adjustments.

Results
There were no significant differences in height, weight or BMI z-scores between groups. At the tibia, when adjusting for maturity offset and gender, children with more sedentary time across childhood had 7.4% lower ToA, 8.0% lower CoA and 9.0% lower SSIP, and 2.0% greater CoD (P < 0.05) compared to their active peers. At the Radius there were no significant differences between groups.

Conclusion
Long-term sedentary behaviour during childhood is associated with decreased total and cortical bone size and strength, which may increase fracture risk at the mid-tibial shaft. Although cortical density at the weight-bearing tibia appears to be greater following long-term sedentary behaviour, this may be due to increased activity resulting in greater bone turnover among the more active children.

Disclosure
The authors declared no competing interests.

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**LB11**

Bone health status as measured by DXA and pQCT in Indian Children with Thalassemia Major

Rubina Mandlik¹, Veena Ekbote², Vijay Ramanan³, Ketki Kelkar³, Jwala Pawar¹, Shashi Chhiponkar¹, Vaman Khadilkar⁴, Anuradha Khadilkar³, Raja Padidela¹ & Zulf Maghul³

¹Hirabai Cowasji Jehangir Medical Research Institute, Pune, Maharashtra, India; ²Royal Manchester Children’s Hospital, Manchester, UK; ³Yashoda Hematology Clinic, Pune, Maharashtra, India.

Objective
Expansion of bone marrow, accumulation of iron, growth failure and delayed puberty affect bone health in thalassemia; data on bone density and geometry are scarce. Our objective was to assess bone density (by Dual Energy X-ray Absorptiometry, DXA) and geometry (by peripheral quantitative computed tomography, pQCT) in children with thalassemia major.

Methods
Children with thalassemia were recruited from a hematology clinic in Pune (India) (Mean age 9.8 ± 3.0 y). History, anthropometry, haemoglobin concentrations, serum ferritin, and lumbar spine and total body bone density (GE Lunar iDXA, Madison, WI) were performed in 71 patients (boys: 38, age 5–18 y.)

Puberty (tanner staging) was classified as pre-pubertal (tanner stage = 1) and entered puberty (tanner stage ≥ 2). pQCT (STRATEC XCT-2000) of the radius of the non-dominant hand at 4% and 66% were performed (n = 21), z-scores were computed (manufacturers data).

Results
Mean height and weight Z-scores were −1.9 ± 1.1, −1.6 ± 1.0 respectively. Mean haemoglobin was 7.0 ± 1.6 g/dl and ferritin was 3005.3 ± 2183.4 ng/ml (range: 14–17.5 g/dl, 11–307 ng/ml respectively) suggesting very poor chelation.

Of the 71 patients, 18(26%) had history of low-impact fractures. LS BMAD and TBLH BMD Z-scores (Crabtree,2016) were −1.6 ± 2.3 (40% < −2) and −1.6 ± 1.4 (34% < −2) respectively. Patients with fractures had significantly (p < 0.05) lower TBLH BMD than patients without fractures (−2.3 ± 1.4, −1.3 ± 1.4 respectively).

At 4% site the mean distal radial trabecular density for age Z-scores were 1.8 ± 1.5 (none below −2), total density for age Z-score was 0.3 ± 1.4 (10% below −2).

The cortical density for age and strength strain index (SSIPol3) for age Z-scores at 66% radial site were −0.8 ± 1.4 (21% below −2) and −1.7 ± 0.7 (37% below −2) respectively.

Mean cortical thickness at 66% radius was 1.3 ± 0.3 mm, the mean periosteal and endosteal thickness was 34.6 ± 3.0 mm (pre-pubertal: 33.8 ± 2.7 mm, entered puberty: 35.7 ± 3.3 mm, NS) and 26.6 ± 3.3 mm (pre-pubertal: 26.4 ± 3.8 mm, entered puberty: 26.8 ± 2.8 mm, NS) respectively.

