Bone Abstracts

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10–13 June 2017, Würzburg, Germany

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Abstract book

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10-13 June 2017
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Best Oral Communication Award

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Michela Rossi (Rome, Italy), P034

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)
2013 Maria Luisa Bianchi (Milan, Italy)
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2007 Ailsa Goulding (Dunedin, New Zealand)
2005 Jean-Philippe Bonjour (Switzerland, Geneva)
2002 John Pettifor (Johannesburg, South Africa)
1999 Francis Glorieux (Montreal, Canada)

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ICCBH 2017

10–13 June 2017

Invited Speaker Abstracts and Biographical Notes

IS01

IS01

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

IS02

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.

DOI: 10.1530/boneabs.6.IS02

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.

IS03

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, osteocyte 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.

Fondazione Cariplo 2013-0612, Telethon GGP13098 and the European Community n. 602300, Care4brittlebones Project 0003, 2014.

Disclosure

The authors declared no competing interests.

DOI: 10.1530/boneabs.6.IS03

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.Reri*o facility to generate zebrafish models of skeletal dysplasias and to start drug screening approach.

IS04

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.

IS05

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 P_i generated intravesicularly by the action of PHOSPHO1 on phosphocholine derived from sphingomyelin by the action of SMPD3, and also via the P_i transporter P_iT-1-mediated incorporation of extracellular P_i 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 PP_i/P_i 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.

DOI: 10.1530/boneabs.6.IS05

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.

IS06

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, allow 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 acts 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 has 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.

DOI: 10.1530/boneabs.6.IS06

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.

IS07

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.

IS08

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-Universitaetsmedizin 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).

IS09

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.

DOI: 10.1530/boneabs.6.IS09

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.

IS10

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 Calcinosis (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 defets 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.

IS11

IS11

Hans van Leeuwen

Biographical details

Hans van Leeuwen

Hans (J.P.T.M.) 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.
- Council member of the American Society for Bone and Mineral Research2015 – present.
- 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.

DOI: 10.1530/boneabs.6.IS11

IS12

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).

IS13

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 histormophometric 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 heath 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.

IS14

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 exceeds 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.

IS15

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 nephrotic syndrome, genetic forms of renal tubular dysfunction, and a host of other diseases affecting the glomerulus and/or the tubule-interstiitum 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

Honoraria for self-created lectures, Alexion. University receives funding for registries. Alexion Consultant. Dicerna.

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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.

IS16

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.

IS17

Factors influencing peak bone mass

Nicholas C Harvey[1,2]

[1]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK; [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.

IS18

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).

IS19

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.

IS21

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.

IS22

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 & Sevgi 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

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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
Trio whole exome sequencing in patients with OI identified, in two patients, compound heterozygous mutations in *NBAS* (*Neuroblastoma amplified sequence*). Patient 1: c.5741G>A p.(Arg1914His); c.3010C>T p.(Arg1004*) 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>A 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 shows 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 nonsense 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 1B (PHP1B or iPPSD3) subtypes

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PHP1B -iPPSD3 per the new proposed classification- is a rare disorder characterized in most patients by proximal tubular resistance to PTH resulting in hypocalcemia, hyperphosphatemia 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 (del*STX16*+) 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 del*STX16*+ ($n=9$) and del*STX16*− ($n=5$) patients; furthermore, sporPHP1B ($n=10$) and healthy controls ($n=10$) were investigated. *STX16* and *GNAS* deletions were excluded in the del*STX16*− patients by MLPA, genomic multiplex and quantitative PCR of the *GNAS* and *STX16* regions.
Results:
1- The mean methylation index at the GNAS-AS2-DMR was significantly higher in del*STX16*− patients (32±14%) than in controls (24±6%), del*STX16*+(5±2%) and sporPHP1B patients (3+1%) ($P<0.0001$).
2- Bisulfite-treated DNA of PHP1B patients with del*STX16*− 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 del*STX16*− 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 del*STX16*+ 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. Our findings reinforce the hypothesis that del*STX16*− patients carry a defect in an element that controls the methylation both at the GNAS-A/B DMR and 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

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Objectives
To determine 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 components analysis, applied to the four Z-scores, generated new integrated aBMD phenotypes. Linear mixed effects models, adjusted for age, Tanner, BMI-Z, dietary 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, $P=3.9\times10^{-10}$); from the GWAS, rs114260199 (*LMO2/CAPRIN1*, $P=3.9\times10^{-8}$) and rs75321045 (*ZMAT4*, $P=2.5\times10^{-8}$, 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*, $P=2.5\times10^{-11}$) 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 rs58649746 (*RAB11FIP5*, $P=4.8\times10^{-9}$, 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.

Conclusion
We identified four integrated pediatric aBMD phenotypes, including non-site-specific (PC1), distal radius-specific (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/CAPRIN1*, *ZMAT4,* and *RAB11FIP5* associated with non-site specific or spine specific phenotypes. These results highlight the utility of an integrated skeletal site phenotyping approach, which may help identify additional genetic loci associated with skeletal development.
Disclosure
The authors declared no competing interests.
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OC4

25-hydroxyvitamin D response to antenatal cholecalciferol supplementation is associated with common vitamin D related genetic variants: findings from the MAVIDOS trial
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Objectives
Single nucleotide polymorphisms (SNP) in genes related to vitamin D metabolism have been associated with 25-hydroxyvitamin D (25(OH)D) status, but these relationships have not been examined in pregnancy or following antenatal vitamin D supplementation. We assessed whether SNPs in *DHCR7* (7-dehydrocholesterol reductase), *CYP2R1* (25-hydroxylase), *CYP24A1* (24-hydroxylase) and *GC* (Vitamin D binding protein) were associated with the response to antenatal vitamin D supplementation.
Methods
MAVIDOS is a randomised double-blind placebo-controlled trial of 1000IU/day cholecalciferol from 14 weeks gestation until delivery in women with a baseline 25(OH)D of 25–100 nmol/l. Anthropometry, serum 25(OH)D (Diasorin Liaison), health and diet were assessed at 14 and 34 weeks gestation. Genotyping of rs12785878 (*DHCR7*), rs10741657 (*CYP2R1*), rs6013897 (*CYP24A1*) and rs2282679 (*GC*) was undertaken by LGC Genomics (Hoddeston, UK) using KASP™ competitive allele-specific PCR. Multiple linear regression was performed using an additive model with the homozygous minor allele as the reference group (beta represents the change in outcome per additional major allele), adjusting for a number of previously identified determinants of 25(OH)D.
Results
712 women (367 placebo, 345 cholecalciferol) were included (95.8% White ethnicity). Only rs12785878 (*DHCR7*) was associated with baseline 25(OH)D ($\beta = 4.1$ nmol/l (95% CI 2.2, 6.1), $P < 0.001$). Conversely, rs10741657 (*CYP2R1*) ($\beta = -4.1$ nmol/l (95%CI −7.1, −1.2), $P = 0.006$) and rs2282679 (*GC*) ($\beta = 4.4$ nmol/l (95%CI 1.2, 7.6), $P = 0.007$) were associated with achieved 25(OH)D after supplementation, but rs12785878 and rs6013897 were not.
Conclusion
Genetic variation in *DHCR7,* which encodes 7-dehyrocholesterol reductase in the cholesterol/vitamin D biosynthesis pathway in the skin appears to modify baseline 25(OH)D, whereas the response to antenatal cholecalciferol supplementation was associated with SNPs in *CYP2R1* and *GC*, which may alter 25-hydroxylase activity and vitamin D binding protein synthesis. Women with more risk alleles may require higher supplement doses to achieve vitamin D repletion in pregnancy.
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
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Objectives
Conventional wisdom suggests that bone is most responsive to physical activity during the growing the 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 (CTh), and tibia torsion strength (pSSI). Physical activity was assessed using the self-report PAQ-A and Actigraph accelerometry (hip worn) analyzed with the Evenson 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 variable (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 ($n = 431$, 215 females, 1582 total records).
Results
PAQ-A was positively associated ($P < 0.05$) with all bone strength measures for males and females with the exception of pSSI for females. VPA was positively associated ($P < 0.05$) with BSI, Z, and CSA in males and BSI, pSSI, 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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Disclosure
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OC6

Lean mass accretion increases during summer and positively associates with vitamin D status in healthy children 2-8 years
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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, NCT02387892) 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 ± 0.60. Vitamin D intake (222 ± 89 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: −12.0 ± 5.5°). 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 ($r^2 = 0.60$, $P = 0.01$). The summer % change 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 ($r^2 = 0.66$).

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

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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 often 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 delivery in mothers and infants (cord blood), and measured haemoglobin concentration and plasma markers of iron status (ferritin, hepcidin), inflammation (C-reactive protein), bone metabolism (FGF23; parathyroid hormone; 25-hydroxyvitamin D [25OHD], total alkaline phosphatase, phosphate) and renal function (cystatin C). For FGF23, we used assays that measured either its intact form (I-FGF23), or both intact FGF23 together with its C-terminal fragment (C-FGF23).

Results

Iron supplementation improved maternal iron status (as seen by effects on haemoglobin and ferritin concentrations), and increased ferritin concentrations in infants (medians: 130 μg/l vs 108 μg/l, $P = 0.008$). In addition, antenatal iron supplementation led to reduced C-FGF23 concentrations in mothers (medians: 105 relative units (RU)/ml vs 399 RU/ml, $P = 0.0001$) and infants (means: 491 RU/ml vs 570 RU/ml, $P = 0.05$); increased I-FGF23 in infants (medians: 7.3 μg/l vs 5.8 μg/l, $P = 0.04$); and reduced concentrations of 25OHD in mothers (means: 93 nmol/l vs 100 nmol/l, $P = 0.01$). There were no evident effects on I-FGF23 in mothers, 25OHD in infants, or other markers.

Conclusions

These findings suggest that iron may play a role on maternal and infant skeletal health through its complex effects on FGF23 expression and downstream catabolism, and its effect on maternal vitamin D metabolism. Mechanisms for these actions require further investigation.

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Disclosure

The authors declared no competing interests.

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OC8

Effective therapeutic control of curve progression using calcium and vitamin D supplementation for adolescent idiopathic scoliosis – a randomized double-blinded placebo-controlled trial

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Objectives

Adolescent Idiopathic Scoliosis (AIS) is associated with low bone mass. This study aimed at evaluating the therapeutic effect and its determinants of Ca + Vit-D supplementation in improving bone strength and preventing curve progression in AIS.

Methods

This was a randomized double-blinded placebo-controlled trial recruiting AIS girls (11–14 years old, Tanner stage < IV) with femoral neck BMDZ-scores < 0 and Cobb angle ≥ 15°. 330 subjects were randomized to Group 1 (placebo), Group 2 (600 mgCalcium + 400 IUVit-D_3/day) or Group 3 (600 mgCalcium + 800 IUVit-D_3/day) for 2-year treatment. Investigations at baseline and 2-year included: (1) Finite Element Analysis (FEA) on HR-pQCT at distal radius, (2) serum 25(OH)Vit-D assay and (3) dietary calcium intake. The SRS guideline was followed for the Latest Follow-up analysis on curve progression defined as Cobb increase ≥ 6°. P-value < 0.05 was considered statistically significant.

Results

270 (81.8%) subjects completed the study. At 2-year, the increases in FEA parameters were significantly greater in the Treatment Group (Table1).

At the Latest Follow-up (N = 132), 21·7% in Group 3 and 24·4% in Group 2 progressed as compared with 46·7% in Group 1 ($P = 0.012$, 0.032). Within-group logistic regression analysis showed in Group 3, increase in FEA parameters of failure load and apparent modulus were significant protective factors against curve progression ($P = 0.043$ & 0.034 respectively).

For those with baseline serum 25(OH)Vit-D ≤ 50 nmol/l (N = 103), 16.2% progressed in Group 3 as compared with 48.6% in Group 1 ($P = 0.003$). For those with 25(OH)Vit-D > 50 nmol/l (N = 29), no difference on curve progression was noted. For those with baseline dietary calcium intake ≤ 1000 mg/day (N = 109), 19.0% progressed in Group 3 as compared with 54.3% in Group 1 ($P = 0.001$). For those with calcium intake > 1000 mg/day (N = 23), no difference on curve progression was noted.

Conclusion

The results of this study provide strong evidences that calcium + Vit-D supplementation can improve bone strength in AIS. Its therapeutic effect on preventing curve progression is correlated with increase in FEA parameters, low baseline 25(OH)Vit-D level and low baseline dietary calcium intake.

Funding Source

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Disclosure

The authors declared no competing interests.

Table 1 Mean changes on serum 25(OH)Vit-D level and Finite Element Analysis (FEA) parameters at 2-year for Group 1, 2 and 3[§].

	Changes from baseline to 2-year[§] mean ± SD			p	
	Gp 1 N = 91	Gp 2 N = 91	Gp 3 N = 88	Gp 1 Vs Gp 2	Gp 1 Vs Gp 3
Serum 25(OH)VitD (nmol/l) [#]	6.3 ± 15.3	20.4 ± 19.6	28.0 ± 23.3	<0.001^	<0.001^
	Gp 1 N = 83	Gp 2 N = 78	Gp 3 N = 72		
FEA: stiffness (kN/mm) [#]	13455 ± 4670	15786 ± 5701	16520 ± 5563	0.048^	0.001^
FEA: failure load (N) [#]	533 ± 193	622 ± 243	658 ± 252	0.094	0.002^
FEA: apparent modulus (MPa) [#]	465 ± 220	591 ± 353	588 ± 289	0.020^	0.028^

§: Changes from baseline to 2-year refers to the parameter at 2-year minus that at baseline.
#: P-value from ANCOVA ^: P-value < 0.05.

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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

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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 mgCa/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 (s.d.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(4.8,5.1), F-P = 118.9(6.4,6.8), M-Ca = 78.1(4.3,4.5), M-P = 67.7(3.4,3.6). Girls had higher IGF-1 than boys in both supplement groups ($P < 0.001$). IGF-1 was lower in F-Ca than F-P (−18 ± 7%, $P = 0.01$) but higher in M-Ca than M-P (+14 ± 8%, $P = 0.05$); 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 ± 7%, $P = 0.02$; M-Ca v M-P = +8 ± 7%, $P = 0.2$), 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.
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Disclosure
The authors declared no competing interests.
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OC10

Inadequate vitamin D status adversely affects trabecular bone mineral density in 14–18 year old adolescents

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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°N)[(1)]. 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 adolescents[(2)], 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 this[(1)]. 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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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

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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, mobility, 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 two boys (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 (+1119 g (+224 to +1744)) compared to controls (+635 g (−951 to +1006)), $P = 0.01$, 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

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

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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 incident rate was 254/10,000 (95% CI 29,887) person-years in GC naïve 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 SDS was -0.7 s.d. (95% CI -1.2, -0.2, $P=0.01$) lower in those treated with daily Deflazacort compared with GC naïve boys, whereas there were no statistical differences in the other GC regimen.
Conclusion
In the largest cohort of boys with DMD to date with longitudinal fracture data, there is an overall 4.2 fold increase in fracture incident rate compared with healthy UK boys (1). Our study showed for the time time that fracture incident rate and growth failure is highest in those treated with daily Deflazacort.
Disclosure
The authors declared no competing interests.
Reference
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OC13

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

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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
Enterococccus 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 Enterococccus faecium ($n=278$; 13%) was 0.25 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 Enterococccus faecium TB-BMD was 0.6s.d. higher per RA unit increase ($P=0.03$), though this effect was no longer significant after adjustment for BMI ($P=0.2$).
Conclusion
Human gut microbiota influences bone mineral density in children. Enterococccus 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 Enterococccus 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 (Enterococccus faeciumI) 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

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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, secreted 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.

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 ($\beta=0.102$, $P=0.010$), homeostasis model assessment for insulin resistance (HOMA-IR; $\beta=0.087$, $P=0.028$), leptin ($\beta=0.275$, $P<0.001$), 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 PBF 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 leptin receptor level had a negative 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

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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 more prone to fracture. Here we aimed to investigate at the population level the influence of sex, ethnic background and bone mineral density (BMD) on the occurrence of bone fractures in children of school age.
Methods
This study ($n=3{,}633$ children with complete information) is embedded in the Generation R Study, a prospective multiethnic pregnancy/birth cohort in Rotterdam, The Netherlands. Children were classified by ethnic background using questionnaire information on parental country of birth and grouped into Europeans and Non-Europeans (Asian and African origin combined). Fractures occurring since birth were registered in questionnaires filled at 9 years of age by parents. Total body (less head) BMD (TB-BMD) and lean mass (LM) were measured using an iDXA densitometer (GE-Lunar) at a mean age of 6 and 10 years. Risk (odds) of fracture was estimated from logistic regression models adjusted for age, height, weight, lean mass fraction and standardized TB-BMD. Statistical significance was set at $P<0.05$.
Results
Among all participants with a mean age of 9.74 years (s.d. = 0.29), 49.4% were males and 87.1% Europeans. Fracture was reported in 521 children (14.3%). As compared to girls, no significant difference in the odds of fractures was seen in boys (OR = 1.04, 95%CI 0.84–1.28; $P=0.74$). Children from European ancestry had almost twice higher fracture risk than non-Europeans (OR = 1.7, 95%CI 1.19–2.30; $P=0.003$). One s.d. decrease in TB-BMD was associated with 26% higher risk of fracture at a mean age of six (OR = 1.26, 95%CI 1.10–1.44; $P=0.001$) and 43% higher risk at a mean age of nine (OR = 1.43, 95%CI 1.24–1.62; $P=4.26\times10^{-7}$) years.
Conclusion
Total body BMD is a determinant of fracture risk in children of both sexes. Odds of fracture did not differ between boys and girls of school age, but are higher in children of European ancestry even after correction for TB-BMD and body composition. Whether this association is due to differences in physical activity between the ethnic groups remains to be determined.
Disclosure
The authors declared no competing interests.

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OC16

Pediatric hypophosphatasia – a retrospective single-center chart review of 50 children

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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. Therefor 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 (in 72%), available low AP activity (88%), accumulating substrates (in 56%) and X-ray findings (in 34%). 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

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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 BMAD) and total body less head (TBLH 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

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

	Odds Radio (96%CI)	Sensitivity (96%CI)	Specificity (96%CI)
Trab vBMD	**3.4 (1.6–7.0)**	**0.29 (0.17–0.41)**	**0.89 (0.85–0.93)**
Hand BHI	**2.8 (1.6–4.9)**	**0.45 (0.33–0.57)**	**0.77 (0.71–0.82)**
Radius BA/MA	**2.3 (1.0–5.3)**	**0.20 (0.09–0.32)**	**0.90 0.86–0.94)**
Corticosteroids	**1.7 (1.0–2.9)**	**0.61 (0.49–0.72)**	**0.53 (0.47–0.59)**
Back Pain	**1.7 (1.0–2.8)**	**0.46 (0.35–0.58)**	**0.66 (0.60–0.71)**
TBLH BMD	1.4 (0.8–2.6)	0.27 (0.17–0.38)	0.79 (0.75–0.84)
LS BMAD	1.3 (0.5–3.2)	0.10 (0.03–0.17)	0.92 (0.89–0.95)
Immobility	1.0 (0.5–2.1)	0.14 (0.06–0.22)	0.86 (0.81–0.90)

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 NIHR 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 platelet derived growth factor type BB attenuates glucocorticoid-induced osteoporosis in growing mice

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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 platelet derived growth factor type BB (PDGF-BB) to promote angiogenesis and osteogenesis during both modeling and remodeling. As glucocorticoid therapy affects both osteoclast bone resorption and osteoblast bone formation, we explored if disruption of osteoclast bone resorption via genetic loss of cathepsin K (*Ctsk*), which increases secretion of preosteoclast PDGF-BB, could protect the skeleton of young mice exposed to prednisolone. We first characterized the temporal osteoclast and osteoblast progenitor populations in global cathepsin K knockout ($Ctsk^{-/-}$) relative to wild type mice in both the primary and secondary spongiosa. Loss of cathepsin K activity resulted in increased trap positive cells in both the primary and secondary spongiosa and correlated with the increased bone volume seen in the $Ctsk^{-/-}$ 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 $Ctsk^{-/-}$ mice and wild type littermates with prednisolone at 10 mg/m^2 per day beginning at 2 weeks of life and continuing 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 $Ctsk^{-/-}$ 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 cathepsin K ameliorated this decrease 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 $Ctsk^{-/-}$ 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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OC19

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

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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 effects 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 osteocarcoma cells which were grown either in adhesion-condition or in non-adherent spheres, favouring proliferation 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 tumor-necrosis-factor related apoptosis inducing ligand (TRAIL).
Results
MSCs secreted factors activated osteosarcoma cell cycle from G_1 to mitosis phases, but did not promote the transition from quiescent G_0 to G_1 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
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OC20

Autoimmune hyperphosphatemic tumoral calcinosis

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Background
Hyperphosphatemic familial tumoral calcinosis (HFTC)/hyperostosis-hyperphosphatemia syndrome (HHS) is an autosomal recessive disorder due to deficiency

of or resistance to intact fibroblast growth factor 23 (FGF23). This leads to hyperphosphatemia, increased renal reabsorption of phosphorus (TRP), and elevated or inappropriately normal 1,25-dihydroxyvitamin D (1,25D). Affected individuals may develop ectopic calcifications and/or diaphyseal hyperostosis. Mutations in *FGF23*, *GALNT3*, or *KLOTHO* have been identified as causative for HFTC/HHS. Here we present the first case of autoimmune hyperphosphatemic 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 hyperphosphatemia (7.2 mg/dl; nl 3–5.7), increased TRP (98%), and inappropriately normal 1,25D (68 pg/ml; nl 24–86). 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 *KLOTHO* or possibly an *FGFR1* mutation. However, no causative mutation was identified in *GALNT3*, *FGF23*, *KLOTHO*, or *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 islet 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, FGFR1, 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 FGFR1 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 hyperphosphatemic 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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OC21

Scoliosis in fibrous dysplasia/McCune-albright syndrome

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Objectives

Fibrous dysplasia is a rare bone and endocrine disorder resulting from somatic activating mutations in *GNAS*. In the skeleton, proliferation of undifferentiated stromal cells results in osseous lesions that are prone to deformity, fracture, and pain. Lesions may affect one bone or many, and may occur in isolation or in association with hyperfunctioning endocrinopathies, termed McCune-Albright syndrome (MAS). Scoliosis is a potentially serious, even lethal complication; however the prevalence, spectrum, and optimal management have not been established.

Methods

Subjects were evaluated as part of a long-standing natural history in fibrous dysplasia/MAS. Clinical, biochemical and radiographic data were analysed from 138 subjects ranging in age from 2.4–80.

Results

Scoliosis was present in 84 subjects (61%), categorized as mild (Cobb angle $>10^\circ$ and $\leq 30^\circ$) in 65% of subjects, moderate (>30–$\leq 45^\circ$) in 13%, and severe ($>45^\circ$) in 21%. Scoliosis was highly correlated with leg length discrepancy ($P<0.002$), impaired mobility ($P<0.0001$), skeletal burden score ($P<0.0001$), and bone turnover markers. MAS endocrinopathies associated with scoliosis included FGF23-mediated hypophosphatemia ($P<0.0004$) and hyperthyroidism ($P<0.0001$). Longitudinal data was available for 69 subjects, including 12 who underwent spinal fusion procedures. In non-surgical subjects, scoliosis was progressive (defined as an increase in Cobb angle $>10^\circ$) in 31% of subjects ($n=18$), with a median increase of 18.3° over a 6.2 year period. In 11/12 surgical subjects, spinal instrumentation remained in place after a mean post-operative period of 15 years (range 2.7–38.0). Only one subject required instrumentation removal due to mechanical failure 3 months post-operatively. Serial radiographs were available from 10 surgical subjects, nine of whom showed no progression over a 5.3 year period (range 0.9–14.7 years). Two non-surgical subjects (aged 19 and 41) died from respiratory complications of progressive scoliosis. No fatalities occurred in surgical subjects.

Conclusion

Scoliosis is common in fibrous dysplasia, particularly in patients with leg-length discrepancies and MAS endocrinopathies. Scoliosis is frequently progressive into adulthood. Long-term outcomes from spinal fusion are favorable in most patients. We report the first cases of scoliosis-related fatalities in fibrous dysplasia patients, demonstrating the critical importance of monitoring and treatment for spinal disease in this population.

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

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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 heterozygous 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 BV/TV. By 6 months, HBM mice hind limb joints fuse with severe osteoarthritis. HBM femora have decreased stiffness, yield and fracture load, which did not improve with age. Their 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 (93%) 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*, *Acp5* and the *Rankl/Opg* 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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OC23

Sustained radiographic and functional improvements with asfotase alfa treatment from up to 7 years in children with hypophosphatasia

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Objective
Children with hypophosphatasia (HPP) treated with asfotase alfa in a Phase 2 study (NCT00952484) 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
1. Whyte et al. *JCI Insight*. 2016;1:e85971.

Table 1

Outcome	Reference range for healthy peers	Median (min, max) Baseline ($n=13$)[a]	Median (min, max) Last observation ($n=12$)[a]	*P* value
RGI-C		NA[b]	2.8 (2.0, 3.0)	0.0005[c]
RSS	0=no rickets	3.0 (0.5, 6.0)	0 (0, 1.0)	ND
Height *Z*-score	−2 to +2	−1.26 (−6.6, 0)	−0.69 (−5.4, 0.4)	0.0007[d]
Weight *Z*-score	−2 to +2	−1.21 (−8.2, 2.3)	−0.15 (−5.4, 2.7)	0.0004[d]
6MWT, % predicted	80% to 120%	61% (29%, 82%)	85% (69%, 104%)[e]	0.0006[f]
BOT-2, Strength and Agility Composite Standard Score	40 to 60	28 (20, 37)	51 (34, 62)[e]	<0.0001[f]
CHAQ-DI[g]	0=no disability	1 (0, 2.25)	0 (0,0.5)	0.0004[f]

[a]One patient withdrew after 1 month for elective surgery. [b]Baseline value not applicable (NA) because RGI-C describes change from baseline; RGI-C range: −3 (severe worsening) to +3 (complete/near complete healing). [c]Wilcoxon signed-rank test assessing if median is 0. [d]Wilcoxon signed-rank test assessing if median change from baseline is 0. [e]n=11 for last observation for 6MWT and BOT-2. [f]*t*-test assessing if mean change from baseline is 0. [g]CHAQ-DI range: 0 to 3, with higher scores indicating greater disability. ND=not determined.

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OC24

KRN23 effects on phosphate and vitamin D dysregulation in children <5 years old with X-Linked Hypophosphatemia (XLH)

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Objectives
XLH features renal phosphate (Pi) wasting, hypophosphatemia, rickets, and skeletal deformities from elevated circulating levels of fibroblast growth factor 23 (FGF23). KRN23, an investigational fully human monoclonal antibody, binds FGF23 and inhibits its action. Our Phase 2 study of KRN23 in XLH children (ages 5–12 years) is demonstrating improvements in serum Pi and rickets. Here we present our Phase 2 trial evaluating the efficacy and safety of KRN23 in younger children with XLH.
Methods
In an ongoing, open-label, multicenter trial, children 1–4 years old with XLH received KRN23 at an initial dose of 0.8 mg/kg subcutaneously every 2 weeks. We evaluated serum Pi, alkaline phosphatase (ALP), $1,25(OH)_2D$, and KRN23 concentrations and safety. This, our initial report, includes BL data for the first 10 children enrolled, and up to 4 weeks of treatment data for the first five subjects.
Results
At BL (N=10), the mean age was 3.0 years and 70% were boys. The median standing height percentile was 9.1%. Complications of XLH included gait disturbance (60%), tibial torsion (60%), knee deformity (40%), and skull malformation (40%). At BL, all had low serum Pi levels (mean (SE) =0.83 (0.025) mmol/l) (Normal (Nl): 1.03–1.97 mmol/l) while ALP was elevated in 8 of 10 subjects. Mean serum Pi increased after KRN23 treatment by 0.41 (0.030) mmol/l at Week 1 and 0.36 (0.070) mmol/l at Week 4. Normal Pi levels were achieved in 100 and 80% of subjects at Weeks 1 and 4, respectively. Mean (SE) serum $1,25(OH)_2D$ levels increased from 118 (17) pmol/l at BL to 256 (38) pmol/l at Week 1 (Nl: 60–220 pmol/l). Serum KRN23 concentrations at Weeks 1 and 4 resembled the values observed in the Phase 2 study in older children. All adverse events (AEs) were mild, and except for upper respiratory tract infections ($n=2$), all other AEs occurred in 1 subject each. No serious AEs occurred.
Conclusions
Initial results in 1 to 4 year-old children with XLH suggest KRN23 pharmacodynamic responses are similar to those of older children. The study is ongoing and will evaluate changes in rickets severity and growth.
Disclosure
Imel: travel and consulting fees from Ultragenyx; Carpenter: grant support and travel from Ultragenyx; Mao, Skrinar, San Martin: employees of Ultragenyx; Whyte: research grant support, honoraria, and travel from Ultragenyx and Alexion.

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OC25

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

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Objective
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 6x/wk (lowest approved dose). The primary safety outcome was tolerability. Coprimary efficacy outcomes included median change at 6 months in plasma inorganic pyrophosphate (PPi) and pyridoxal-5'-phosphate (PLP) levels. Other measures 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

injection-site reactions. Decreases in PPi were numerically greater and decreases in PLP were statistically significantly greater at 6 months in treated patients ($n=13$) vs controls ($n=6$): PPi, −2.2 μM (−4.4, 0.3) vs −0.2 (−6.8, 1.1; $P=0.0715$); PLP, −255 ng/ml (−1467, −17) vs 11 (−374, 346; $P=0.0285$). Decreases were sustained through 5 years ($n=16$): PPi, −3.0 μM (−5.2, 7.8); PLP, −284 ng/ml (−1580, −25). Distance walked in 6MWT improved from 355 m (10, 620; $n=19$) before treatment to 450 m (280, 707; $n=13$) after 5 years of treatment with asfotase alfa, increasing from 76% predicted (42, 101; $n=15$) to 88% predicted (62, 137; $n=11$). BOT-2 Running Speed and Agility total point score was 6.5 (0, 39; n=16) before treatment and improved by 4.0 (−5, 18; $n=11$) after 5y, and BOT-2 Strength total point score was 13.5 (0, 33; $n=18$) before treatment and improved by 3.5 (−9, 9; $n=12$) after 5y.

Conclusion

Asfotase alfa was generally well tolerated, numerically decreased circulating PPi and PLP, and improved physical function in adolescents and adults with HPP.

Disclosure

This study was sponsored by Alexion Pharmaceuticals, Inc. PSK is a clinical trial investigator and has received honoraria and travel support from Alexion Pharmaceuticals, Inc., for consulting and participation on advisory boards. AED and SM are employees.

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OC26

A randomized, open-label Phase 2 study of KRN23, an investigational fully human Anti-FGF23 monoclonal antibody, in children with X-linked Hypophosphatemia (XLH)

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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, ≤Tanner 2).

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

P001

Abstract withdrawn.

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P002

A 3-year longitudinal study of skeletal effects and growth in children after kidney transplantation

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Objectives
Children and adolescents with chronic kidney disease (CKD) are at risk of developing CKD-mineral bone disorder (CKD-MBD). This study was designed to follow Swedish pediatric patients prospectively for 3 years after kidney transplantation regarding growth and skeletal development.
Methods
The study group comprised 13 patients (4 females), 4–15 years of age. Growth, bone mineral density (BMD) and markers of bone and mineral metabolism were investigated at start, and after 3, 12, 36 months after kidney transplantation.
Results
Median glomerular filtration rate was 63 (range 37–96) ml/min/1.73 m^2 after 3 years. The median height standard deviation score (SDS) increased from -1.7 to -1.1 after 3 years, $P=0.0012$, and median BMI SDS increased from -0.1 to 0.6 after 3 years, $P=0.013$, which implies that transplantation had a favorable outcome on growth. Total BMC increased at all time points in comparison with the initial values at study start, $P<0.001$. No change was observed for total BMD, calcaneal BMC and BMD over the study period. A delayed median bone age was found at start and after 3 years post-transplantation. Parathyroid hormone, phosphate and magnesium decreased at 3 months, and decreased further during the study period. The bone resorption markers tartrate-resistant acid phosphatase isoform 5b (TRACP5b) and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) decreased initially after 3 months ($P<0.05$), and remained stable throughout the study period. The bone formation markers alkaline phosphatase, intact amino-terminal propeptide of type I procollagen (PINP) and osteocalcin decreased initially, but successively increased over the study period.
Conclusion
This study demonstrates that height SDS and BMI SDS increased, along with the increased markers of bone formation that reveals a positive bone acquisition after kidney transplantation, which was reflected by the significant increase in total body BMC.
Disclosure
The authors declared no competing interests.

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P003

Abstract withdrawn.

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P004

Bone mass tracks into teenage years

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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 pubertal according to self-assessed tanner-stage. *Z*-scores were calculated for all three bone parameters (Total Body Less Head (TBLH) Bone Mineral Content (BMC), TBLH Bone Mineral Density (BMD) and TBLH Bone Area (BA)) adjusted for sex and age.
Results
We found the correlation between TBLH BMC Z-score at baseline and at the last follow up to be 0.79 (CI 0.73–0.84). The correlation between *Z*-score TBLH BMD and TBLH BA were comparable (BMD 0.77 (CI 0.71;0.82) and BA 0.81 (CI 0.75;0.85)). For TBLH BMC and BMD we found a higher correlation coefficient for girls than boys (ex. TBLH BMC 2008–2015 girls: 0.86 (CI 0.79;0.90), boys: 0.71 (CI 0.60;0.80)). Dividing the participants into quintiles depending on *Z*-scores in 2008 respectively 2015 we found that 85–87% were in the same quintile or the neighbour quintile at follow up. No one moved from the lowest to the highest quintile or opposite.
Conclusion
We found a high degree of tracking in bone mass (TBLH BMC, BMD and BA) in children from 8 to 17 years. The strong tracking may be useful in early identification of individuals at risk of osteoporosis later in life.
The study has been supported by the Danish Foundation TrygFonden
Disclosure
The authors declared no competing interests.

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P005

Muscular fitness, bone mineral density and hip geometry in young males: the PRO-BONE study

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Objective
The main aim was to evaluate associations between muscular fitness indices 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 tests (vertical jump and standing long jump) with bone outcomes were analysed using three regression models: Model 1, adjusted by age and stature; Model 2, model 1 + vigorous physical activity (VPA); Model 3, model 2 + lean mass. Bonferroni correction was applied and only values of $P<0.006$ were considered statistically significant.
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.
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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

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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 followed by cross 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 GnRH 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 +1.0 SDS respectively. The iDXA recorded 5.4% more body fat (mean 4.6 kg, SD. 3.2) and 8.2% less lean mass (mean 7 kg, SD. 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 in females and males respectively. The longitudinal study included 32 patients (11 male, 21 female), with repeat scans done at a mean of 1.1 years after commencing GnRHa. The mean (SD.) difference in bone density scores between the pre and post GnRHa scans was: LSBMD −0.66 (1.1), BMAD −0.68 (1.0), TBBMD −0.2 (1.0), TBLH +0.0 (1.1). The BMI Z-score increased by a mean of 0.1 SDS. The fat mass increased by a mean (SD.) of 6.2% (8.0), lean mass and BMC decreased by 4.3% (7.9) increased by 0.4% (0.7) respectively.
Conclusions
The Tanita body composition scales underestimate the body fat and overestimate lean mass compared to the iDXA. In post-pubertal adolescents, GnRHa for one year increases the BMI marginally but increases fat mass and reduces lean mass and BMC. The clinical significance of these short term changes remains to be determined.
Disclosure
The authors declared no competing interests.
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P007

Effects of a 8-month physical activity and nutrition-induced weight loss program on bone health of obese adolescents.

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Objective
This work investigated the changes of bone hip structural parameters in obese adolescents enrolled in a successful weight loss program.
Methods
Thirty-one obese adolescents (age 13.61 ± 1.27) enrolled in a 8-month weight loss program combining physical activity and nutrition were compared with normal-weight (NW) matched peers (age 15.9 ± 0.43). Investigations were performed at baseline and 8 months. Bone geometric and strength indices were quantified by DXA using hip structural analysis at the narrow neck (NN) and femoral shaft (FS).
Results
At the end of the interventional program, obese adolescents had geometric bone indices significantly lower than controls at NN for endocortical diameter (ED) ($P=0.005$) and width ($P=0.003$). At FS bone density ($P=0.004$), ED ($P=0.006$) and cortical thickness (CT) ($P=0.001$) were significantly reduced compared with normal peers. Also, compared with unchanged loading (NW), reduced load (obese from the intervention group) significantly increased axial strength (cross sectional area (CSA); section modulus (Z)) at NN and FS and bending strength (cross sectional moment of inertia (CSMI)) at FS leading to similar raw data compared with NW. However, at the end of the intervention obese adolescents had higher bulking ratio at NN (8.25 ± 2.00 vs 6.92 ± 1.0; $P=0.008$) and at the shaft (2.73 ± 0.54 vs 2.27 ± 0.47; $P=0.004$). After adjusting for body weight changes, bone density (NN $P=0.007$; FS $P<0.001$) and CT (NN $P=0.05$; FS $P=0.009$) became significantly lower in the obese population. Also, obese adolescents displayed lower bending and torsional (CSMI $P<0.001$; Z $P<0.001$) strength, lower resistance to axial stresses (CSA $P<0.001$; Z $P<0.001$) at both sites but higher bulking ratio ($P=0.027$) at NN only. Finally, after adjustment for fat mass changes, lower bone density ($P=0.003$) and CT ($P=0.011$), higher ED ($P=0.018$) and bulking ratio (2.87 (95%CI 2.396–2.961) vs 2.32 (2.035–2.600) $P=0.004$) were observed at the shaft in obese adolescents.
Conclusion
The present results suggest that, after a weight loss, geometric indices of bone strength at weight bearing site remain unadapted to excess body weight despite 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.
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P008

Cardiorespiratory fitness, bone mineral density and hip geometry in young males: the PRO-BONE study

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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 were analysed using three regression models: Model 1, adjusted by age and stature; Model 2, model 1 + vigorous physical activity (VPA); Model 3, model 2 + lean mass. Bonferroni correction was applied and only values of $P<0.006$ were considered statistically significant.
Results
performance 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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Disclosure
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P009

Sexual dimorphism in cortical bone morphology during adolescent growth in Chinese