Conclusion
In poorly controlled short Indian thalassemia major patients, bone health was affected as judged by history of fractures, low lumbar spine and total body bone density, cortical density, strength strain index and poor bone accrual during puberty.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.LB11

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**LB12**

Correlation of the first fracture time and COL1A1/2 mutations in patients with Osteogenesis Imperfecta

Lidia Zhytnik1, Katre Maasalu1,2, Binh Ho Duy1,4, Ene Reimann3, Ele Prans1, Sulev Kõks3 & Aare Märtson1,2

¹Department of Traumatology and Orthopaedics, Tartu University, Tartu, Estonia; ²Clinic of Traumatology and Orthopaedics, Tartu University Hospital, Tartu, Estonia; ³Department of Pathophysiology, University of Tartu, Tartu, Estonia; ⁴Hue University of Medicine and Pharmacy, Hue University, Hue, Viet Nam.

Osteogenesis Imperfecta (OI) is a brittle bone disease, characterized with reduced bone mass and bone fragility of different severity due to collagen type I defects. Patients suffer from recurrent spontaneous fractures starting in the early childhood or before birth.

The main aim of the study was to relate time of the first fracture with COL1A1/2 mutations in patients with Osteogenesis Imperfecta.

Patients and methods
Total number of 167 unrelated OI patients from the Osteogenesis Imperfecta database of Tartu University Department of Traumatology and Orthopedics, were included in the study. Data of OI genotypes was recruited from previous Sanger sequencing mutational analysis of the COL1A1/2 genes. Time of the first fracture was recorded from patients’ words and divided into categories: intrauterine, perinatal, 0-6 and ≥ 7 years old. Significance of correlations
between time of the first fracture and COL1A2 mutations was tested with Fisher’s test. Among 167 analysed patients, 29 (17.37%) had intratable fractures, 21 (12.57%) had perinatal fractures, 106 (63.47%) had fractures at the age 0–6 years old, 8 patients (4.79%) at ≥7 years old, and 3 (1.80%) patients had no fractures. Number of patients harbouring collagen I mutations was 103 (61.68%). The statistically significant correlation between the first fracture time and COL1A1/2 mutations was highlighted during the study (P=0.0288). Exploring of the relation between type of the COL1A1/2 mutations and time of the first fracture, showed connection between quality collagen mutations and earlier time of the first fracture (P=0.0414). Intratable fractures did not reveal correlation with presence of the COL1A1/2 mutations (P=0.8339), however showed correlation with collagen I quality mutations (P=0.0270). Perinatal fractures, represented mostly with fractures happened during delivery, did not reveal correlations with presence (P=0.8180) or type (P=1) of the COL1A1/2 mutations. Our results show general correlation between time of the first fracture and presence of the COL1A1/2 mutations. Quality collagen mutations are related to the earlier time of the first fracture. Despite the clear genetic background of bone fragility phenotype in OI patients, minor variations in time of the first fracture in OI patients might come from additional non-genetic factors.

Disclosure
The authors declared no competing interests.

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**LB13**
Low dose of intravenous pamidronate therapy in quadriplegic children with osteoporosis
Soonjung Moon, Sookni Kim, Youngse Kwon & Jieun Lee
Pediatrics, School of medicine, Inha University, Incheon, Republic of Korea.

Objectives
Quadriplegic children are more susceptible to osteoporosis because of various risk factors for the inhibition of bone metabolism. The importance of bone metabolism is being emphasized on extending life span of the patients but research on this area is insufficient. Intravenous (IV) pamidronate is well known as an effective treatment for pediatric osteoporosis, but there are no treatment guidelines for accurate dose capacity and duration in quadriplegic children with osteoporosis. We aimed to evaluate the efficacy of low doses of IV pamidronate in those patients.

Methods
Nine quadriplegic patients (6 male, 3 female; mean age 10.6±6.0) who were taking antiepileptic drug in one institution were treated with pamidronate (0.25–1.0 mg/kg/day) for 8–12 weeks. The patients were receiving calcium and vitamin D before the treatment. BMD Z-score, blood and urine biochemical markers of bone metabolism were measured periodically before and during treatment.