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Objective
Previous study in Hong Kong reported boy-to-girl ratio of limb fracture was 5.5:1 in the adolescent group. Chinese children have increased risk for forearm fracture during puberty. This study aimed to investigate cortical growth in healthy Chinese adolescents during pubertal growth.
Methods
214 boys and 219 girls aged between 7 and 17 years old with no bone diseases were recruited. Maturity was assessed by self-reported Tanner staging. Images of non-dominant distal radius were obtained using high-resolution peripheral quantitative computed tomography (HR-pQCT) at 5 mm proximal to growth plate. Proximal 40 slices of 110 slices were analysed by StrAx1.0. Cortical cross-sectional area (CSA), cortical porosity, volumetric bone mineral density (vBMD), trabecular bone volume fraction (BV/TV) and matrix mineral density were measured. ANCOVA was used to identify sex difference on bone parameters after adjustment for age, height and weight, dietary calcium intake (Ca) and physical activity level (PA).
Results
After adjusting for all confounders, boys had 11.1–12.8% larger total CSA than girls across puberty, but boys had 1–4% lower cortical CSA/total CSA in Tanner stage 2–5. In Tanner stage 1, cortical thickness was 6.1% greater in boys but cortical porosity did not differ from girls. From Tanner stage 2, cortical thickness did not differ by sex while boys had 8.4–12.6% higher cortical porosity. Boys had 0.7–1.6% lower matrix mineral density in Tanner stage 3–5 after adjusting for age, Ca intake and PA. In boys, 15.1–21.3% lower total vBMD and 14.3–23.8% lower cortical vBMD were found in Tanner stage 2–5 compared to girls. Boys had 13.8–15.2% greater trabecular vBMD after adjusting for age, Ca and PA.
Conclusion
Total and cortical CSA increase during pubertal growth in both sexes and are larger in boys. Cortical porosity was higher and matrix mineral density was lower in boys, perhaps because their skeletal maturing takes longer to complete. Transient physiologically changes of the cortical morphology, reduced bone density and higher cortical porosity in Tanner stage 2 boys might partly decrease bone mechanical strength and contribute to the increased risk of distal forearm fracture.
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P010

Transient hyperphosphatasemia in a child with nephrolithiasis And history of severe prematurity

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Background
Transient hyperphosphatasemia of infancy and early childhood (THI) is a benign, usually accidentally detected condition characterized by transiently increased activity of serum alkaline phosphatase (S-ALP), its bone or liver isoform, in children under five years of age, without signs of metabolic bone disease or hepatopathy. When encountered in a child with either chronic bone, liver or kidney disease, THI might concern the physician. We present a patient with urolithiasis and THI.
Case presentation and clinical management
13-months old boy with a complicated perinatal history (severe prematurity – 26th week of gestation, respiratory distress syndrome, reanimation, neonatal sepsis, pneumonia, artificial ventilation) resulting in bronchopulmonary dysplasia and osteopathy of prematurity, was hospitalised because of hematuria. Abdominal ultrasound revealed renal stones in each kidney, diameter, 3 mm on the left and 6 mm on the right, respectively. The serum values of blood urea nitrogen (BUN), creatinine, potassium(S-K), sodium(S-Na), calcium(S-Ca), phosphate(S-P), magnesium(S-Mg), alanin-aminotransferase(S-AST), apartate-aminotransferase(S-ALT) were all within normal reference range, same as urinary concentrations of Ca, P, Mg and urinary calcium/creatinine ratio (U-Ca/U-cr). S-ALP was 34 μkat/L (normal 2.5–9.5 μkat/l). Wrist X-ray was normal without any signs of rickets. Hematuria resolved within 3 days. As there were neither laboratory or clinical signs of liver or bone disease, THI was considered as the most likely diagnosis. The boy was checked 28 days.later, and at that time the S-ALP dropped to normal value 9.2 μkat/l.
Discussion
Patient with bilateral nephrolithiasis and a history of severe prematurity presented with high S-ALP, initially suggestive of disturbed bone metabolism. However, the normal values of S-Ca,P,Mg,PTH,U-Ca/U-cr and normal wrist X-ray ruled out this possibility and pointed to the diagnosis of THI, which was further confirmed by the normalisation of S-ALP within one month. The present nephrolithiasis was considered as a result of previous hypercalciuria in osteopathy of prematurity, that has already resolved without causal relationship to transiently increased S-ALP. There were no further increases in S-ALP and the patient, who is currently 25 months old, remains stable. THI is a benign condition with good prognosis. Children with THI should be spared from unnecessary early diagnostic procedures and therapeutic interventions.
Disclosure
The authors declared no competing interests.
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P011

Neonatal hypocalcemia – transient neonatal pseudohypoparathyroidism

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Background
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 mays 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 asfyxia or sepsis) with mild hypotonia, where S-Ca in the range of 1.67–1.9 mmol/l was detetectd within the first three days of life, together with hyperphosphatemia (S-P 2.5–2.6 mmol/l), normomagnesemia (S-Mg 0.77–0.88 mmol/l), normal alkaline phosphatase activity (S-ALP 2.8–4.5 μkat/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, asfyxia, 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.

Dg	S-Ca	S-P	S-Mg	S-ALP	S-PTH
Asfyxia	↓	↔ ↓	↔	↔	↑
Transient hypoparathyroidism	↓	↑	↔	↔	↓
Primary hypoparathyroidism (Di George syndrome)	↓	↑	↔	↔	↓
Hyperphosphatemia	↓	↑	↔	↔	↑
Hypomagnesemia	↓	↑	↓↓	↔	↓
Maternal Ca/D-vitamin deficiency	↓	↔ ↓	↔	↔↑	↑
Pseudohypoparathyroidism I,II	↓ ↔	↑	↔	↔	↑
Transient pseudohypoparathyroidism	**↓**	**↑** **↔**	**↔**	**↔**	**↑↑**
Sepsis	↓	↔	↔↓	↔	↑
Hepatopathy	↓	↓	↔	↑	↑

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

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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. Dual energy X-ray absorptiometry (DXA) measured aBMD at lumbar spine, femoral neck (FN) and total body. Hip structural analysis evaluated hip geometry estimates at the FN. Multiple linear regression examined the contribution of the sports practised, stature, lean and fat mass, serum calcium and vitamin D, moderate to vigorous physical activity (MVPA), vertical jump and cardiorespiratory fitness (CRF) with aBMD and hip geometry estimates.
Results
Region specific lean mass was the strongest positive predictor of aBMD ($\beta = 0.614–0.931$) and football participation was the next strongest predictor ($\beta = 0.304–0.579$). Stature ($\beta = 0.235–0.380$), fat mass ($\beta = 0.189$), serum calcium ($\beta = 0.103$), serum vitamin D ($\beta = 0.104–0.139$) and vertical jump ($\beta = 0.146–0.203$) were associated with aBMD across various specific sites. All hip geometry estimates were associated with lean mass ($\beta = 0.370–0.568$) and stature ($\beta = 0.338–0.430$). Football participation was associated with hip cross-sectional area ($\beta = 0.322$) and MVPA ($\beta = 0.140–0.142$). CRF ($\beta = 0.183–0.207$) was associated with section modulus and cross-sectional moment of inertia.
Conclusion
Region specific lean mass is the strongest determinant of aBMD and hip geometry estimates in adolescent male athletes. Football participation and stature were important determinants for aBMD and hip geometry estimates while the contribution of the other predictors was site specific.
Funding sources
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Disclosure
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P013

Longitudinal evaluation of bone mass, geometry and metabolism in adolescent male athletes. The PRO-BONE study

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Objectives
Cross-sectional studies show that exercise may have positive effects on bone outcomes in youth. However, there is no evidence from longitudinal studies, which type of sports can induce improvements in bone acquisition in adolescent athletes. 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 aminoterminal propeptide (P1NP), bone resorption using isomer of the Carboxi-terminal telopeptide of type 1 collagen (CTX-I) 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 accelerometers. 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
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Disclosure
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P014

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

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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-osteogenic sports.
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Disclosure
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P015

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

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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 was observed in 251 children (13.7%). A significant increase in the odds of fracture was observed for every standard deviation (SD) decrease in TBLH-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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P016

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

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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 (TBLH) BMC were significantly lower among NEC-children ($P<0.001$). Regarding TBLH BMD, no significant differences were found. TBLH 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 BMD Z-score ($P<0.05$).
Conclusion
Children with NEC during their neonatal period were shorter, had lower body weight, a decreased TBLH 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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P017

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

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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 $\pm$ S.D., 11.6$\pm$4 years). Bone mineral apparent density (BMAD) and trabecular bone score of the lumbar spine were also obtained.
Results
In our cohort areal BMD (aBMD) *Z*-score was below the mean in 88% of patients at lumbar spine (LS, 70% after correction for bone size) and in 86% considering total body (TB) scans. However, aBMD *Z*-score was < -2 in 14% (12% after correction for bone size) and 12% patients at LS and TB, respectively. Though BMD at all sites was higher in older and pubertal patients, LS aBMD *Z*-score ($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 ($P = 0.007$, $P = 0.02$, $P = 0.01$, respectively), as suggesting that patients failed to gain as much as expected for age. Hypovitaminosis D was highly prevalent, as 98% patients had 25OHD 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 timing frame to implement prevention strategies to allow a correct bone accrual and reduce future fractures risk. Trabecular bone score, providing information on bone quality even in the absence of age and gender adjusted normative data, could be useful during longitudinal follow-up for better characterizing bone impairment in these patients.
Disclosure
The authors declared no competing interests.

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P018

Abstract withdrawn.

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P019

Zebrafish as model organism for craniosynostosis

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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 staining methods 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. More 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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P020

Age at onset of walking affects bone mineral content in early childhood

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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 D_3 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/cm^3 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

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).

Conclusions

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.

	BOYS			GIRLS		
	Adjusted	CI 95%	*P*	Adjusted	CI 95%	*P*
Weight (kg)	3.047	2.109; 3.985	<0.001	3.829	3.011; 4.647	<0.001
Walking age (months)	−1.153	−1.892; −0.415	0.002	−0.553	−1.094; −0.011	0.046
Serum 25-OHD	0.020	−0.045; 0.085	0.543	0.010	−0.043; 0.064	0.704

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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

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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 strength, size, 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 radius shaft 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

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Backgound

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 40th 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 39.7±1.0 weeks of GA underwent a total body DXA scan less head (TBLH, LUNAR Prodigy, Infant software) at 40.9±1.8 weeks of GA. Bone mineral content (BMC, g), bone mineral density (BMD, gr/cm^2), FM (gr and %) 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±534.7 vs 3465±260.1 gr, $P<0.0001$) and shorter (42.4±5.1 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±5.1% vs 16.7±6.3%, $P=0.19$) and similar FFM. Moreover, preterm infants had a lower BMD (0.207±0.048 vs 0.275±0.011 gr/cm^2, $P=0.0004$), BMC (25.6±6.3 vs 48.2±6.8 gr, $P<0.0001$) and area (126.6±28.5 vs 175.0±20.1 cm^2, $P<0.0001$) 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 (71.0±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's<0.05) and GA (r's range=0.17–0.37, all P's<0.05) in preterm infants. However, multiple regression analysis showed that in preterm FFM was a positive (β 4.736e-5, $P=0.0305$) and %FM an negative predictor (β −0.003, $P=0.0078$) of TBLH BMD (adj.$R^2=0.646$, $P<0.0001$), 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

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Objectives

The skeleton is not a single functional unit but consists of different, well-organized and mineralized 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 cartilaginous endplate, which typically shows important species differences. This study aims to describe and compare histological, histomorphometric and regulatory characteristics in the GP of the spine and long bones in a porcine model.

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 Insuline-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 mechanism 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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P024

Spondyloepiphyseal dysplasia: A rare cause of short stature

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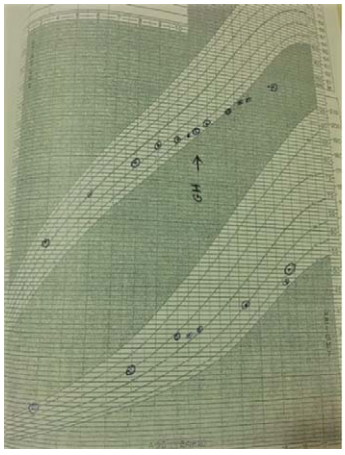
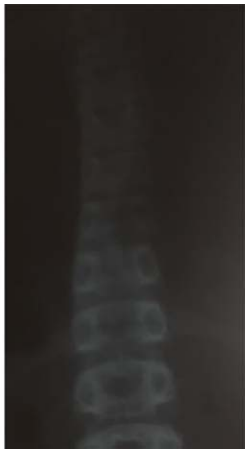
Background
Short stature is a very common reason for referral to a pediatric endocrinologist. Endocrine and non endocrine causes are involved. Short stature can be classified as symmetrical or non-symmetrical. Numerous monogenic causes of growth disorders have been identified.
Presenting problem
To present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

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P025

The impact of Haemophilia A on bone health

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Objectives
Haemophilia A (FVIII deficiency) is an X-linked disorder of haemostasis with bleeding tendency, mainly in joints and muscles. Recurrent haemarthroses, subsequent 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/uCr), 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, octeocalcin 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/uCr (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/uCr 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, $p<0.05$). No significant differences were detected in uCa/uCr and boneTRAP5b. Low vitamin D levels (mean 27.9 ± 19.33 ng/ml, normal values >20 ng/ml) were found in 36.7% of the patients and there was no correlation with BMD.
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

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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 Fundation) 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 (P1NP), state osteo resorption reflect the level of β-CTx in blood serum. The level of osteocalcin (OC) - the rate of bone remodeling.
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 of 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) 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.

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P027

Sex and iron modify fibroblast growth factor 23 concentrations in 1-year-old children

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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 D_3 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.
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P028

Abstract withdrawn.

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P029

Web-based surveys using Patient-Reported Outcome Measurement Information System (PROMIS) instruments allow documentation of important components of the disease experience among individuals with Osteogenesis Imperfecta

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Objectives
Two important goals of the Rare Diseases Clinical Research Network Brittle Bone Disorders Consortium (RDCRN BBD) are i) to perform collaborative clinical research in brittle bone disorders including a longitudinal observational study driven by genotypic association and ii) to explore use of the PROMIS tool to provide valid quality of life (QOL) measures in individuals with Osteogenesis Imperfecta (OI). This work has been motivated by the fact that all current outcome measures in OI have been developed by medical experts without input from patients; yet, patients and clinicians often disagree on level of disease burden. As the possibility of new medical, genetic and surgical treatments for OI become available, it is imperative to develop clinical scoring instruments which capture the disease characteristics of importance to individuals with OI in order to fully compare and contrast the impact of new treatments as well as determine future needs and research topics.
Methods
Using a web-based platform, 300 individuals with self-reported OI, representing a wide range of self-reported disease severity, were recruited from the RDCRN BBD Contact Registry to respond to a survey utilizing Patient-Reported Outcomes Measurement Information System (PROMIS) instruments focused on a wide range of health issues including mobility, anxiety, and fatigue. Parent proxy surveys were provided for children.
Results
290 individuals completed the survey including 92 children represented by parent proxy. 94% self-identified as white. Fewer than 30% 78/290 had had their diagnosis/type confirmed by skin biopsy or DNA. Nearly half reported having affected family members. 56% walked unaided while 23% used a wheelchair; 26% reported difficulty with breathing and 38% percent reported hearing loss. 52% reported undergoing rodding surgery while only 13% have required spine surgery. 53% of women over 18 had been pregnant. PROMIS score variations suggest that the instruments used can appropriately pick up changes in QOL measures. We will validate survey results by including data from the natural history portion of the BBD study.
Conclusion
Adults with OI vary from the general population in QOL measures. Our survey experience supports an internet-based strategy for successful patient-centered outcomes research in rare disease populations. The Brittle Bone Disorders Consortium (U54AR068069-01) is a part of the NCATS RDCRN. RDCRN is an initiative of the Office of Rare Diseases Research (ORDR), NCATS, funded through a collaboration between the NCATS, the National Institute of Dental and Craniofacial Research (NIDCR), the National Institute of Child Health and Human Development (NICHD) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).
Disclosure
The authors declared no competing interests.
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P030

Outcomes of zoledronic acid use in paediatric conditions

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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 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.

Table 1

Indication for ZA use	*n*	Outcomes
Bone abnormality indications		
Avascular necrosis (AVN)	36	3/36 collapse within 12 months
Bone metastases	8	4/8 significant pain relief
Fibrous dysplasia	11	8/11 significant pain relief, no lesion size change
Non-union	3	2/3 complete union within 6 months, 1/3 partial healing at 12 months
Massive sacral bone erosion by neurofibroma	1	Significant pain relief but no size change
Aneurysmal bone cysts	4	2/4 significant reduction in lesion size after 12 months
Chronic recurrent multifocal osteomyelitis (prior to availability of biology)	3	2/3 significant pain relief and no major change in lesion size
Other indications		
Osteogenesis imperfecta	117	Median 73.4% (Interquartile range 35%, 97%) increase in lumbar spine bone mineral density from baseline after 1–12 years of treatment
Osteoporosis inclusive of steroid dependent non DMD neuromuscular diseases	57	Median 44.2% (Interquartile range 23%, 72%) increase in lumbar spine bone mineral density from baseline after 1–10 years of treatment
Duchenne Muscular Dystrophy (DMD)	16	Median 21% (Interquartile range 11%, 32%) increase in lumbar spine bone mineral density from baseline after 1–7 years of treatment
Immobility	55	Median 39% (Interquartile range 24%, 57%) increase in lumbar spine bone mineral density from baseline over 1–10 years of treatment
Hypercalcaemia	2	Normalization of serum calcium, with reactive hypocalcaemia for 24–48 hours 2–4 days post dose

Disclosure
The authors declared no competing interests

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P031

Valproic acid induces Fanconi syndrome and reversible hypophosphataemic rickets via upregulation of fibroblast growth factor 23

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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
P2: Menkes disease
P3: Mowat-Wilson Syndrome
P4 and P6: Quadriplegic cerebral palsy secondary to hypoxic ischaemic encephalopathy
P5: Lissencephaly
All children were developmentally delayed, at least partially immobile and presented with low trauma fractures, all were gastrostomy fed and on VPA for epilepsy management (Table). The diagnosis of FS was established by confirming hypophosphatemia, phosphaturia and aminoaciduria. All had elevated alkaline phosphatase activity and normal 25 OHD levels, apart from P1 (29.2 nmol/l). X rays confirmed marked osteopenia.

Table Clinical and biochemical characteristics at presentation

	P1	P2	P3	P4	P5	P6
Age in years, Gender	8, F	5, M	6, M	6, F	9, M	6, M
Duration on VPA	7y	4.5y	4.8y	5y	7.5y	3.5y
Immobility	Yes	Yes	No	Yes	Yes	Yes
Long bone fractures	1	3	1	3	3	1
Serum phosphate (1.0–1.8 mmol/l)	0.29	0.81	0.38	0.34	0.37	0.74
Serum alkaline phosphatase U/L	2219	1170	3375	1197	934	615
PTH ng/l (10–60)	12	21	6.7	12.1	54	52
25OHD (>50 nmol/l)	29.2	139	118	117	69	82.9
TmP GFR (1.14–2.44)	0.6	0.8	0.03	0.15	–	0.56
FGF23 (0–99 RU/ml)	–	–	195	–	219	136

VPA, Valproic acid; TmP GFR, renal tubular maximum reabsorption of phosphate per litre of glomerular filtration rate; FGF23, Fibroblast growth factor 23.

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 (osteomalacia). In addition, immobility in this severely disabled subgroup of children on VPA, contributes to poor bone health.
Disclosure
The authors declared no competing interests

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P032

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

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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, *z*-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 pmol/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 (SSIPol3) at 66% for age Z-score were -1 ± 1.0 (15% <-2), -0.7 ± 1.0 (7% <-2), -0.1 ± 1.3 (10% <-2) and -1.3 ± 0.71 (20% <-2) respectively, and less 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 ± 0.73) than 2.3–4.5 years (-1.07 ± 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.

Disclosure

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

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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 health center after reconstructive hip joint surgery. Anthropometric measurement carried out using WHO Anthro, WHO AnthroPlus (2009) soft ware. Following parameters were taken into account: weight/age, weight/height, height/age, body mass index (BMI)/age. Dual energy X-ray absortiometry 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 ± 2.6 years old. Eight girls and ten boys. Anthropometric measurement of researched group of patients: average body weight 21.47 ± 6.9 kg, average body height 124.5 ± 15.6 sm., average body mass index 13.7 ± 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 absortiometry 15 patients (83,3%) showed a decrease in bone mass compared with the age norm (Z-criterion is less than -2.0 S.D.), the average value is 3.5 ± 1.04. At the same time, the average value of Z-criterion, BMD L1-L4 vertebrae were within the age norm and made -0.91 ± 0.1 a positive correlation between the Z-criterion of BMD at the Whole body and BMI ($r=0.622$; $P=0.02$).

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 preoperative preparation to reduce the risk extravertebral fractures.

Disclosure

The authors declared no competing interests.

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P034

Identification of bone remodelling alterations in Gorham-Stout disease

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Objectives

Gorham-Stout disease (GSD) is a very rare disorder characterized by extensive angiomatous 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 ethiology 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.

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 ± 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 *TCIRG1*, *CTSK* and *MMP9* genes. pOCs from healthy donors treated with serum from patients showed an increase of osteoclastogenesis 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

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P036

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

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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].

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 [−5.6; −0.1] to −2.05 [−3.8; 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] mM/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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P037

Early fragility fractures in Zellweger syndrome spectrum – peroxisome dysfunction affecting osteogenesis?
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Background
Peroxisomal Biogenesis Disorders (PBD) is a group of rare metabolic diseases in which peroxisomal function is disrupted. PBD encompasses Zellweger Syndrome Spectrum (ZSS) disorders, which range in severity from classical ZS with severe neurological impairment and markedly reduced life expectancy to Refsum Disease presenting later in childhood. Recent fragility fractures in our ZSS patients in very early childhood prompted case series review.
Presenting problem
Amongst eight current ZSS patients (5M:3F, median age 2.3 years (range 0.7–19.4 years)), there were five fractures (at ages 0.5, 1.7, 4.5, 5 and 14.5 years): four femoral fractures (one patient had two femoral fractures) and one tibial and fibular following negligible force including normal handling. Patients presenting with fractures tended to have more severe neurological impairment.
Clinical management
All fractures were radiologically confirmed, minimally displaced and conservatively managed. Additional radiographic findings were osteopaenia ($n=5$), epiphyseal flattening ($n=2$), broad metaphyses ($n=1$), patellar stippling ($n=1$) and vertebral clefts ($n=1$). Those with fractures and older than 3 years had DEXA scans showing lumbar spine Bone Mineral Density (BMD) z scores −3.1 and −1.2. Intravenous bisphosphonates were commenced in the former (aged 14.5 years) and are under consideration for the latter (5 years). While the 14.5 year old had additional risk factors for reduced BMD (prolonged immobility, hypogonadism, anticonvulsants), three patients had femoral fractures at ages younger than expected purely from immobility.
Discussion
This case series demonstrates a high prevalence of fragility fractures in ZSS. A separate subgroup of PBD, rhizomelic chondrodysplasia punctata (RCDP), shares epiphyseal stippling as a common feature with ZSS. However fragility fractures are not a feature in RCDP. Reduced BMD in Peroxisomal Biogenesis Disorders can be multifactorial: hypotonia, immobility and impaired gonadal function. Peroxisomal dysfunction affecting bone formation is an additional possibility. Low BMD has been reported in ZSS, but fragility fracture before 5 years (our three ZSS patients) seems early to attribute to 'simple osteopaenia', supporting bone abnormality intrinsic to the PBD as the aetiology. This highlights the need for careful assessment of children with ZSS who sustain early fractures to determine the contributing factors and to inform judicious use of bisphosphonates.
Disclosure
The authors declared no competing interests.
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P038

Hypophosphatasia associated with acute disseminated encephalomyelitis (ADEM): causal relationship or coincidence?
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Background
Hypophosphatasia 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 PLP/PA 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 Hypophosphatasia 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 difficultly 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/l (range 20–140) with a PA of 39 nmol/l (9–60) giving a PLP/PA ratio of 8 (normal non-supplemented 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
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Objective
Cystinosis is a rare lysosomal storage disorder caused by *loss-of-function* mutations of the *CTNS* gene, encoding for cystinosin symporter that mediates cysteine efflux from lysosome. ~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-lysosomal 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 % WT: 16.43±0.62; KO: 12.22±0.59; $P<0.05$) and bone mineral density in 1-month-old KO mice (BMD mgHA/cm^3 WT: 194.01±4.04; KO: 158.02±6.00; $P<0.05$) with a decrease of trabecular number and thickness.

P1NP serum levels were reduced in KO samples compared to WT (P1NP ng/ml WT: 1130±42.48; KO: 931.6±40.52; $P<0.05$). Furthermore a reduction of *alp* and *col1a2* expression in femurs of KO mice was observed. *In vitro* analysis showed a reduced ALP positivity of KO osteoblasts with a decrease of *alp*, *osterix* and *col1a2* gene expression compared to WT cultures.
Conclusions
Our study shows that cystinosin deficiency could primarily affects bone cells, leading to bone loss phenotype of KO mice, independently from renal tubulopathy.
Disclosure
The authors declared no competing interests.
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P040

Atypical femoral fractures in 2 children treated with bisphosphonates
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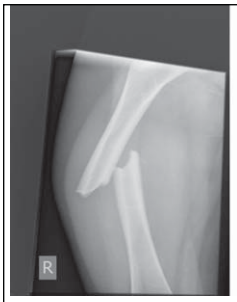
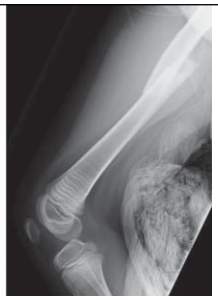
Background
Atypical Femur Fracture (AFF) has become widely reported as a complication of bisphosphonate therapy in adults since the first case report in 2005. A trend towards a similar pattern of fractures has been reported in children in Sheffield in 2012. A 13 year old boy was reported in 2014 with 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>G 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 children with milder bone disease.



AP radiograph of the 7 year old girl's femur showing a complete, short oblique fracture in the mid-shaft of the femur typical of an AFF.


Lateral radiograph of the same femur showing the fracture and 'Zebra' lines from pamidronate infusions.

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
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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 (25OHD) 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 Paulo, 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 25OHD 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 25OHD and HR-pQCT parameters. After 24 weeks, higher 25OHD 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 [ΔTb.N: 0.16(0.24) vs. 0.03(0.19), $P=0.024$] and a decrease in trabecular separation [ΔThSp: -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
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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. 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 (p.Thr196Valfs*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
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P043

Pseudohypoparathyroidism type IB: A cause of late hypocalcemia
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Background
Hypocalcemia presenting in adolescence is rare. Most common etiology is autoimmune hypoparathyroidism. Pseudohypoparathyroidism (PHP) is a rare group of disorders characterized by end-organ resistance to parathyroid hormone (PTH), and other hormones, such as TSH, with or without features of Albright's hereditary osteodystrophy.
Case presentation
A 14-year-old boy presented complaining of fatigue and spontaneous carpal spasms in association with a febrile viral illness. Past medical history was significant for an episode of asymptomatic hypocalcemia treated with calcium and alphacalcidol. He had discontinued therapy and he was lost to follow-up. Family history was remarkable for episodes of hand numbness for the mother, muscle cramps for maternal grandfather and carpal spasms and multiple fractures for maternal great grand mother. Physical examination revealed an adolescent with no dysmorphic features, normal height and weight, fully pubertal, with no skeletal abnormalities except for mild genu valgum. He had positive Chvostek and Trousseau sign. Laboratory investigation revealed markedly low serum calcium (5.3 mg/dl), phosphate (5.6 mg/dl), magnesium, albumin ALP and TSH were normal. PTH was markedly elevated (299.4 pg/ml). and he had vitamin D deficiency(16.5 μg/lt). ECG showed prolonged corrected QT interval. The patient was initially treated with calcium and alphacalcidol. In summary, this patient presented with PTH resistance, and no phenotypic signs of Albright's osteodystrophy, normal puberty, thyroid function and cortisol production, consistent with the diagnosis of PHP-I. A molecular genetic analysis was performed that revealed loss of methylation at the GNAS locus consistent with the diagnosis of PHP type Ib.
Conclusion
PHP-Ib is the result of defects in the methylation pattern of the complex GNAS locus and it can be inherited in an autosomal-dominant manner or may occur sporadically. The human GNAS gene is located on chromosome 20q13 and defects are heterogeneous. Genetic counselling is important for the patient and the family, as well as the need for life-long treatment with calcium and activated vitamin D.
Disclosure
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P044

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P045

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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
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Background
Congenital multiple hormone deficiency (CMHD) is a rare condition caused by mutations in transcription factors involved in pituitary ontogenesis[1]. 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 level[3].
Clinical management
After investigations for hypoglycemic hypotonia, deficit of growth and cranio-facial dismorfism 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 hypoplasic at brain MRI. The patient underwent periodic 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 expecially during stressfull 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 litterature 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 evalutation 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

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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 childhood cancer patients. The aim of the present study was to evaluate the serum level of (25(OH)D) in children treated for childhood cancer before 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 χ^2 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 (22.25 ± 11.91 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

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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.

Table 1 Clinical and biochemical characteristics of patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Gestational age	31w	27w	33w	39w+3	31w	term	34w+4
Diagnosis	Presumed cow's milk allergy	GORD, cow's milk intolerance	Pierre-Robin Syndrome, cleft palate, CP, GORD, NJ fed	CHARGE syndrome, GORD, PEG fed	FTT, GORD, PEG-NJ fed	SCID, BMT, GVHD on steroids, refusing feed	SOD, GORD, 8q12.3del, tectal plate glioma, NJ fed
Age at presentation	6 months	10 months	11 months	18 months	9 months	3 years 2 m	5 months
Exclusively on Neocate before presentation (duration)	3 months	10 months	>8 months	>6 months	5 months	15 months	5 months
Ca(mmol/L)	2.48	2.52	2.6	2.49	2.57	2.47	2.38
Phosphate (mmol/L)	1.23	0.68	0.83	1.04	0.84	1.34	0.63
ALP (IU/L)	1000	1000	3469	431	1653	419	2206
PTH (ng/L)	24	19.8	17	5.6	21	31	Normal
25-OH-VitaminD (nmol/L)	59.7	86	88	347	76	131	110
TRP (%)	99.5	–	99.8	>97	NA	NA	NA

Abbreviations: GORD: gastro-oesophageal reflux disease; CP: cerebral palsy; NJ: naso-jejunal; PEG: percutaneous endoscopic gastrostomy; FTT: failure to thrive; SCID: severe combined immunodeficiency; BMT: bone marrow transplant; GVHD: graft versus host disease; SOD: septo-optic dysplasia;.NA: not available.

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P049

Difficulties in diagnostics and clinical classification of osteogenesis imperfecta in Poland

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Introduction

Osteogenesis imperfecta (OI) is a genetically determined bone dysplasia characterised predominantlyby recurrent fractures, reduced bone mineral density and some clinical features connected with colagenopathy. However, not all patients have exhibit all this 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 ($P<0.05$).

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.

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Disclosure
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P050

The prevalence of fragility fractures in children with cerebral palsy in Greater Manchester, UK-a cross-sectional survey
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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 ($x^2=7.14$, 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)
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Background
Trisomy 9p is a rare abnormality caused by duplication of the short arm of chromosome 9. Translocation *t*(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/hand anomalies, delayed bone maturation. Low bone mineral density (BMD) or fragility fractures have never been reported.
Presenting problem
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 calciuria (boy: 56 mg/24 h; girl: 58 mg/24 h; normal range 100–250 mg/24 h) with normal phosphaturia. 25OH-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
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P052

Hypophosphatasia - from symptom to diagnosis - case report
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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 (1p36.1-34). 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
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P053

Determinants of bone density in Duchenne muscular dystrophy

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Objectives
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 age 9.9 ± 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 BMAD ≤ 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 25OH-vitamin D levels < 20 ng/ml. Bone formation markers (OC, BSAP) were within normal range for age, while bone resorption markers (CTx) 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 25OH-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 25OH-vitamin D status, IL-6 levels, imbalance between RANKL and OPG with insufficient compensation due to Dkk1 reduction.
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Disclosure
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P054

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

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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.
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.
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.
Results
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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P055

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P056

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

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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
The conservative management of the 39 patients with rickets-like diseases involved 4 stages (tab1). 1st stage included complete examination of the patient referring determination of the calcium and phosphorus in blood and urine, calcidiol and calcitriol in blood, parameters of parathyroid hormone and osteocalcin as well as marker of bone formation P1NP and that of bone resorption B-CTx. At the first stage, it was obligatory for all children to go through genetical testing. The goal of the study was detection of alterations (polymorphism) in the alleles of receptors to vitamin D (VDR) and to collagen type 1 (COL1). Examinations and correlations (tab. 2,3) at the next stages were conducted completely except genetical studies.
Results
Comprehensive study of vitamin D metabolism and biochemical parameters of osseous tissue's vital activity among patients with VDDR type 2 allowed us to have fundamentally studied some points in the pathogenesis and nature of osteomalatic and subsequently osteoporotic alterations at the deferent extent.
Summary & Conclusion
On the ground of biochemical parameters' study among the patients with vitamin D-dependent rickets type 2 we developed pathogenetically-substantiated effective pharmacological therapy of orthopedic manifestations. The therapy is based upon administration of higher doses of vitamin D (up to 70000 U/month at the first

stage, then down to 45000 U/month) and alfacalcidol (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 in rickety process level of the hormonal form of vitamin D (calcitriol) should be limited within range 250–350 ng/ml ($P < 0.05$). There is no reason to use alfacalcidol 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.

	Stage of treatment			
Indices of bone metabolism	Stage 1 M±m	Stage 2 M±m	Stage 3 M±m	Stage 4 M±m
Ca+	1.24±0.0097	1.28*±0.0104	1.28*±0.0154	1.32*±0.0087
P	1.71±0.0309	1.637368±0.0583	1.724±0.0336	1.79±0.1579
Ca	2.51±0.0158	2.57**±0.0257	2.58**±0.0250	2.60**±0.0452
25(OH)D	34.51±3.3952	68.05*±4.9992	77.60*±8.3518	73.18*±20.2909
1,25(OH)$_2$D	149.92±8.8943	276.79*±22.6938	296.60*±24.7957	306.50*±35.7736
PTH	32.87±2.6910	25.20**±3.2285	23.45**±2.9311	24.125±4.0828
Osteocalcin	33.90±4.9894	16.19*±2.0041	15.65**±2.0247	19.45±4.1949
Urine calcium (daily)	1.74±0.1288	2.047895±0.3555	1.4±0.2658	1.4225±0.4731
Urine phosphorus (daily)	17.16±1.6125	14.72105±1.5853	11.55±0.8034	9.05**±0.9350
P1NP	895.82±41.2641	662.95*±46.7270	567.6*±35.3347	583.25*±31.7579
B-CTx	1.51±0.0685	1.14*±0.0910	1.01*±0.1072	1.10*±0.1871

* - 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).

Correlations (marc vdzr2.sta)
Marked correlations are significant at p <,05000
N=39 (Casewise deletion of missing data)

	Ca+	P	Ca	25(OH)D	1,25(OH)2D	PTH	osteocalcin	urine calcium (daily)	urine phosphor (daily)	P1NP	B-CTx
Ca+	1,00	0,28	0,32	0,40	0,33	-0,12	0,01	-0,06	-0,10	-0,14	-0,10
P	0,28	1,00	-0,01	0,21	0,31	-0,36	0,02	0,09	-0,25	0,05	-0,52
Ca	0,32	-0,01	1,00	0,26	0,16	-0,23	-0,05	-0,11	-0,25	-0,06	0,08
25(OH)D	0,40	0,21	0,26	1,00	0,67	-0,20	-0,23	0,03	-0,31	-0,10	-0,24
1,25(OH)2D	0,33	0,31	0,16	0,67	1,00	-0,14	-0,06	-0,03	-0,17	0,05	-0,18
PTH	-0,12	-0,36	-0,23	-0,20	-0,14	1,00	0,18	0,26	-0,54	0,29	0,28
osteocalcin	0,01	0,02	-0,05	-0,23	-0,06	0,18	1,00	0,24	0,09	0,03	0,32
urine calcium (daily)	-0,06	0,09	-0,11	0,03	-0,03	0,26	0,24	1,00	0,21	-0,27	-0,25
urine phosphor (daily)	-0,10	-0,25	-0,25	-0,31	-0,17	-0,54	0,09	0,21	1,00	0,10	0,01
P1NP	-0,14	0,05	-0,06	-0,10	0,05	0,29	0,03	-0,27	0,10	1,00	0,41
B-CTx	-0,10	-0,52	0,08	-0,24	-0,18	0,28	0,32	-0,25	0,01	0,41	1,00

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

Correlations (marc vdzr2.sta)
Marked correlations are significant at p <,05000
N=19 (Casewise deletion of missing data)

	Ca+	P	Ca	25(OH)D	1,25(OH)2D	PTH	osteocalcin	urine calcium (daily)	urine phosphor (daily)	P1NP	B-CTx
Ca+	1,00	0,03	0,49	0,28	0,44	-0,57	-0,20	0,03	-0,54	0,02	-0,10
P	0,03	1,00	-0,03	-0,01	0,09	-0,05	-0,01	0,09	-0,60	0,11	-0,52
Ca	0,49	-0,03	1,00	0,43	0,51	-0,61	-0,55	-0,03	-0,37	-0,05	-0,27
25(OH)D	0,28	-0,01	0,43	1,00	0,67	-0,53	-0,23	0,01	-0,32	-0,09	-0,32
1,25(OH)2D	0,44	0,09	0,51	0,67	1,00	-0,34	0,01	0,02	-0,21	0,07	-0,40
PTH	-0,57	-0,05	-0,61	-0,53	-0,34	1,00	0,72	0,06	0,62	0,22	0,40
osteocalcin	-0,20	-0,01	-0,55	-0,23	0,01	0,72	1,00	-0,06	0,32	0,09	0,19
urine calcium (daily)	0,03	0,09	-0,03	0,01	0,02	0,06	-0,06	1,00	0,54	0,10	0,69
urine phosphor (daily)	-0,54	-0,60	-0,37	-0,32	-0,21	0,62	0,32	0,54	1,00	0,08	0,38
P1NP	0,02	0,11	-0,05	-0,09	0,07	0,22	0,09	0,10	0,08	1,00	0,38
B-CTx	-0,10	-0,52	-0,27	-0,32	-0,40	0,40	0,19	0,69	0,38	0,38	1,00

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P057

Zebrafish as a model for hypophosphatasia

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Objectives

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 isoforms 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 RNAs 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.

Disclosure

Franz Jakob received honoraria for lectures and advice from Alexion.

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P058

The relationship between maternal and child bone density in Nigerian children with and without nutritional rickets

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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 multivariate 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 ($P=0.48$).

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

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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 activities. Most of these had transient hypophosphatasemia. However, 32 (6.1%) patients were identified as study-positive for persistent low ALP activity. Of these, four patients had been evaluated for rheumatologic problems, 12 patients with orthopaedic problems (unexplained fractures), two patients for endocrine problems (short stature), two patients for respiratory failure in the first years of age, three patients for neurosurgical problems (craniosynostosis and intracranial hypertension), one patient nephrology problems, two patients poor feeding and failure to thrive, and six patients for neurological problems (recurrent seizures). No patient had been recalled for such low values in suspicion of hypophosphatasia.

Conclusions

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, with the availability of enzyme replacement therapy, can be life-saving or avoid years of undiagnosed morbidity. In our patients, a retesting with pyridoxal-5'-phosphate and phosphoethanolamine concentrations and subsequently for ALPL gene mutations will be necessary for confirm the suspicion of hypophosphatasia.