Results
All patients were quadriplegic state graded at Level V using gross motor function classification system. The main underlying disease was perinatal hypoxic brain damage (44.4%, 4/9). The mean cumulative dose of IV pamidronate was 3.39±1.08 mg/kg/year, and the mean treatment period was 12.0±6.36 months. There was significant increase in BMD Z-score of the lumbar spine after the treatment. (from −4.01±1.26 to −2.35±1.53, P=0.018). Urine NTX (cross-linked N-terminal telopeptide of type 1 collagen) and alkaline phosphatase were significantly decreased during treatment (P<0.05). 55.5% (5/9) of them experienced a fracture before treatment, but no fracture occurred after treatment. No significant adverse effects were observed.

Conclusion
Low dose of IV pamidronate therapy improved BMD of lumbar spine and biochemical markers of bone metabolism in quadriplegic children with osteoporosis. Appropriate guidelines including the optimal dose and duration are required for it.

Disclosure
The authors declared no competing interests.

DOI: 10.1530/boneabs.6.LB13

**LB14**
P4HB recurrent missense mutation causing Cole-Carpenter syndrome: exploring the underlying mechanism
Meena Balasubramanian1,2, Raja Padidela2, Rebecca Pollitt1,3, Nick Bishop1, Zulf Mughal1, Amaka Offiah1, Bart Wagner2, Janine McAughley3 & David Stephens1
1Sheffield Children’s NHS Foundation Trust, Sheffield, UK; 2Royal Manchester Children’s Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK; 3Academic Unit of Child Health, University of Sheffield, Sheffield, UK; 4Royal Hallamshire Hospital, Sheffield, UK; 5School of Biochemistry, University of Bristol, Bristol, UK.

Cole-Carpenter syndrome (CCS) is commonly classified as a rare Osteogenesis Imperfecta disorder. This was following the description of two unrelated patients with very similar phenotypes who were subsequently shown to have a heterozygous missense mutation in P4HB. Here, we report a 3-year-old female patient who was diagnosed with a severe form of OI. Exome sequencing identified the same missense mutation in P4HB as reported in the original cohort, thus reinforcing a recurrent missense mutation in P4HB as the underlying aetiology of Cole-Carpenter syndrome.

We discuss this patient with particular emphasis on the phenotype and similarities with the previously reported patients with CCS. The clinical phenotype appears consistent in patients reported so far but interestingly, there also appears to be a definitive phenotypic clue (crumpling metaphysics fractures of the long tubular bones with metaphyseal sclerosis which are findings that are uncommon in OI) to the underlying genotype (P4HB variant). P4HB (Prolyl 4-hydroxylase, beta subunit) encodes for PDI (Protein Disulfide isomerase A1) and in cells, in its tetrameric form, catalyses formation of 4-hydroxyproline in collagen. The recurrent variant in P4HB, c.1178A>G, p.Tyr393Cys, sits in the C-terminal reactive centre and is said to interfere with disulphide isomerase function of the C-terminal reactive centre.

P4HB catalyses the hydroxylation of proline residues within the X-Pro-Gly repeats in the procollagen helical domain. Initial experiments on patient fibroblasts showed no major difference in extracellular collagen type I deposition as judged by immunofluorescence. While there was a minor trend towards an increase in extracellular collagen type I compared to control, this was not statistically significant. Given the inter-dependence of ECM components in assembly of a functional matrix, our data suggest that it is the organisation and assembly of the functional ECM that is perturbed rather than the secretion of collagen type I per se. This will require further functional analysis of whole ECM, which is ongoing.

We discuss the genetic heterogeneity of Cole-Carpenter syndrome as originally described and the underlying mechanism of P4HB in collagen production and how this recurrent variant causes a rare form of osteogenesis imperfecta.

Disclosure
The authors declared no competing interests.