Disclosure

The authors declared no competing interests.

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P060

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P061

Renal tubular acidosis with an elevated urinary β-2 microglobulin in a boy presenting with sporadic hypophosphataemic rickets and intellectual disability (Dent's Disease)

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Background

X linked hypophosphataemic 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 phosphatase (524 u/l), and a decreased tubular reabsorption of phosphate (TRP) of 85%.

Clinical management

With a clinical picture suggestive of X linked hypophosphataemic 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 β-2 microglobulin 27200 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[1]. 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 hypophosphataemic 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

1. Devuyst O and Thakker RV. Dent's Disease. Orphanet Journal of Rare Diseases 2010; 5:28.

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P062

Lysinuric protein intolerance presenting with short stature and osteoporosis

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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 (<3.p), height 135 cm (<3.p), 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 hemogram, hepatic and renal function tests, serum calcium, phosphorus, and parathormone were normal. His serum alkaline phosphotase was

461 U/l, 25(OH)D 15.7 ng/ml, trigliseride 156 mg/dl, ferritin 336 ng/ml, and LDH 499 U/L, and levels of ammonia were variable. Hypercalciuria or proteinuria was not detected. Lumbar spine DXA Z-score was −3.6 and X-ray revealed vertebral compression fracture. His nutrition patern with osteoporosis causing us to suspect LPI, but plasma and urine concentrations of cationic amino-acids were found normal twice.
Clinical management
His osteoporosis was 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. *SLC7A7* is the only gene in which mutation is currently known to cause LPI.
Disclosure
The authors declared no competing interests.

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P063

Effects of KRN23, a fully human anti-FGF23 monoclonal antibody, on functional outcomes in children with X-linked hypophosphatemia (XLH): results from a randomized, open-label Phase 2 study

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Objectives
In XLH, musculoskeletal outcomes of current treatment with oral phosphate (Pi)/active vitamin D are suboptimal for many patients. In a Phase 2, open-label study, we tested the hypothesis that KRN23 improves rickets and functional outcomes in XLH children.
Methods
Fifty-two children with XLH (ages 5–12 years at baseline) received KRN23 subcutaneously biweekly (Q2W) or monthly (Q4W). At study entry, most participants had received oral Pi/active vitamin D for a mean duration of ∼7 years. Rickets severity score, walking ability (six-minute walk test [6MWT]), and patient-reported pain and functional disability (Pediatric Outcomes Data Collection Instrument [PODCI]) were assessed at baseline and at Week 40 (Wk40).
Results
Serum Pi was increased significantly through 40 weeks. Mean Thacher Rickets Severity Score improved from baseline by 61% for Q2W, 37% for Q4W, and 50% overall (each $P<0.001$). Walking ability also improved from mean distance by +23 m at Baseline to Wk40 by ($P=0.0037$). At Baseline, 24/52 (46%) children had walking impairment defined as 6MWT distance <80% predicted for age. Among those with impaired walking, 6MWT distance increased from 70% of that predicted to 80% (Q2W dosing, $n=14$) and from 66% to 71% (Q4W dosing, $n=10$). KRN23 treatment was also associated with significantly improved pain and functional ability. Overall at Wk40, the Sport/Physical Function domain improved by +9.5, Pain/Comfort by +7.5, and Global Functioning by +8.6 (each $P<0.0001$). Substantial functional impairment at baseline (PODCI Global Functioning score <40) was present in 28/52 (54%) children (mean score of 25.2; >2 SD below the normal mean of 50, 1 SD=10). The domains of Sports/Physical Function and Pain/Comfort were particularly affected (Baseline means of 22.0 and 23.9 respectively). At 40 weeks of KRN23 treatment, the Global Functioning, Sports/Physical Function, and Pain/Comfort domain scores increased by +17.0, +16.7, and +17.4, respectively (each $P<0.0001$), bringing values into or near the normal range.
Conclusion
In children with XLH, impairments in walking ability, physical function, and pain persist despite standard of care treatment. Our data suggest that KRN23 substantially improves these key functional outcome measures.

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; Padidela: consulting f.

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P064

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

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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, ulnar, 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

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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 D_2/D_3 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 6 00 000 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 isoleucine 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

1. Roizen J., *et al.*, Presentation No. 1063 ASBMR Annual Scientific Meeting, Atlanta, GA, USA, September 2016.

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P066

Unusual presentation of acquired hypophosphataemic rickets

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Background

Acquired hypophosphataemic rickets is an unusual presentation in children and usually consequent on renal tubular damage. One of the factors important in phosphate homeostasis is FGF23. Healthy individuals maintain normal phosphate homeostasis by coupling FGF23 production with proteolytic cleavage. Iron deficiency stimulates FGF23 transcription and is a novel mechanism of FGF23 elevation. We present two children who presented with iron deficiency anaemia and hypophosphataemic rickets, which we postulate was due to elevated FGF23 levels.

Presenting problem

Two children, 5 year old male (Case 1) and 7 year old female (Case 2), presented with rachitic deformities of the lower limbs and a history of pica. Both children were stunted (HAZ score -2.59 and HAZ score -3.16) with genu valgum deformities at the knees, had frontal bossing and pallor. Radiological findings confirmed the presence of active rickets and biochemical findings confirmed hypophosphataemic rickets and anaemia in both cases (Table 1).

Clinical management

Table 1 Biochemical findings of case 1 and case 2.

Laboratory investigations (normal range)	Case 1 Initial	Case 1 Follow-up 9 months	Case 1 Follow-up 18 months	Case 2 Initial	Case 2 Follow-up 9 months	Case 2 Follow-up 18 months
Total Calcium (2.10–2.60 mmol/l)	2.13	2.20	2.33	2.24	2.49	2.33
Phosphate (1.4–1.8 mmol/l)	1.15	1.45	1.51	0.97	1.89	1.77
Alkaline phosphatase (<350 IU/l)	454	382	324	623	311	314
Parathyroid hormone (<6.4 pmol/l)	5.1	1.6	2.1	3.0	2.8	2.4
Haemoglobin (>12 g/dl)	5.0	11.7	12.3	5.4	11.5	11.7
MCV (77.1–91.5 fL)	58	79.4	80.5	–	74.7	73.7
MCH (25.8–31.7 pg)	14.3	26.2	26.6	–	24.1	24
Thacher Xray score (maximum of 10)	7	0	–	9	0	–

Both cases were treated with oral phosphate supplements, one alpha vitamin D and ferrous gluconate. The rickets healed and anaemia 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 anaemia 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 hypophosphatasia

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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).

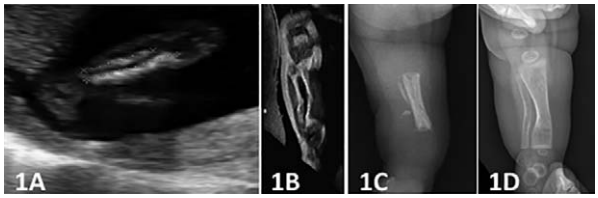

Figure 1 Right tibia and fibula. A: Normal at 20 weeks GA; B: Fractures and rickets-like changes at 31 weeks GA; C: At birth, shows shortened and hypomineralised bones; D: Improvement in bone mineralisation following 5 months treatment with asfotase alfa.

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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P068

Craniosynostosis can occur in children with nutritional rickets

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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 craniosynostosis 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: Afrocarribean or Asian ethnic backgrounds with darker skin pigmentation ($N=5$); multiple food intolerances ($N=2$) and prolonged breast feeding with picky 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 <20 nmol/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 33–44 nmol/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.

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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)

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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 (pyrodoxal-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, 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 *varum*, 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/cm^2. 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.

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P070

The abnormally high and heterogeneous bone matrix mineralization after childhood solid organ transplantation is not further increased by bisphosphonate treatment

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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 transiliac bone biopsies obtained from kidney ($n=9$), liver ($n=9$) and heart ($n=5$) transplant recipients (aged 7.6–19.7 years, 6.0 ± 5.6 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, the 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 (fivefold; 10-fold, both $P<0.0001$), in heterogeneity of mineralization ($+26{,}5\%$ and $+27.8\%$, both $P<0.0001$), 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.

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P071

In search of hypophosphatasia: a need to establish normative data for low alkaline phosphatase in pediatric population

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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 under treatment ($n=14$), 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.
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P072

Raised intracranial pressure in a boy with Pycnodysostosis with open fontanelles

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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.
Presenting problem
We describe a 13-year-old boy with PDO who developed raised intracranial pressure (ICP) which led to visual deterioration and a fracture of the cribriform plate.
Clinical management
The patient presented at the age of 6 years with insidious visual deterioration. The CT brain showed papilloedema secondary to raised ICP, which was confirmed by invasive manometry. He was treated with acetazolamide 100 mg three times a day. At the age of 8 years, he had developed persistent rhinorrhoea from his left nostril, which was confirmed to be cerebrospinal fluid. Follow-up CT scan of the head showed open fontanelles (Fig. 1A and B) and a 3.8 mm defect in the left cribriform plate (Fig. 1C), which was repaired after insertion of a ventricular peritoneal shunt.
Discussion
It is intriguing that some patients with PDO develop raised intracranial pressure in spite of open fontanelles. In our patient it led to visual deterioration and fracture of the to cribriform plate fracture.
Conclusion
Patients with PDO can develop raised ICP in spite of open fontanelles. Therefore regular visual examinations should be undertaken and enquiry about the symptoms of raised ICP should be sought during clinical reviews.
Disclosure
The authors declared no competing interests.

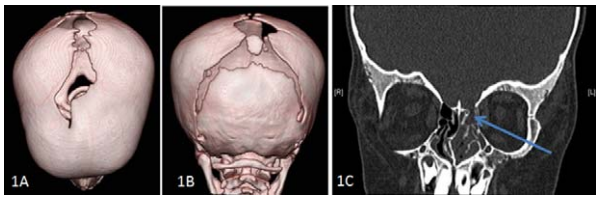

Figure 1 (A and B) Three-dimensional reconstruction of cranial CT scans of the patient showing open anterior (A) and posterior (B) fontanelles. (C) Coronal section of the CT scan showing the 3.8 mm defect in left cribriform plate.

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P073

Spectrum of paediatric hypophosphataemic rickets in a tertiary centre

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Background
Hypophosphataemic rickets is a rare form of rickets characterised by hypophophatamaemia 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 chidren with hypophosphataemic rickets, both their clinical and bichemical 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 (ENNP1) mutation. One child had craniosynostosis, one had coeliac disease. All children were treated with a combination of phosphate sandoz and alfacalcidol. 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.
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P074

High bone turnover markers and disturbances of bone mineral density in children with hypophosphataemic rickets

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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 pirydynolin 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 (QUS) of the tibia and radius was also performed.
Results
Hyperphosphaturia was observed in all cases (6/6), 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.

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Disclosure
The authors declared no competing interests.

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P075

Improvement of bone density in eating disorders correlates with improvements in growth

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Introduction
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) cm^2 at baseline to 1875(1510–2321) cm^2 at follow-up ($P<0.001$), and LS BA increased from 36.3(28.1–44.2) cm^2 at baseline to 37.7(31.8–47.2) cm^2 at follow-up ($P=0.002$). TB percent predicted BMC for BA decreased from 98.2(89–108)% 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. Faltering 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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P076

Abstract withdrawn.

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P077

The role of bone age in the evaluation of trabecular bone score (TBS) of children and adolescents 5–19 years old

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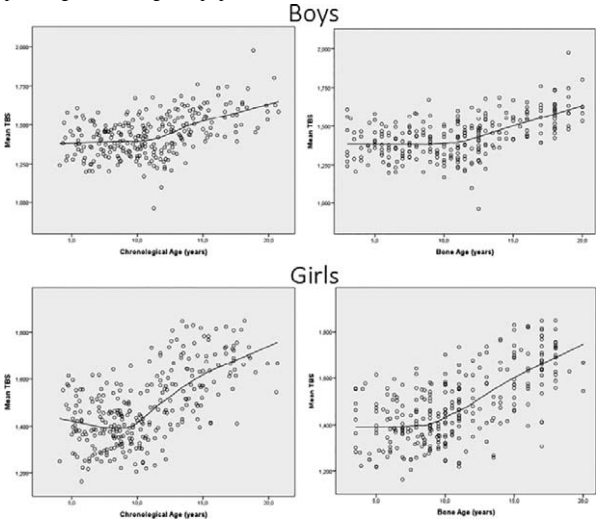
Rationale
Trabecular Bone Score (TBS) is a texture-based tool analyzing DXA images in order to assess bone microarchitecture in the lumbar region. In pediatric population, definition of normative values has remained elusive due to the disparities of results in normal population, probably link to uncontrolled 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.

Results
When chronological age was used, girls' curve showed decreasing phase delineating a 'U' shape1. 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.



Disclosure
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P078

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P079

Assessment of a semi-automated software program for the identification of vertebral fractures in children
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Purpose
We aimed to assess observer reliability and diagnostic accuracy in children, of a semi-automated 6-point technique developed for vertebral fracture diagnosis in adults, which records percentage loss of vertebral body height.
Methods
Reading 137 spine radiographs of children and adolescents, diagnostic accuracy (sensitivity, specificity and 95% confidence interval) calculations of five observers' for SpineAnalyzer were calculated. Comparison was made with a previously established consensus arrived at by three experienced pediatric radiologists using a simplified algorithm based qualitative scoring system (sABQ).
Results
Of a total of 1781 vertebrae, 1187 (67%) were adequately visualized by 3 or more observers. Overall, 20 (15%) patients had one or more VF (vertebral height loss 20% or more). Interobserver agreement in vertebral readability for each vertebral level for five observers ranged from 0.05 to 0.47 (95% CI, −0.19, 0.76). Intraobserver agreement using the intraclass correlation coefficient (ICC) ranged from 0.25 to 0.61. Overall sensitivity and specificity were 18% (95% CI, 14–22) and 97% (95% CI, 97–98) respectively (Fig. 1).

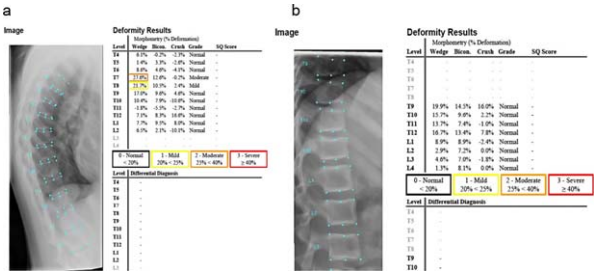

Figure 1 (a) False positive SpineAnalyzer result. Wedging of T7 and T8 as indicated by SpineAnalyzer was reported by the consensus expert panel as physiological, rather than pathological wedging. (b) False negative SpineAnalyzer result. T11, T12 and L2 were reported by the consensus expert panel as fractured 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.
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P080

Bone age assessment using Greulich and Pyle and Tanner-Whitehouse methods: a systematic review
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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.

Disclosure
The authors declared no competing interests.
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P081

Bone age determination using dual-energy X-ray absorptiometry
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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. $\pm$3.8) and 9.8 (S.D. $\pm$3.2) years for girls and boys respectively. A significant difference between DXA scans and radiographs ($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
This study was funded by a grant from the Skeletal Dysplasia Group for Teaching & Research.
Disclosure
Dr Amaka Offiah 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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P082

Impact of age, sex, location of injury, physical activity, vitamin D and calcium intake on the injury outcome of wrist and ankle in children
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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 it 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 facture) 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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P083

Schmorl's node and vitamin D deficiency: cause or coincidence
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Pathologist Christian Georg Schmorl described a specific type of vertebral lesion, which is now known as Schmorl's node. A Schmorl's node or intradiscal herniation is herniation of nucleus 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-male 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.

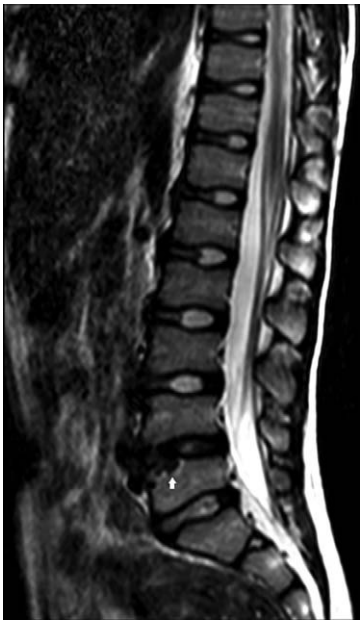

Figure 1 A Schmorl's node in the superior endplate of L5 vertebra.

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 nodule 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 factor on developing of Schmorl's nodes.

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?

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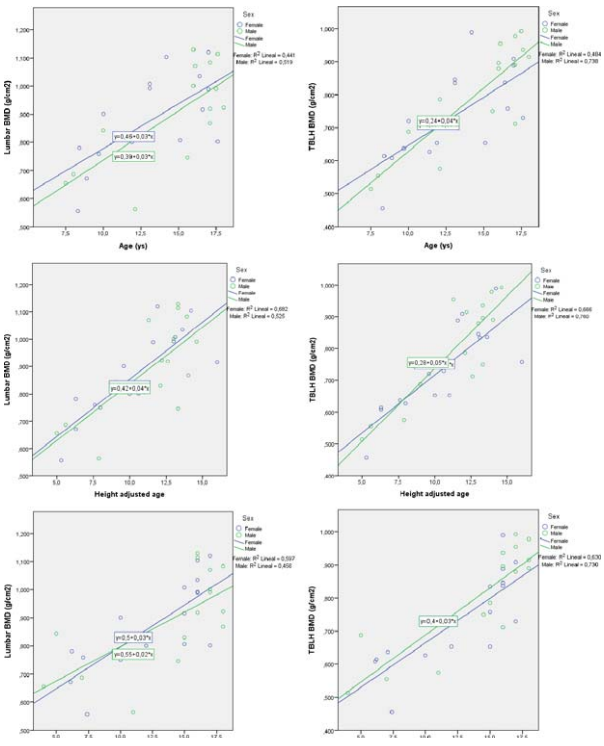
Chronic kidney disease in children causes multiple bone alterations, particularly renal osteodystrophy, which affects both bone quality and size, in turn causing short stature, bone deformities and brittleness. Once they get a transplant, this process starts to revert, and although mineral alterations 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, R^2 values were obtained.



Results

R^2 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.

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P085

Cumulative radiation exposure from diagnostic imaging and associated lifetime cancer risk in children with Osteogenesis Imperfecta

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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 ($P=0.004$) but not different to Type IV ($P=0.059$). Across OI types, fracture-negative X-rays and cumulative E was 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.

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P086

Feasibility and reproducibility using HRpQCTII in children and adolescents

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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 criterion (≤ 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 I ≤ 2 I ≤ 3).

	5-10 years (N=21)	11–15 years (N=22)	16–21 years (N=17)	All Ages (N=60)
Distal Radius	52% I 71% I 76%	50% I 64% I 95%	65% I 88% I 100%	55% I 73% I 90%
Distal Tibia	43% I 62% I 71%	82% I 96% I 100%	71% I 88% I 100%	65% I 82% I 90%
30% Radius	48% I 62% I 76%	68% I 82% I 91%	71% I 94% I 100%	62% I 75% I 88%
30% Tibia	71% I 86% I 95%	100% I 100% I 100%	88% I 94% I 94%	87% I 93% I 97%

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

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

	BMD	Ct.BMD	Ct.Th	Ct.Po	Tb.BM D	Tb.N	Tb.Th	Failure Load
Distal Radius	0.40%	0.83%	1.27%	16.3%	0.55%	1.35%	0.86%	3.7%
Distal Tibia	0.27%	0.61%	1.57%	7.4%	0.43%	1.47%	0.78%	3.4%
30% Radius	0.18%	0.23%	0.38%	5.59%	-	-	-	0.38%
30% Tibia	0.46%	0.35%	0.94%	5.07%	-	-	-	0.70%

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P087

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

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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.

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P088

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

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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 ($R^2 \geq 0.66$) as well as whole body and lumbar spine BMD measurements ($R^2 \geq 0.72$).

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.

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P089

Cross Calibration of GE Lunar DPX Pro and GE Lunar iDXA

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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 measurements as the dependent variable and iDXA as the predicting variable. These equations have been represented in the following figures: Figure 1a and 1b log transformed values of TBLH BMC of DPX against iDXA for males and females respectively; Figure 2a and 2b log transformed values of TBLH BA of DPX against iDXA for males and females respectively. The observed DPX values and predicted values were similar ($P > 0.1$). The TBLH BMC and TBLH BA z-scores were calculated for both values. The z-scores were tested on the validation sample and it was found that the bone status as defined by z-score remained similar after the use of predicted BMC values.
Conclusions
In the absence of reference data for the iDXA, the z-scores from the predicted TBLH BMC and TBLH BA may be used to assess Indian children's bone status.
Disclosure
The authors declared no competing interests.

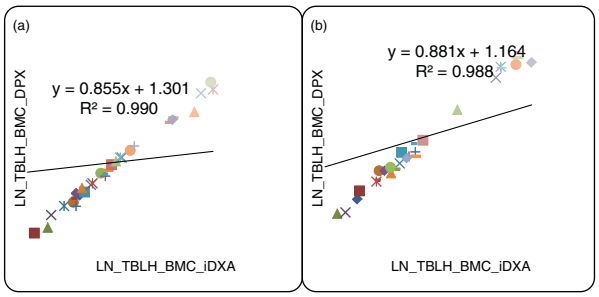

Figure 1

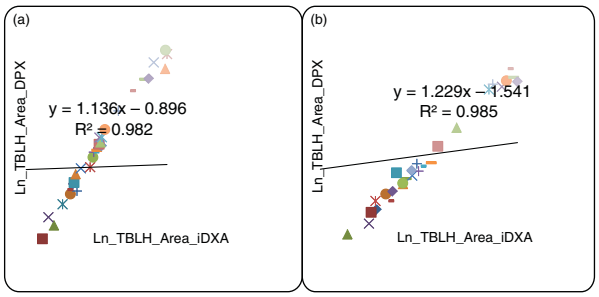

Figure 2

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P090

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

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Background
The role of untrained observers in evaluating vertebrae 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 vertebra 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 (κ) 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 (T5-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 height ratio 7.9 and 9.7% on SXR and DXA. Intra-observer agreement of VF grade from was substantial in both imaging modalities' (iDXA, κ = 0.61; SXR, κ = 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.

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P091

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

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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).
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 clinic 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 0.991 to 0.995), indicating almost perfect reliability. Mean difference of DXA Ht SDS with clinic measurements was −0.099, with upper

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 −0.393, 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

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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 phalanxes 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 phalanxes by Bone Profiler-IGEA (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

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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

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Objectives
Bone disease is a long-term complication in patients with thalassaemia and therefore current UK guidelines recommend biannual 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

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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 between 4 and 8 years, LCPD is of unknown ethology. An increased incidence has been stated in case of GH 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: 8 years 8 months old boy, addressed for growth retardation. At 8 years 3 month he was diagnosed with LCPD of the right hip. He was small for his age (115 cm, −3.2 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, −2.54 S.D.) and significantly delayed bone age (2 years) and GHT was started: Somatropin 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-up 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

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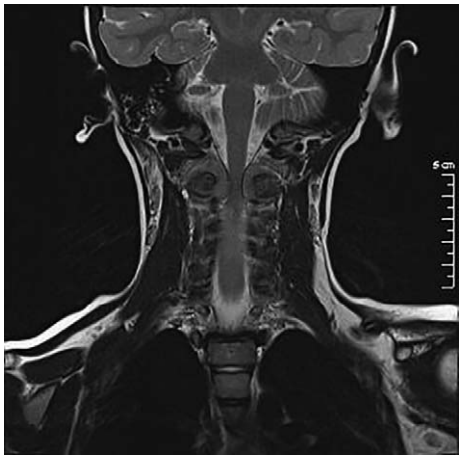
Background
Torticollis in toddlers is most frequently a manifestation of traumatic atlantoaxial rotatory displacement or oropharyngeal inflammation, and rarely due to retropharyngeal abscesses and pyogenic cervical spondylitis. Rarely, intermittent torticollis may be caused by posterior fossa tumors. 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, encasing 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, phenothiazine-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

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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 QCT CHOP protocol and pQCT standard analysis between baseline (age 8.0 ± 1.2 years) and follow-up (age 11.4 ± 1.1 years). Results of the two methods correlated highly (all $r > 0.7$ and $P < 0.001$), although there was a mean offset in values attributable to the 0.06 g/cm 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 min 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

Marjanovic EJ, Ward KA, & Adams JE. The impact of accurate positioning on measurements made by peripheral QCT in the distal radius. *Osteoporosis International* 2009 **20** 1207–1214.

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P098

Preliminary precision-error estimates of bone mineral density in children with cerebral palsy

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Objectives

Dual Energy X-ray Absorptiometry (DXA) is commonly used to monitor changes in bone mineral density (BMD). Small changes in BMD can be clinically meaningful; therefore precision-error calculations are needed to estimate true changes in BMD. The few published studies of precision errors in children with cerebral palsy (CP) are diverse in terms of ethnicities and medical comorbidities. Application of these estimates is inappropriate to our particular Midwest American cohort. Statutes in our state also limit precision studies in children. Therefore, a research project was undertaken to calculate the BMD precision error at multiple skeletal sites in the CP patients seen at our institution.

Methods

Ten of the required 30 participants aged 3–18 years with CP (GMFCS I-III) underwent two DXA scans (Hologic) by the same technologist with repositioning. BMD was measured at spine, hip, left/right distal femur, and whole body. Precision error and least significant changes were calculated according to ISCD recommendations.

Results

Ten participants (15 ± 4 years, range 7–18 years, 6 male) have undergone repeated DXA scans. An additional 10 patients are scheduled, and all 30 subjects are expected to be complete by April. Three patients had low bone mineral density for age. Preliminary calculations of Least Significant Change using bootstrapping approach indicated a value of 0.034 g/cm^2 for the whole body (minus the head) and 0.178 g/cm^2 for the lateral distal femur. These preliminary values are expected to change once we reach the required 30 patients, and also allow for error calculations at each skeletal site.

Conclusion

Despite the significant heterogeneity in our Midwest CP population, especially in regard to their neuromuscular effects upon bone health, useful estimates of precision error were obtained at a number of regions of interest. This will allow us to continue to use DXA as a tool for long term bone health management**.** We conclude, therefore, that DXA BMD precision estimates can (and should) be done on the typical cerebral palsy pediatric population seen at each center or use estimates from geographically similar sites. We anticipate the need for similar studies in children with GMFCS classification IV–VI. This work was supported by Gillette Children's Hospital Foundation.

Disclosure

The authors declared no competing interests.

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P099

Bone strength and microarchitectural deficits in children with cystinosis

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Children with cystinosis have numerous risk factors for impaired bone accrual. We used state-of-the-art quantitative imaging of bone micro-architecture (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. Micro-finite element analysis (μFEA) was applied to estimate bone strength. One-way ANOVA regression, adjusting for age and sex was used to 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 (-25%, $p < 0.008$) 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

	Distal Radius			Distal Tibia		
N	CYST 18	CTRL 32	*P*-value*	CYST 20	CTRL 34	*P*-value*
Total-Area	172 ± 81	193 ± 67	0.04	628 ± 239	717 ± 215	0.06
BMD	310 ± 53	313 ± 53	0.91	222 ± 31	261 ± 47	0.002
Ct.Ar	40 ± 14	46 ± 17	0.007	61 ± 16	78 ± 29	0.003
Ct.BMD	788 ± 76	763 ± 83	0.13	771 ± 62	757 ± 83	0.28
Ct.Po	0 ± 0	0.01 ± 0	0.003	0 ± 0	0.01 ± 0.01	0.003
Ct.Th	1.0 ± 0.2	1.1 ± 0.3	0.46	0.8 ± 0.1	0.9 ± 10.3	0.02
Tb.Ar	134 ± 71	149 ± 54	0.12	572 ± 228	639 ± 197	0.17
Th.BMD	153 ± 32	167 ± 34	0.15	161 ± 32	198 ± 31	0.0002
BV/TV	0.2 ± 0.05	0.23 ± 0.05	0.09	0.23 ± 0.04	0.28 ± 0.05	0.0001
Th.N	1.4 ± 0.2	1.5 ± 0.2	0.29	1.5 ± 0.2	1.6 ± 0.2	0.002
Th.Th	0.21 ± 0.02	0.22 ± 0.02	0.08	0.23 ± 0.02	0.25 ± 0.02	0.009
Th.1/N.SD	0.27 ± 0.09	0.24 ± 0.07	0.35	0.27 ± 0.08	0.23 ± 0.04	0.01
AppMod	1505 ± 364	1660 ± 315	0.13	1106 ± 246	1479 ± 412	0.0006
FailureLoad	2695 ± 1076	3135 ± 1564	0.08	6353 ± 2189	8227 ± 5044	0.08
Ct.LF.Dist	46 ± 8	39 ± 10	0.02	34 ± 10	26 ± 7	0.001

Table 2

	Diaphyseal Radius			Diaphyseal Tibia		
N	CYST 17	CTRL 31	*P*-value*	CYST 20	CTRL 32	*P*-value*
Total-Area	65 ± 20	81 ± 24	< 0.0001	233 ± 76	279 ± 68	< **0.0001**
BMD	820 ± 101	855 ± 78	0.06	704 ± 61	707 ± 76	0.83
Ct.Ar	53 ± 15	69 ± 21	< 0.0001	165 ± 56	203 ± 58	0.0003
Ct.BMD	991 ± 76	1017 ± 52	0.01	967 ± 55	960 ± 51	0.59
Ct.Po	0.03 ± 0.02	0.02 ± 0.01	0.002	0.03 ± 0.03	0.04 ± 0.03	0.19
Ct.Th	2.7 ± 0.5	3.1 ± 0.6	0.0004	4.3 ± 0.9	4.9 ± 1.1	0.01
Tb.Ar	13.0 ± 7.4	13.6 ± 4.7	0.56	71 ± 27	79 ± 23	0.09
AppMod	7904 ± 677	8442 ± 691	**0.002**	7516 ± 654	77091681	0.22
FailureLoad	3219 ± 1031	4243 ± 1376	< **0.0001**	10116 ± 3667	12388 ± 4074	0.0006

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P100

Decreased bone turnover in HIV-infected children on antiretroviral therapy

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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), procollagen type-1 *N*-terminal propeptide (P1NP), 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 CD4% was 37%; 94% had viral suppression (HIV-1 RNA <400 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 P1NP (584 vs 634 ng/ml, $P<0.01$) concentrations were lower in HIV-infected than uninfected children. Osteocalcin was also lower in HIV-infected children than uninfected children, although not significantly (64.1 vs 69.2 ng/ml, $P=0.22$). CTX (1.70 vs 1.75, $P=0.5$) and P1NP (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 (P1NP) 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.

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P101

Establishing the clinical need for a dedicated service for children and adolescents with Osteogenesis Imperfecta in the Republic of Ireland

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Objectives
Research studies confirm that children with osteogenesis imperfecta (OI) benefit from co-ordinated multidisciplinary services to optimise outcomes and reduce morbidity and mortality. Implementation of a tertiary paediatric centre of excellence within the Republic of Ireland is contentious due to the absence of data on the prevalence, service needs and medical requirements of these patients.
Methods
A retrospective chart review of all patients with OI attending a general endocrine clinic staffed by a consultant with an interest in metabolic bone disease at Children's University Hospital, Temple Street, Dublin from September 2012 to 2016 was performed. Data extracted included demographics, genotype, phenotype, bisphosphonate use and need for additional specialist services.
Results
Thirty-four children (male = 18) with OI have attended this service. The median age of this cohort is 4.5 years (Range 0.67–17.3 years). Severity of OI was classified as mild ($n=16$), moderate ($n=9$) or severe ($n=9$). Thirteen patients are receiving bisphosphonate therapy with the majority ($n=11$) requiring intravenous infusions through day ward or inpatient care on a three to 6 monthly basis. One third of the clinic cohort have a genetic diagnosis (Col1A1, Col1A2, LEPRE) with the remainder receiving a diagnosis based on clinical and radiological findings. Three of our patients were born to mothers with severe OI. Five patients are accessing additional care at a centre of excellence abroad. At present, 22 children require orthopaedic input, 26 children attend ophthalmology services, 27 attend audiology and 20 have required the input of the paediatric dentist. From an allied health perspective, local children ($n=14$) are followed in our hospital with the remainder referred to local intervention teams.
Conclusion
For a relatively small country (population 4.75 million) this indicates a larger than cohort within a short time period. We predict the total number of cases of OI in children <18 years in Ireland to be more than three times this number. This is a young complex cohort with multiple needs and underlines the growing requirement for a centrally funded, dedicated multidisciplinary service to deliver the level of care required. Further work is required to establish the true incidence and natural history of OI in this country.
Disclosure
The authors declared no competing interests.

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P102

Do measures of adiposity and muscle cross-sectionally predict the health of weight-bearing bones in 11–12 year old Australian children?: A population-based study

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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.
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
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Disclosure
The authors declared no competing interests.
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P103

Femoral fractures in infants – comparison of a population-based and an osteogenesis imperfecta-cohort

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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.
Grants
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Disclosure
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P104

Dietary calcium deficiency contributes to the causation of nutritional rickets (NR) in the United Kingdom (UK): data from the British Paediatric Surveillance Unit (BPSU) NR survey

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Background
Rickets is a disorder of the growing child arising from impaired mineralisation of the growth plate and osteoid. The most common cause of NR in the UK is thought to be secondary to vitamin D deficiency [VDD; serum 25-hydroxyvitamin D (25OHD) <25 nmol/l], although in some African & South Asian countries dietary calcium deficiency (DCaD) by itself, or together with VDD is an important cause of NR (Ann Trop Paediatr. 2006;26:1–16). Currently, the data on new cases of NR is being collected monthly (March 2015–March 2017) from 3500 Paediatricians, using the BPSU reporting methodology.
Objective
Eight cases of NR who did not meet the criteria for VDD (serum 25OHD <25 nmol/l) but had clinical and radiological features of rickets/osteomalacia have been reported to BPSU, during 22 months of surveillance (Table). In these patients, DCaD is likely to have contributed to the causation of NR.
Results
The table summarises the patient characteristics and biochemical features of NR.

Age in months and gender	Ethnicity	Ca mmol/l	P mmol/l	ALP IU/l	PTH	25OHD Nmol/l
9; M	A&C	2.2	1.06 (1.3–1.9)	1794 (<300)	19.8 pmol/ (1.3–1.9)	27.3
17; M	U	2.53	1.22 (0.9–1.8)	1634 (110–440)	16.5 pmol/l (1.6–6.9	27
22; F	SA	2.21	1.2 (0.9–1.8)	914 (60–425)	24.6 pmol/l (1.6–6.9)	29
10; F	A/C	2.29	0.84 (0.81–2,26)	911 (126–524)	247 ng/ml (10–70)	31
25; M	A/C	2.23	1.15 (0.8–1.4)	1096 (0–281)	389 ng/ml (5–50)	33
20; M	A-C	1.88	0.5 (0.8–1.4)	6000 (0–281)	1051 ng/ml (5–50)	44
27; F	A-C	2.09	0.97 (0.9–1.8)	>1500 (60–435)	89.4 pmol/l (0–6.4)	48
176; F	SA	1.39	1.0 (0.8–1.5)	539 (50–390)	87.1 pmol/l (1.5–7.6)	28

Legend: M- male; F- female; A&C – African & Caucasian; A/C – African or Afro-Caribbean; U-Unknown.
SA- South Asian; Ca – serum calcium; P- serum inorganic phosphate; ALP – serum alkaline phosphatase; PTH-plasma parathormone; 25OHD – serum 25-hydroxyvitamin D. Age related reference range from local laboratories given in parentheses.

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 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.
Disclosure
The authors declared no competing interests.
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P105

Low serum alkaline phosphatase is often not recognised by clinicians

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Objective
The significance of low alkaline phosphatase (ALP) is often not recognised by clinicians. It is the hallmark of hypophosphatasia and this oversight leads to delays in diagnosis, inappropriate treatment and potentially harm. Using the standard that an abnormal result should be recognised by the clinician and the potential cause and need for further investigation documented in the medical records we conducted an audit of our practice at the Royal National Orthopaedic Hospital.
Methods
The biochemistry database was searched to identify patients aged less than 18 years with an abnormally low ALP. The medical records of those identified were reviewed to identify if the abnormal result was recognised, the relevant medical history and any further investigation.
Results
A search of 3031 ALP assays performed over 3 years identified 71 abnormal results and 28 patients with a persistently low ALP. None of the medical records showed any recognition of the abnormal result, consideration of its potential cause or plan for further investigation. Subsequently, one inpatient with acute disseminated encephalomyelitis with persistently low serum ALP was found to have a pathogenic heterozygous mutation in exon 5 of *ALPL*: c.346G>A, pAla116Thr.
Conclusions
Our findings correspond to existing reports (Saraff *et al*. J Pediatr. 2016;172:181–186.e1) and show that clinicians miss low ALP results, an omission that has and

will continue to lead to undiagnosed cases of hypophosphatasia. This is crucial for the paediatric population as a specific treatment is licensed and can help prevent associated dental problems and reduce fracture risk. It is important that awareness amongst clinicians is raised and that ALP values are reported using an age adjusted lower limit of normal.

Disclosure

The authors declared no competing interests.

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P106

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

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Objectives

Turner syndrome (TS) affects 1:2000 girls and is mainly characterised by short stature and ovarian failure. Increased fracture risk 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 estradiol.

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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P107

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

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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.

Presenting 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.1097_1101delACTTA). 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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P108

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

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Background

OI-V is an autosomal dominant type of OI, which is characterized by recurrent fractures, hyperplastic callus formation and forearm interosseous membrane calcification. Less than 5% of OI patients are diagnosed with OI-V. The 5'-UTR *IFITM5* 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 occured at 13 months, suggesting a clinical diagnosis of Osteogenesis imperfecta (OI) type 1. However, molecular analysis of the type I 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 Neridronate 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 *IFITM5* 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 *IFITM5* 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 *IFITM5* 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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P109

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

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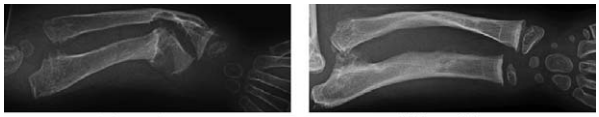
Background
Parathyroid hormone-like hormone (PTHLH) is an important regulator of endochondral bone development. Mutations of the *PTHLH* gene can cause a variety of different skeletal dysplasias, with duplications of the *PTHLH* gene resulting in a phenotype characterized by endochrondomatosis, metaphyseal dysplasia and osteolysis.
Presenting problem
Our patient presented at the age of 4 months, given concerns regarding lower 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 PTH pathway were found on exome sequencing.
Clinical management
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.