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**LB15**
Consensus paper – Physiotherapy in children with OI
Oliver Semler1, Brigitte Mueller1 & Dagmar Mekking2
1University Cologne, Cologne, Germany; 2Foundation Care4BrittleBones, The Hague, The Netherlands.

Physiotherapy is one of the most important therapeutic approaches in Osteogenesis imperfecta (OI) besides medical and surgical treatment. At the moment there are no guidelines and no consensus about appropriate physiotherapeutic concepts for children with OI. In each country different preferences regarding the therapeutic approaches (neuro-developmental techniques, active and passive training, treadmill training, pool therapy etc) are used. There are hardly any scientific research projects performed to investigate different therapeutic approaches regarding their effectiveness and safety in OI.

The aim of this project is to develop a consensus paper for physiotherapeutic approaches globally for OI-children.

The project will consist of different parts:
1) Preparatory work involving screening of relevant literature, identifying physiotherapists who are experts in the field of OI worldwide and collecting physiotherapeutic approaches and techniques used in different countries. Care4BrittleBones will have an active role to provide input from an OI Community perspective.
2) Forming a consensus group of experienced physiotherapists from different countries, which will prepare a consensus statement with “recommendations for physical therapy in children with OI”. The experts will attend a consensus conference prior to the “13th International Conference on Osteogenesis
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Imperfecta 2017” in August. The resulting consensus paper, which will be submitted to international journals.

3) When the guideline has been developed it will be actively promoted to be used (communication, networking with OI Community Groups and the professional OI-community, supporting materials) for maximum positive impact on the quality of life of people with OI.

Expected outcome:
In the short term there is an expectation that the consensus statement will provide help for patients and local physiotherapists working with OI children irrespective if they are living in an area without many other people with OI. It makes access to best practices easy and therefore should make a positive impact on quality of life of people with OI.

In the long term this consensus-statement should encourage physicians, physiotherapists and researchers to initiate clinical trials investigating ad comparing different therapeutic approaches to find the most effective way to train mobility and increase independency of children with OI.

Disclosure
The authors declared no competing interests.

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LB16
Implementing an osteoporosis disease management program: what works and what doesn’t work
Kathy Williams
Kaiser Permanente, Pasadena CA, USA.

Objectives
To identify and determine the extent to which effective/ineffective steps in the implementation of the Kaiser Permanente Southern California Healthy Bones Model of Care were perceived by physician champions and Healthy Bones Care Managers.

Methods
The subjects in the study included 20 Physician Champions and 35 Healthy Bones Care Managers employed in the Kaiser Permanente Southern California Healthy Bones Model of Care.25 have been employed in their current role since the implementation of the program. Of those, 16 agreed to participate. The instrument for interviewing was an email interview.

Results
Each participant was asked to respond to a set of nine standard questions. Examination of qualitative data resulted in eight major findings. As a result ten best practices for creating change efforts when implementing Disease Management Programs emerged.
1. Relentlessly informing, advocating, and networking.
2. Balancing the merits of consistency gained by centralized control, with the merits of creativity and innovation, guided by autonomous flexibility.
3. Creating strong multi-disciplinary champions.
4. Providing hands-on monitoring and management of change.
5. Creating inclusive feedback systems.
6. Leveraging external forces and available data to support change.
7. Rewarding meritorious or noteworthy behaviors, innovations, and ideas.
8. Personalizing interactions with potential change agents.
9. Providing adequate resources and administrative support.

Conclusions
This study utilized e-mail-based interviews to assess perceptions of the participants who were involved in the implementation of the Healthy Bones Program. These steps will greatly increase the likelihood of success and long-lasting sustainability of a Disease Management Program. The results of the study also support effective guides for healthcare reform initiatives at the national, corporate, and medical center levels. Proponents of improvements to any healthcare system can use recommendations from this study to remove obstacles and barriers to change and foster supportive participation from involved health care professionals.

Disclosure
The authors declared no competing interests.

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LB17
Bone health among boys with duchenne muscular dystrophy after initiation of glucocorticoids
Joanna Yueung Tung & Sophelia Hoi-shan Chan
Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong, Hong Kong.