R forearm 2 ½ yrs
Pre-bisphosphonate

R forearm 5 ½ yrs
Post-bisphosphonate

Disclosure
The authors declared no competing interests.
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P110

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

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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 = −4.4), 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 Enrichment library preparation protocol and the NextSeq500 desktop sequencer (Illumina). Data analysis was conducted with GensearchNGS, Exomiser and Phenomizer. 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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P111

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

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Background
Vitamin D-dependent rickets type 1 (VDDR-1) is a rare autosomal recessive disorder caused by mutations in *CYP27B1*. This gene encodes the 1α-hydroxylase enzyme which converts 25-hydroxy vitamin D to the active form 1,25-dihydroxyvitamin D.
Objective
To describe a case of VDDR-1due 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 alfacalcidol, calcium and phosphor supplements and finally was extubated successfully.

Conclusion
Here we report a novel *CYP27B1* mutation in child that presented with severe rickets and respiratory failure. Treatment with high doses of alfacalcidol lead to clinical improvement and allowed a successful extubation.
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
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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 > G(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.
Disclosure
The authors declared no competing interests.
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P113

A challenging case of hyperphosphatemic tumoral calcinosis
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Hyperphosphatemic tumoral calcinosis (HTC) is a rare autosomal recessive metabolic disorder characterized by ectopic calcifications due to progressive deposition of basic calcium phosphate crystals 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 *FGFR1* 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 (5N). 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 CGH analysis and direct sequencing. However, the first 99 amino acids of the KL gene could not be sequenced due to the high GC dinucleotide content. Exome sequencing might allow us to find a possible mutation of *KL* and this analysis is underway. In summary, we identified a patient with HTC suspected biochemically of having a Klotho mutation (would be only second patient to be reported), but more genetic investigations are required to localize the mutation.
Disclosure
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
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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_005032.6) and a 1.6-Mb deletion affecting the entire ETV1 gene and in part *DGKB*. Mutations in *COL1A2*, encoding the α2 chain of type I 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.
Disclosure
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P115

Expanding the genotype–phenotype correlation of osteogenesis imperfecta with a novel mutation in Col1A2 gene
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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 femori. 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 femori. 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 now 6yo, 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

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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 tibulae 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 dental abscesses, with abnormal tooth architecture. Exome sequencing identified a *de novo* heterozygous missense mutation in exon 6 of *SATB2* (c.1169 C>T, p.Thr390Ile), confirmed by Sanger sequencing.
Discussion
This case provides further evidence for the association between *SAT2B* 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
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P117

Uptake of influenza vaccine in UK patients with fibrodysplasia ossificans progressiva

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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 flare-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 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 $\geq$ 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 maturation

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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 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 $P<5\times10^{-8}$. 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*; $P=1.1\times10^{-7}$) and 17p11.2(*SREBF1*; $P=9.1\times10^{-8}$) loci associated with advanced DA. Significant evidence for replication of both GWAS signals was observed in the previous NT15M meta-analysis (*IRX5*: $P=2.7\times10^{-5}$ and SREBF1: $P=0.001$). In the combined meta-analysis, the top-associated marker in the IRX5-region reached GWS ($P=2.1\times10^{-9}$). Also, alleles of these markers associated with higher DA were nominally associated with earlier teeth eruption in the AFTE meta-analysis (*IRX5*: $P=1.5\times10^{-5}$ and *SREBF1*: $P=0.002$). Furthermore, after genome-wide meta-analysis we identified variants in three novel loci: 1q32.1(*ASCL5;* $P=3.2\times10^{-8}$), 7p15.3 (*IGF2BP3*; $P=2.87\times10^{-8}$) and 14q13.3 (*PAX9;* $P=3\times10^{-8}$); on top of replicating seven previously reported loci: 2q13 (*EDAR*), 10q22.2 (*ADK*), 12q14.3 (*HMGA2*), 14q22.2 (*BMP4*), 14q24.1 (*RAD51B*), 17q21.32 (*IGF2BP1*), 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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P119

Phenotypic spectrum in Weyers acrofacial dysostosis: A case report
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Background
Weyers acrofacial dysostosis (WAD, OMIM 193530) is a rare autosomal dominant disease, characterized by mildly short stature, postaxial polydactyly, nail dystrophy and dental anomalies. WAD should be distinguished from Ellis-van Creveld syndrome (OMIM 225500), a similar but more severe disease, comprising chondrodysplasia, orofacial anomalies and, in a proportion of patients, cardiovascular malformations. Both diseases are caused by mutations in either EVC or EVC2[1,2]. We report the case of a patient with WAD who presented altered bone metabolism and severe obesity.
Presenting problem
The patient came 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 ECV2 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 WAD[3]. 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[3]. 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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P120

Vitamin D-dependent rickets – a rare form of rickets – diagnostics and therapeutic problem
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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 L 1-hydroxylase and abnormal hydroxylation of *25-hydroxycholecalciferol* (25OHD) at C 1 which constitutes the last (renal) stage of transformation of vitamin D to 1,25dihydroxycholecalciferol ($1,25(OH)_2D$). The clinical picture includes features characteristic for deficiency rickets such as: prominent frontal eminences, costochondral swelling, chest abnormalities, widening of epiphyses of long bones, varus knees. Other symptoms involve hypotonia, muscle weakness, growth retardation and in severe cases *hypocalcaemia*-induced *seizures*, in particular in infancy. The diagnosis is made on the basis of history, physical examination and laboratory tests, with hyperphosphatasia, hypophosphatemia, and hypocalcaemia and secondary hyperparathyroidism, the serum concentration of 1,25 $(OH)_2D$ is undetectable or decreased, while 25OHD is normal or above the norm.
Aim of study
We present a case of an almost 2-year-old boy with type 1 vitamin D-dependent rickets (VDDR1), which is also known as pseudovitamin D deficiency rickets or vitamin D 1α-hydroxylase deficiency vitamin D-dependent rickets type I (VDDR I). The clinical course, imaging and laboratory tests results, applied treatment and differentiation with other calcium and phosphate metabolism disorders have been discussed.
Conclusions
1. Due to non-specific clinical picture, the diagnosis of vitamin D resistant rickets forms is complicated and often time consuming, and patients with the diagnosis of v*itamin D-dependent rickets* type 1 require constant multispecialistic medical care.
2. Optimal treatment is difficult to establish and should be based on supplementation of 1,25-dihydroxycholecalciferol or alphadiol in individually adjusted doses, as treatment with cholecalciferol is not effective.
3. After normalisation of biochemical tests results patients require systematic follow-up (physical examination and laboratory tests) as they may require modification of treatment and determination of the lowest effective dose of drugs.
Disclosure
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P121

Abstract withdrawn.

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Bisphosphonate treatment initiated in the newborn period – our experience
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Background
Osteogenesis imperfecta (OI) is a clinically heterogenous 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 roding 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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The treatment of Hyperphosphatemic Familial Tumoral Calcinosis
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Background
Hyperphosphatemic familial tumoral calcinosis (HFTC) is characterized by hyperphosphatemia and the formation of tumor-like extra-osseous 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
A 5-year-old African boy with a loss-of-function mutation in the *GALNT3* gene presented with bilateral elbow TC and underwent surgical excision at 7 years. At 9 years, he presented with a diaphysitis of the left tibia and recurrence of TC at the left elbow. Combination therapy with ACTZ and sevelamer was initiated. Biochemistry prior to medical therapy was as follows: serum phosphate 2.01 mmol/l (N: 1.2–1.8), 1,25-dihydroxyvitamin D_3 65 pmol/l (N:39-193), c-terminal FGF23 1435 RU/ml ($N: <=230$) and serum bicarbonate 27 mmol/l. After 2 years of therapy, his biochemical panel was as follows: serum phosphate 2.34 mmol/l (N:1.05–1.75), 1,25-dihydroxyvitamin D_3 225 pmol/l (N:48–190) and serum bicarbonate 22 mmol/l. The tumor regressed substantially with little residual signs on plain radiograph and clinically with no limitation of activity. There have been no side effects to the therapy.
Case Report #2
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 hyperphoshatemic at presentation (phosphate 1.82 mmol/l), 1,25-dihydroxyvitamin D_3 143 pmol/l (N:39–193), c-terminal FGF23 60 RU/ml ($N: <=230$). After 2 years of therapy, the patient remained normophosphatemic with serum phosphate level at 1.27 mmol/l despite maintaining mild metabolic acidosis with serum bicarbonate 18 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 tolerated well 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
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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 microRNAs 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 phalloidin, 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
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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.

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. He had significant derangement of his biochemical parameters (see table) including a positive urine myoglobin. 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 lymphocytosis 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[1-3]. 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.

Bloods	Na	K	Ur	Cr	CK	Adj Ca	Phos	AST	ALT	ALP	LDH	Ferritin
Result (inital)	154	2.3	18	53	30553	1.79	1.48	994	310	185	3909	2681
Result (after 1 wk)	139	4.1	2.9	19	868	2.68	0.73	54	84	530	-	664

Disclosure
The authors declared no competing interests.
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P127

Growth hormone treatment in two short peri-pubertal brothers with X-linked hypophosphatemic rickets

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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)); pubertal 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

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Background
Asfotase alfa therapy improves clinical outcome in young children with severe form of hypophosphatasia (HPP). Treatment outcome in older children ($\geq$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/cm^2 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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P129

The case of severe osteoporosis in patient with recessive dystrophic Epidermolysis Bullosa

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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 deformities 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 18 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 (1800 IU) supplements. In seven months after examination one of the patients who had normal BMD (aBMD *Z*-score at total body −1.7 S.D. and −1.8 at the level L1-L4) and degraded 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, P1NP - 160.3 ng/ml, alkaline phosphatase - 79.72 Un/l (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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P130

The impact of intravenous bisphosphonate on vertebral morphometry in children with secondary osteoporosis and vertebral fractures

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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 VFs.
Disclosure
The authors declared no competing interests.

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P131

The treatment of Camurati-Engelmann disease with Losartan: a case report

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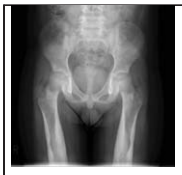
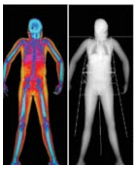
Background
Camurati-Engelmann disease (CED) is a rare bone dysplasia characterised by hyperostosis and sclerosis of the diaphyses of the long bones and skull. It is caused by autosomal dominant gain-of function mutations within *TGFB1*, which result in increased activity of transforming growth factor 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 TGFB1 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.



Pelvis and Leg radiograpghs showing severe CED changes with patchy sclerosis and cortical thickening along the diaphyses



Whole Body DEXA

Time	Baseline	1 month	2 months	3 months	
Pain	0.6	0.9	0.9	0.2	
CHAQ	1.5	1.8	1.6	1.9	
Global CHAQ	1.5	1.2	1.2	0.9	
6-min walk (m)	372	386	453	468	

Disclosure
The authors declared no competing interests.

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P132

Anti-RANKL treatment in a murine model of fibrous dysplasia

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Fibrous dysplasia of bone (FD) is a crippling skeletal disease caused by activating mutations (R201C, R201H) of the $G_s\alpha$ gene. We recently generated $G_s\alpha^{R201C}$ 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 maintained 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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P133

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

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Presenting problem
Severe forms of juvenile Paget's Syndrome (JPD) result in extreme bone turnover, necessitating long-term treatment with anti-resorptive drugs to control for 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 JPD 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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P134

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

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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.
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P135

Continuous subcutaneous PTH infusion in autosomal dominant hypocalcaemia

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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 hypercalciuric hypocalcaemia, 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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Preliminary results for a ramping model of pamidronate administration

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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 10.8%, 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.

Schedule	Dose	Number of courses*	Total drug
Ramped dose (Group 1)	0.5 mg/kg per day×3 days	1	18.5 mg/kg over 24 months
	1 mg/kg per day×3 days	4	
	1 mg/kg per day×2 days	2	
	1 mg/kg per day×1 day	1	
Uniform dose (Group 2)	1 mg/kg per day×3 days	5	15 mg/kg over 13.6 months

*3–4 months between courses.

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

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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 ($P<0.01$).

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
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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 ($\sim$13% of the world's known FOP population) is representative. The results also indicate that lesional 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
Dr 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
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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/m^2) 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, ß: 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 lumber spine aBMD (ß: 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 (ß: 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
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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
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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: NCT02097160, NCT02387892) 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

229 ± 120 IU/d, 80% (16/20) of children maintained serum 25(OH)D ≥ 50 nmol/l over the 12 mo and 95% (20/21) of children were physically activity (60 min/d). Height velocity was not different between summer and winter (0–6 mo: 0.61 ± 0.09 cm/mo, 6–12 mo: 0.57 ± 0.13 cm/mo). The % change in whole body BMC increased ($P < 0.01$) during winter (0–6 mo: 0.4 ± 3.4%, 6–12 mo: 6.5 ± 2.8%). Bone formation biomarkers P1NP and osteocalcin increased from 6 to 12 mo ($P < 0.05$) whilst bone resorption marker CTx, and PTH did not change. Using linear regression, for every 5% increment in summer change in lean mass, there was a 1.5% higher winter lumbar spine bone mineral accretion ($r^2 = 0.70$) and a 1.0% higher winter whole body BMC % change ($r^2 = 0.66$, $P < 0.05$). Twelve month changes in 25(OH)D were relatively homogenous, which may explain why 25(OH)D did not explain changes in BMC.

Conclusion

Winter bone mineral accretion in children 2–8 years may be positively and temporally related to lean mass accretion in the preceding seasons. (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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P141

Unique correlation pattern between cortical trabecular bone qualities and standard dynamometer handgrip strength in girls with adolescent idiopathic scoliosis (AIS)?

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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 and trabecular vBMD, trabecular plate structure (including pBV/TV, pTb.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

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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

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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/cm^2, $P = 0.023$). 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

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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.

Method

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, mm^3), Total Bone Area (TBA, mm^2), Functional muscle bone unit (FMBU: (SSI/bone length) and Robustness (SSI/bone length^3). 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
DCD participants were younger (mean = 14.4 years S.D. = 1.3) than the non-DCD group (15.3 years S.D. = 1.8) ($P = 0.007$), gender was equally represented for radius (54.1% male) and tibia (53.2% male), although sample size differed for each bone site due to motion artefact (DCD radius $n = 26$, tibia $n = 39$, non-DCD radius $n = 96$, tibia $n = 147$). DCD participants had lower scores for tibial SSI, TBA and Robustness, and no gender-group interaction was observed. A significant gender-group interaction was found for tibial FMBU ($P = 0.021$) with DCD males having lower tibial FMBU scores (mean = 36.7 S.D. = 8.0) than the non-DCD group (41.7 S.D. = 6.5) ($P = 0.004$). 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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P144

Soft tissues, areal bone mineral density and hip geometry estimates in active young boys: the PRO-BONE study

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Objectives
Soft tissues, such as fat mass (FM) and lean mass (LM), play an important role in bone development but this is poorly understood in highly active youths. The objective of this study was to determine whether FM or LM is a stronger predictor of areal bone mineral density (aBMD) and hip geometry estimates in a group of physically active boys after adjusting for height, chronological age, moderate-to-vigorous physical activity (MVPA), FM, and LM.
Methods
Participants included 121 boys (13.1 ± 1.0 years) from the PRO-BONE study. Bone mineral content (BMC) and aBMD measured at total body, femoral neck and lumbar spine using dual-energy X-ray absorptiometry (DXA), and hip structural analysis was used to estimate bone geometry at the femoral neck. Body composition was assessed using DXA. The relationships of FM and LM with bone outcomes were analysed using simple and multiple linear regression analyses.
Results
Pearson correlation coefficients showed that total body (less head) aBMD was significantly correlated with LM but not FM. Multiple linear regression analyses showed that FM, after accounting for height, age, MVPA and LM had no significant relationship with aBMD or hip geometry estimates, except for arms aBMD. By contrast, there were positive associations between LM and most aBMD and hip geometry estimates, after accounting height, age, MVPA and FM.
Conclusion
The results of this study suggest that LM, and not FM, is the stronger predictor of aBMD and hip geometry estimates in physically active boys.
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Disclosure
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P145

Longitudinal growth and bone development in glucocorticoid treated boys with Duchenne muscular dystrophy

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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 (TBLH-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 Deflazacort, 3/15 (20%) daily Prednisolone, 1/15 (6.7%) pulsed Deflazacort 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 (0.3, −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 TBLH-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
1. Crabtree NJ et al J Bone Miner Res 2017 Jan [Epub ahead of print].
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P146

Stature and longitudinal growth in glucocorticoid naïve boys with Duchenne Muscular Dystrophy

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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, 6.9). 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
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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
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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/m^3 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/m^3 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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P150

DXA based evaluation of the bone mass and body composition in a group of Romanian cystic fibrosis children

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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/m^2 and a height centile (WHO reference) of 28.6 ± 23. Mean values of measured parameters were 77.9 ± 19.8 for FEV% predicted, 81.4 ± 23.6 for FEV1% predicted, 11.98 ± 2.24 years for bone age, -0.93 ± 1.01 S.D. for the total body less head bone mineral density (TBLH-BMD) Z score, 18.44 ± 8.81% for total body fat, 12.51 ± 1.09 g/m^2 for LMI (lean mass index). Two patients had short stature, one was overweight and one underweight. Vitamin D insufficiency was found in 14 patients, defficiency 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 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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P151

Characterisation of skeletal developmental in mouse models of Duchenne Muscular Dystrophy

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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 tibialis 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. CK assay results indicated significantly higher serum values from *mdx* ($P < 0.02$) and *mdx:utr* ($P < 0.002$) mice, compared to WT. Muscle histology was consistent with these observations.
Growth
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.
Bone
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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P152

Seasonal variation in internet searches for vitamin D

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Objective
Over the last decade, there has been increasing scientific interest in vitamin D, and it is now advised that all pregnant women and infants should receive vitamin D supplementation. Despite of this, it is recognized that knowledge of vitamin D in the general public is limited. The internet is now an important source of health care information and analysis of internet search activity rates can provide information on disease epidemiology, health related behaviors and public interest. We therefore explored internet search rates for "vitamin D" to determine whether this reflects the increasing scientific interest in this topic.
Methods
Google Trends is a publically available tool that provides data on internet searches using Google. Search activity for the term "vitamin D" from 1st January 2004 until 31st October 2016 was obtained. Comparison was made to other bone and nutrition related terms.
Results
Worldwide, searches for "vitamin D" increased from 2004 until 2010. Thereafter a statistically significant ($P < 0.001$) seasonal pattern with a peak in February and nadir in August was observed. This seasonal pattern was evident for searches originating from both the USA (peak in February) and Australia (peak in August), $P < 0.001$ for both. Searches for the terms "osteoporosis", "rickets", "back pain" or "folic acid" did not display the increase observed for vitamin D or evidence of seasonal variation.
Conclusion
Public interest in vitamin D, as assessed by internet search activity, did increase from 2004 to 2010, likely reflecting the growing scientific interest, but now displays a seasonal pattern with peak interest during late winter. This information could be used to guide public health approaches to managing vitamin D deficiency and increasing uptake of supplementation in at risk groups.
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

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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 I^2 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, <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 (I^2=73%). Once adjusted for dose, heterogeneity was low (I^2=0%). Study designs were qualitatively high and 97% had Jadad scores ≥4. The 25(OH)D weighted mean difference (26.5 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.

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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

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Suppression of parathyroid hormone (PTH) has been suggested as a potential biochemical outcome measure for determining the optimal serum 25-hydroxyvitamin D (S25(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 S25(OH)D concentration at which PTH plateaus (40–90 nmol/l), with some reporting no plateau. The aim of this study was to identify the S25(OH)D concentration at which PTH is suppressed (the inflection point) in 14–18 year old white male and female adolescents in the UK. S25(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 S25(OH)D concentration at which PTH plateaued and a linear model was selected based on best fit. Mean S25(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 S25(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 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 S25(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 basis for determining optimal vitamin D status in healthy adolescents.