Objectives
Poor bone health in boys with Duchenne Muscular Dystrophy (DMD) due to muscle weakness and glucocorticoid treatment is a major concern. This study assesses the bone health status in DMD boys with glucocorticoid treatment in a single centre.

Methods
A retrospective chart review from January 2009 to January 2017 was undertaken on all DMD cases with active follow-up in a single paediatric unit in a University-affiliated hospital. We assessed their vitamin D (25, OH) levels, areal bone mineral density (BMD) with DXA total body less head (TBLH) and lumbar spine (LS), and fracture frequency.

Results
Fifteen DMD boys were included (mean age 7.0 years, range: 3.9–12.9 years). Before glucocorticoid initiation, thirteen were at their good ambulatory state, one was at late ambulatory and one was at non-ambulatory state. None reported any fracture. All had BMD assessment with DXA. Mean Z-scores of LS (n=11) and TBLH (n=6) were −1.2±0.8 and −2.1±1.8 SD respectively. Six (40%) had low BMD for age (Z-score ≤ −2.0 SD) and it was not associated with age (P=0.71) or vitamin D level (P=0.62). The mean vitamin D (25, OH) levels was 38.7±12.1 nmol/L. Twelve (80%) had vitamin D insufficiency (<50 nmol/L). All were started on prednisolone at 0.5±0.1 mg/kg/day. At a mean follow-up of 2.9±1.4 years, eleven were still at their good ambulatory state, one was at late ambulatory and three were at non-ambulatory state. None reported any long bone fracture but one developed grade 3 vertebral fractures. DXA was repeated in nine and seven had low BMD for age. Two showed improved BMD Z-score at LS despite steroid treatment.

Conclusions
Vitamin D insufficiency and low BMD are common among DMD boys even before initiation of glucocorticoids. The use of long-term steroid further compromises their bone health status. The low baseline BMD noted at very young age and the difference observed in LS and TBLH Z-scores raised concern of the interpretation complexity of the DXA findings. A better bone imaging measure is needed for this group of children.

Disclosure
The authors declared no competing interests.

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LB18
Occurrence of vitamin D and vitamin K deficiency in children with low-energy fractures
Michal Karpinski1, Sylwia Chojnowska2, Katarzyna Maresz3, Robert Milewski1, Janusz Popko2 & Vladimir Badmaev4
1Department of Pediatric Orthopedics and Traumatology Medical University of Białystok, Białystok, Poland; 2Medical Institute Łomza State University of Applied Sciences, Łomza, Poland; 3International Science & Health Foundation, Krakow, Poland; 4American Medical Holdings Inc, New York, USA.

Objective
Bone fractures are very common in children and their number is growing every year. Vitamin D has a proven role in the prevention of fractures. In the recent study, we have shown that children with low-energy fractures have significantly lower vitamin D blood levels compared to the children without fractures. Our data indicate that higher levels of vitamin D reduced the risk of fracture by 1.06 times (P<0.0005).

Past decade has seen increased interest in the role of vitamin K, especially K2 menaquinone-7, in bone health and prevention of bone fractures. There is a scarcity of research examining the effects of vitamin K deficiency on bone health in children and adolescent populations. The aim of the current study was to evaluate the vitamin D and K status in healthy children with low-energy fractures and in the control group without fractures.

Methods
The study group of 20 children aged 5 to 15 years old, with clinically confirmed low-energy fractures was compared with the control group of 19 healthy children, aged 7 to 17 years old, without fractures. Total vitamin D [25(OH)D3 plus 25(OH)D2], calcium, BALP (bone alkaline phosphatase), NTx (N-terminal telopeptide) and undercarboxylated (ucOC) and carboxylated osteocalcin (cOC)
selenium concentrations were evaluated in every patient. Ratio of serum under-carboxylated osteocalcin to serum carboxylated osteocalcin ucOC:OC - UCR - was used as an indicator of vitamin K status. Logistic regression models were created to establish UCR influence for odds ratio of low-energy fractures in both groups.

Results

There were no statistically significant differences in the serum calcium, NTx, BALP or vitamin D levels between the groups, however the statistically significant difference in the UCR was observed. The median UCR in the fracture group was 0.4709 compared with the control group value of 0.2445 (P<0.0000004). In the logistic regression analysis, the odds ratio of the low-energy fractures for the UCR was calculated, with the increased risk of fractures by 9.62 times (P<0.003).

Conclusions

In this small sample study, the better vitamin K status expressed as the ratio of ucOC:OC - UCR - have positively and statistically significantly correlated with lower rate of low-energy fracture incidence. 

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.LB18

Selected risk factors of fractures in children -own observation

Elzbieta Jakuchowksa-Piekiewicz1, Wojciech Młynarski1, Danuta Chlebna-Sokoł1 & Paweł Matsik2

1Medical University of Lodz, Lodz, Poland; 2Medical University of Silesia, Katowice, Poland.

Bone fractures may depend on Vitamin D Receptor Gene (VDR), bone mineral density, bone turnover markers. Patients and methods About 161 patients were recruited and underwent: skeletal densitometry (DXA) method and bone turnover studies (Osteocalcin and NTx). The study group was evaluated using restriction enzyme digestion at BsmI (rs2228570), FokI (rs7975236), ApaI (rs7041732) and TaqI (rs731236), polymorphic sites of the VDR gene. Multivariate logistic regression was used to assess factor significance. The model included variables with sex- and age-standardized parameters, VDR genotypes, and bone metabolism marker levels.

Results

Factors associated with fractures were: osteocalcin concentration and Z-score BMDt. Odds Ratio (OR) values equaled: 1.01 (95%CI 0.95–1.07) for osteocalcin (P = 0.006), and 0.66 (95%CI 0.42–1.03; P = 0.07) for Z-score BMDt. In patients with reduced bone mass, factors related to fractures were: osteocalcin (0.04) and carriage of BsmI b (0.07) or ApaI alleles (0.08). ORs were 1.01 (95%CI 1.00–1.02) for OC, 0.29 (95%CI 0.07–1.14) for BmI, and 2.13 (95%CI 0.91–4.99) for ApaI polymorphic allele carriage.

Conclusions

1. Carriage of BsmI b allele reduces, while carriage of ApaI a allele and heightened osteocalcin level increase the risk of fractures in study children with reduced bone mass.
2. VDR polymorphism, bone mineral density and bone formation’s marker – osteocalcin maybe considered as risk factor for fracture in children from Central Poland.

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.LB19

COL2A1 c.1609G> A (p.Gly537Ser) a pathogenic variant causing multiple skeletal abnormalities and severe short stature

Elpis Vlachopapadopoulou1, Irene Dikaiakou1, Emmanouil Manolakos2, Ioannis Panagiotopoulos1 & Stefanos Michalacos1

1Children’s Hospital P.A. Kyriakoy, Athens, Greece; 2Access To Genome Clinical Laboratory, Athens, Greece.

Background

Skeletal dysplasias include many pathological conditions that involve bone metabolism and health and most of them are associated with short stature. 211 genes are associated with bone dysplasia and short stature.

Presenting problem

To present a boy with severe short stature and skeletal abnormalities. He was born at term AGA. Growth failure was noted from the age of 8 months. RGE-I levels were low and he was tested for growth hormone deficiency (GHD). GHD was diagnosed, so he was treated with GH. He had poor response and GH therapy was discontinued. On physical examination a significant lordosis was appreciated and bone x-rays revealed flattening of the pelvic bones and flattening and deformities of the vertebral. Family history was significant for short stature of the father (150 cms) with bone deformities.

Methods

NOS exome study was carried out on DNA obtained from peripheral blood, in order to identify genomic variants in 211 genes associated with bone dysplasia and short stature. The panel used for the preparation of the library has been designed by SureSelectXT Human All Exon V5 (Agilent Technologies), it captures (> 19000 genes, > 35000 exons, > 85% of the alterations responsible for genetic diseases) and the splicing flanking (5 bp) regions, its size is ~50Mb. Sequencing was performed with the HiSeq2000 SystemTM (llumina) sequencer. The reads obtained were filtered, based on quality parameters, and aligned to the reference genome (build 37 of genome Hg19), using the BWA (version 0.7.12) alignment program.

Results

Genetic variants identified in this study are listed in the following table:

<table>
<thead>
<tr>
<th>Genomic position and gene</th>
<th>Gene/Name</th>
<th>Aminoacid Change</th>
<th>Nucleotide Change</th>
<th>Genomic Transcript</th>
<th>Exon</th>
<th>Effect</th>
<th>Zygosity(2)</th>
<th>Variant Freq. (3)</th>
<th>Categorization of the variant(6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr12:46273982CT</td>
<td>COL2A1</td>
<td>NM_001844.4</td>
<td>c.1609G&gt;A</td>
<td>p.Gly537Ser</td>
<td>6</td>
<td>Missense</td>
<td>Het 42%</td>
<td>Pathogenic</td>
<td></td>
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</table>

Segregation studies in order to determine the inheritance pattern of the identified variant were done and the same variant was identified at the father.

Conclusions

The presence of mutations in COL2A1 has been associated, with an autosomal dominant inheritance pattern, with different chondrodysplasias and spondyloepiphyseal dysplasia. The variant COL2A1 c.1609G>A (p.Gly537Ser) identified in heterozygosity in the patient, is considered a pathogenic variant and has been previously registered in HGMD associated to spondyloepiphyseal dysplasia (accession:CM052184) [1].

Disclosure

The authors declared no competing interests.

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Morbid obesity and respiratory failure in a child with pseudohypoparathyroidism type 1A

Moran Gal1, Ifat Sarouk1 & Yael Levy-Shraga1

1Edmond and Lily Safra Children’s Hospital, Sheba Medical Center, Tel-Hashomer, Ramat Gan, Israel; 2Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Background

Pseudohypoparathyroidism type 1A (PHP1A) is a rare genetic disorder caused by mutations in the gene GNAS. It is characterized by multi-hormone resistance, obesity, cognitive impairment and the Albright hereditary osteodystrophy phenotype. A recent study found a 4.4-fold increase risk of sleep apnea in children with PHP1A compared with similarly obese children.

Objective

To describe a case of morbid obesity and respiratory failure in a child with PHP1A.

Presenting problem

A 4-year-old boy was admitted to the Emergency department due to respiratory failure and somnolence. He was diagnosed during infancy with PHP1A and was treated with levotiroxine, calcium carbonate, alfalcacidol and vitamin D. He also had history of asthma and night-time snoring and was treated with inhaled therapy as needed.

Couple of days prior to the admission, he had cough without fever. At arrival to the Emergency department, his temperature was 36.5°C, blood pressure 98/65 mmHg, saturation of 82% and his weight was 40.5 kg (5.3 SDS). On physical examination, the patient appears exhausted and drowsy, with poor respiratory effort, and minimal breath sounds. Venous blood gases revealed a metabolic acidosis with a pH of 7.35 and a bicarbonate of 22 mmol/L. 

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pH-7.031, pCO2-100 mmHg, pO2-30 mmHg, HCO3-26.1 mmol/l BE-7.3 mmol/l. He had marked leukocytosis of 35,560/microl and C-reactive protein of 40.1 mg/l (normal range of 0.08–5).

Clinical management
He was treated with 100% oxygen mask, albuterol, ipratropium and budesonide inhalations, IV methylprednisolone, ceftriaxone, furosemide and magnesium. Due to poor response to treatment he received continues terbutaline infusion and high flow nasal canulla (HFNC) with improvement.

Conclusion
Asthma and sleep apnea may be severe complications of obese PHP1A patients. Early detection and intervention could improve health outcome of this vulnerable population.

Disclosure
The authors declared no competing interests.

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