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Disclosure

The authors declared no competing interests.

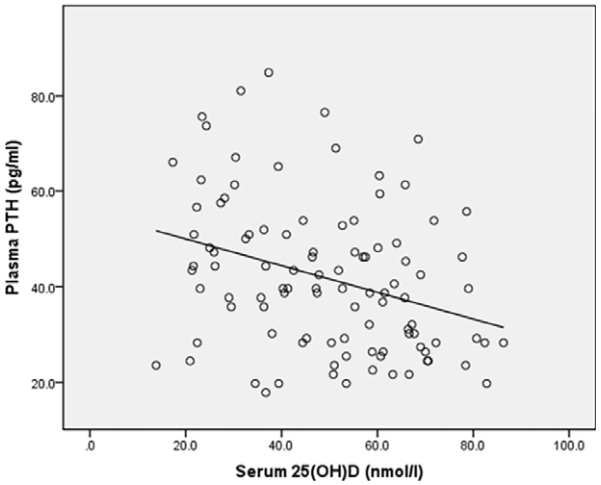

Figure 1 Linear regression association of serum 25(OH)D with plasma PTH.

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P155

Dietary protein is associated with bone adaptations and performance of pre-adolescents

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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 body composition as well as body 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 correlational 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

performance markers in both boys and girls. Furthermore, PA was positively associated with BMD (0.68, $P<0.05$). A significant interaction between nutrient intake, PA and BMD was revealed with children of average and high PA and protein intake exhibiting a greater BMD than those with high PA and low protein intake independent of sex.
Conclusion
The results of this investigation suggest that protein intake may be important for growth-related bone and performance adaptations.
Disclosure
The authors declared no competing interests.
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P156

Bone health status of underprivileged Indian adolescent girls
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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) by 24-hr diet recall and nutrient intakes were computed using (c-diet software-Xenios technologies). 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 mins/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 mm^3 and mean *Z*-score was -1.4 ± 2.5; SSI was below -2 in 33% girls. All macro (protein $=29.0\pm11.3$ gm/day) and micronutrient (calcium $=302\pm113$ 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?
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Background
While the relationship between visceral (VFAT) and subcutaneous (SFAT) fat mass with cardiometabolic risk has been demonstrated in adults, fat mass evolution during teenagehood 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 android-gynoid 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 approximatively at the level of the 4th lumbar vertebra.
Results
Ninety-two volunteers were included: 44 girls (G) and 48 boys (B), age: 13.9 ± 1.9 and 13.6 ± 2.4 yrs, body weight (BW): 49.6 ± 10.0 and 47.9 ± 14.4 kg, height: 158.8 ± 9.0 and 159.3 ± 15.0 cm, BMI: 19.4 ± 2.5 and 18.4 ± 2.9 kg/m^2, respectively. Age, Height, BW and BMI were not significantly different between genders in the total cohort. In the Tanner 5 sub-group, age, BW and BMI were not different between genders; in contrast, height, FM, LM, And/Gyn and BMD were all significantly different (Table 1). Interestingly, VFAT and SFAT were significantly different between genders from Tanner 2 stages onwards: VFAT was significantly greater in boys whilst SFAT was significantly greater in girls.
Discussion
Using a non-irradiative and inexpensive technique, VFAT and SFAT are significantly different between genders as early as Tanner stage 2 in healthy teenagers. The clinical consequences of such differences should be determined, but could explain some of the differences in cardiovascular risk observed between genders later in life.
Disclosure
The authors declared no competing interests.

Table 1.

Tanner stage (Boys: N/Girls:N)	Tanner 1 (B:14/G:2)	Tanner 2 (B:8/G:7)	Tanner 3 (B:5/G:13)	Tanner 4 (B:7/G:7)	Tanner 5 (B:10/G:19)
Height	−5.5	−1.1	0.1	−5.5	−6.7*
Weight	−2.1	3.1	−8.6	−7.7	−10.9
BMI	10.0	5.6	−7.6	3.0	2.6
Lean Mass (LM)	−7.7	−5.2	−6.6	−19.0*	−23.1**
Fat Mass (FM)	13.5	42.8	−21.8	52.4	54.9*
Mass (M)	−3.1	3.3	−10.5	−8.3	−11.6
Android Gynoid Ratio (And/Gyn)	6.5	−2.0	−13.1	−18.2	−18.5*
BMD	−5.3	−0.3	1.5	−6.7	−11.9*
Visceral fat (VFAT)	−31.1	−44.0*	−50.5*	−55.6*	−35.2*
Subcutaneous fat (SFAT)	108.6	196.4**	10.0*	129.1*	111.7*

**$P\leq0.001$; *$P\leq0.05$

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P158

Bone metabolism in adolescent girls with anorexia nervosa
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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 auxological parameters on markers of bone metabolism, bone mineral density in girls with anorexia.
Patients and methods
In groups of 45 girls with anorexia nervosa ($x=16.15\pm2.67$ 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, IGF1). 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 – lumbal spine (LS) was -0.8 ± 1.27, 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, $P \leq 0.05$), estradiol (19.48 vs 27.50 pg/ml, $P \leq 0.05$) 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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Maternal calcium supplementation in a rural Gambian population associated with reduced blood pressure among adolescent female, but not male, offspring

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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).[1,2] There was no effect on maternal blood pressure, the primary outcome of the original trial.[3] In this study, we aim to investigate effects on offspring blood pressure (BP) in the same cohort. We recruited children (205 female, 182 male) born following a randomised, placebo-controlled trial of calcium supplementation during pregnancy (1500 mg daily from gestational week 20 until delivery, ISRCTN96502494). Offspring BP was measured using the Omron 705IT monitor, at mean(S.D.) age 13.8(1.2) years. Outcomes were resting sitting systolic and diastolic BP (SBP, DBP). Linear regression was used to determine the effects of maternal calcium supplementation on an intention-to-treat basis, separately among boys and girls, first adjusted for age only and then further adjusted for height and BMI. Boys and girls were then pooled to test for a sex-supplement interaction. Mean(S.D.) SBP/DBP was 108(11)/62(9) mmHg among girls and 103(11)/59(10) mmHg among boys. Girls whose mothers had received the calcium supplement during pregnancy had lower BP than those whose mothers received placebo (mean(95%CI) difference in SBP: $-4.4(-7.1,-1.7)$ mmHg; DBP: $-2.5(-4.9,-0.1)$ mmHg). These differences were partly attenuated when height and BMI were included in the models (SBP: $-2.8(-5.3,-0.3)$ mmHg; DBP: $-1.6(-3.9,+0.7)$ mmHg). No significant differences in BP were observed among boys (SBP: $+1.3(-1.7,+4.4)$ mmHg; DBP: $+0.7(-2.2,+3.5)$ mmHg). In the pooled analysis, the sex-supplement interaction was significant for SBP ($+5.7(+1.6,+9.7)$ mmHg) but not for DBP ($+3.2(-0.5,+6.9)$ mmHg). Maternal calcium supplementation was associated with reduced BP among girls during adolescence, but not among boys. A previous analysis in the same cohort at mean age 7 years showed no result of maternal calcium supplementation on offspring BP,[4] suggesting that these differences may develop with age. Further follow-up will be required to determine whether the difference persists into adulthood and to investigate potential long-term effects on cardiometabolic health.

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Disclosure

The authors declared no competing interests.

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(1) Ward *et al. JBMR* 2015 **30** (S1) OP1096.
(2) Ward *et al. OI* 2016 **27** (S1) P585.
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(4) Hawkesworth *et al. AJCN* 2010 **92** 741–7.

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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

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We have previously reported sex-specific effects of prepubertal calcium supplementation on the timing of adolescent growth,[1] 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 children (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. Mixed effects models were used to determine the effects of maternal calcium supplementation on offspring growth trajectories of height, weight and BMI, on an intention-to-treat basis, separately among boys and girls. The outcomes were log-transformed to allow interpretation of the coefficients as percentage differences, fixed effects were fitted as natural cubic splines, and random effects were included to model between-subject variation in the intercepts and slopes. Boys and girls were then pooled to test for a sex-supplement interaction. Girls whose mothers had received the calcium supplement during pregnancy were shorter and lighter than those whose mothers received placebo (mean(95%CI) difference in height: $-1.1(-2.1,-0.2)$%; weight: $-3.4(-6.8,-0.0)$%). There were no significant supplement-age interactions, indicating that the difference was consistent across the age range studied. There was no difference in BMI ($-1.3(-3.6,+1.0)$%) among girls, and there were no differences among boys (height: $+0.4(-0.7,+1.4)$%; weight: $+1.6(-1.7,+4.8)$%; BMI: $+1.0(-1.1,+3.1)$%). The pooled analysis identified a significant sex-supplement interaction for weight only (height: $+1.3(-0.2,+2.7)$%; weight: $+4.9(+0.2,+9.6)$%; BMI: $+2.2(-0.9,+5.4)$%). Maternal calcium supplementation was associated with reduced height and weight among girls, but not among boys, with a significant sex-supplement interaction effect on weight. Despite low habitual calcium intake in this population, there was no evidence that maternal supplementation promoted offspring growth. Instead, these data suggest that calcium supplementation may have reduced growth among girls. It remains to be seen whether these differences are transient, e.g. as a result of differences in the timing of growth, or will be sustained into adulthood.

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Disclosure

The authors declared no competing interests.

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(3) Ward *et al. OI* 2016 **27** (S1) P585.

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P161

Early-life vitamin D status and bone mass at five years in a prospective birth cohort study

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Objective

We aimed to investigate associations between early-life vitamin D status, mode of infant milk-feeding and bone outcomes at five years.

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 mol/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, $P=0.046$). 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
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Nutritional rickets presenting to secondary care in children (<16 years) – A UK surveillance study
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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 paediatricians 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 sequelae. 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.
Disclosure
The authors declared no competing interests.

Table 1 Demographics and clinical characteristic.

Sex ($n=89$)	***n***	**%**
Male	46	52
Female	42	48
Ethnicity ($n=89$)		
African	27	30
Arab	2	2
Caribbean	6	7
South Asian	32	35
Other Asian background	3	3
Other Black/African/Caribbean background	7	8
Other White background	5	6
Other mixed/multiple ethnic background	5	6
Not known	3	3
Age at Presentation ($n=89$)		
<1 year	22	20
1–5 years	61	69
5–15 years	8	9
Feeding practices ($n=84$)		
Exclusively breastfeeding	15	18
Exclusively formula fed	1	1
Mixed	8	10
Solids (with dairy $n=50$)	60	71
	42	84
Clinical Presentation ($n=89$)		
Bony Sign (in 8, the only abnormality)	69	85
Radiological Abnormalities	65	73
Neuromuscular Abnormalities	40	45
Incidental Blood test or X-ray	13	15

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Vitamin D insufficiency and inadequate bone mineral status in newcomer immigrant and refugee children in Canada
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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 Saskatoon 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 three 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
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Stable and functional osteosynthesis with intramedullary growing rods: results of surgical correction in eleven patients with systemic skeletal disease

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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 wich 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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Cortico-cancellous bone allografting in treatment of children with orthopedic diseases

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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 osteomyelitis, 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 occured 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 alloplasty 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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Orthopaedic management of leg length discrepancy in Proteus syndrome: a case series

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Background
Proteus syndrome (PS) is a rare mosaic disorder comprising asymmetric bony and soft tissue overgrowth leading to significant morbidity. Placement of guided

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, distorting, 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 HG200388).
Disclosure
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Physical activity is negatively correlated with circulating sclerostin in 6–12 year-old children

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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: $n=101$; 9–12 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 correlational 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 (-0.58, $P<0.05$), muscle power (-0.61, $P<0.05$) and endurance (-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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Review of lower limb range of movement following intramedullary fixation in children with Osteogensis Imperfecta

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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 35 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 tibiae. 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.
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Bone health at 11–12 years, physical activity and sedentariness: a cross-sectional Australian population-based study

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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 periosteal 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 11.4 years (s.d. 0.5); 49% 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
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Disclosure
The authors declared no competing interests.
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P173

Results of surgical treatment of tibia deformity in patients with Campanaccis disease

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Relevance of the study due to progressive and opportunity of Campanaccis disease to relapse after surgical treatment. Analysis of surgical treatment of seven patients with osteo-fibrous dysplasia of tibia (four male, three – female) aged 2–17 years who performed 13 surgeries. Bone grafting after curettage residual cavities made in two of seven patients, including allografts used in one patient, ceramic hydroxyapatite 'Kerhap' – in two patients, with one patient in three cases. Two teenage patients with missing tibia deformation and small in size cells made in two cases, marginal resection. In two patients with lack of tibia deformation and small in size cells made marginal resection. Corrective osteotomy of the tibia performed in four patients aged 3–10 years (mean age 5 years), including combined with osteosynthesis with plate fixation in three patients, one patient made primary osteosynthesis with intramedulary telescopic rod. Two patients with recurrent deformity outside of the plate held osteosynthesis with telescopic intramedullary rod. Due to the high probability of relapse osteoplasty have not done. Analysis of long-term results of surgical treatment of osteo-fibrous dysplasia found that resection of pathological cells and bone grafting is not prevented relapse. The use of intramedullary osteosynthesis with telescopic rod helped allowed to maintain the correct axle of the limbs and prevent recurrence deformity of the tibia. Violation growth of the bone after osteosynthesis with intramedullary telescopic rods are not observed in any case. Fusion after corrective osteotomy was in ordinary terms under the age of all patients. Support function and gait in patients was restored in time from 2.5 to 3.5 months. The use of intramedullary telescopic rods in patients with osteo-fibrous dysplasia in childhood can prevent recurrence of deformity of the tibia and provide recovery of moves in optimal time.
Disclosure
The authors declared no competing interests.
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P174

Maffucci syndrome – as an extremely rare form of Ollier disease

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Present a clinical case of Maffuchchi Syndrome and highlight its clinical and orthopedic features and differences from the Ollier disease Methods: Risk of secondary chondrosaroma higher at Maffuchchi syndrome – 46%. Almost 100% extraskeletal malignant transformation compared to Ollier disease which, according to various estimates, is 5–43% of all cases. Maffuchchi 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 Maffuchchi Syndrome (with almost 100% of likelihood of cancer complications) causes necessity of careful monitoring of patients with multiple enhondromatosys, 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 Maffuchchi Syndrome.
Disclosure
The authors declared no competing interests.
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P175

Management of Gorham disease in the cervicothoracic spine with mobile gravity traction and Sirolimus

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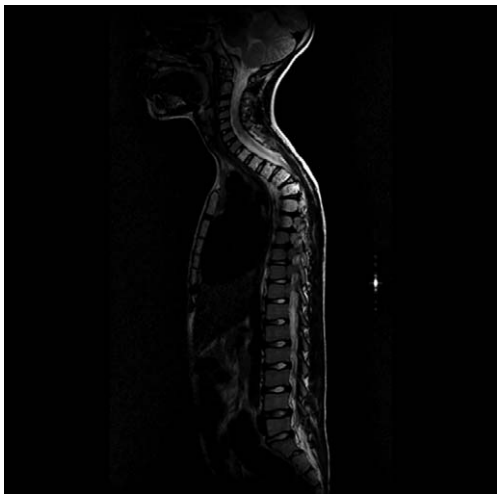
Background
Gorham Disease is a rare condition characterised by massive osteolysis. The pathophysiology is related to angio/lymphatic proliferation within bone. No genetic transmission has been identified and onset occurs in patients of all ages.

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 Sirolimus 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
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P176

System epidermal nevus with hyperkeratosis and violations of bone tissue metabolism – therapy of drug of pamidrinic acid and surgical orthopedic treatment. Case from practice

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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-epiphyseal 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 mkg 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?

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Objectives
Type V osteogenesis imperfecta (OI) results in abnormal modelling of the ulna, dislocation of the radial head and interosseous membrane calcification (IOM). 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 ($\leq 120°$) and supination ($\leq 60°$). Radiographic images were double reported, consensus reached in cases of discrepancy. Earliest age of onset was determined for radiographic features: IOM; subluxation (SUBL) or complete dislocation (DISL) of radial head; abnormal modelling of proximal ulna (ULNMd); and ulna bowing $\geq 15°$ (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 (6/12 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 $\leq 120°$, 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 3.6yrs (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
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P178

Abstract withdrawn.

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P179

Skeletal health of young patients with perinatal HIV infection: Experience from a reference center

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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 25(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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P180

Abstract withdrawn.

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P181

Generalized arterial calcinosis of infancy: a case of a new mutation with central nervous system involvement and good response to bisphosphonates

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Background
Mutations in the ENPP1 gene have been identified in individuals with generalized arterial calcification of infancy (GACI), a life-threatening disorder characterized by 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 n suspicion of GACI he was started on IV infusions of pamidronate (0.1 mg/kg/week for 4 weeks), followed by oral risedronate (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 homozygote 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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P182

Osteonecrosis results in significant long term morbidity in patients with acute lymphoblastic leukaemia

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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

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P183

The treatment of severe pain in melorheostosis with daily walking program only: a case report

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Bacground

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 temporarly by several drugs including local anastethics but fully stopped by daily walking only.

Presenting Problem and Clinical management

A 16 years- old female had chronic hard pain around her foots and hands, and had been hurt in shoe gear. The physical exam revealed a severely 'C' shaped right and left foots 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 mounts) for 9 mounts. At the end of this period, swelling in her hands and foots decreased and the pain abated, but when biphospanate treatment was stopped, severe bone pain on her legs and arms began again. She was taking local anasthetics 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 mounts. 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

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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 Questionnaire 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/starting 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

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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°N). 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

infection-related symptoms was collected fortnightly for 6 months using a validated questionnaire administered to parents and children. Biochemical assessments included serum 25OHD and zinc.
Results
After removal of outliers, final results have been presented on 361 children – 119 (vitamin D + Calcium group), 118 (zinc group) and 124 (placebo group). All the three groups had similar anthropometric characteristics, environmental characteristics, personal hygiene habits and nutritional intakes at baseline and endline. 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 56.5 ± 15.5 nmol/l in the zinc group). There was no significant difference noted in the serum zinc levels among the theree 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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P186

Is cherubism a systemic disease? Prospective study about 9 patients
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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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P187

Acroosteolysis presenting as nail resorption
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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 feet 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.

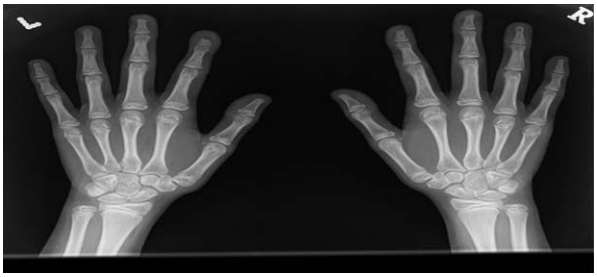

Figure 1 Hand X-ray showing resorption of distal phalanges of the index and middle fingers of both hands.

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P188

Paradoxical response of serum parathyroid hormone concentration in response to vitamin D and calcium supplementation in undernourished Indian children
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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 (1000IU-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 mmol/l), phosphorus (1.7 ± 0.2 mmol/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 phosphate 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
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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 $Clcn7^{G213R}$ 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 *Glo1* 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 perivascular fibrosis in ADO2 kidneys (+4.4-fold; $P < 0.0001$). Moderate perivascular fibrosis was also observed in lungs (+1.5-fold; $P < 0.001$), which in homozygous $Clcn7^{G213R}$ mice was more pronounced and associated with severe atelectasis and airway closure. Interestingly, perivascular fibrosis was confirmed in muscle, a tissue that does not express ClC7 but that it is often damaged in ADO2 patients. ADO2 spleens did not show fibrosis but had elevated number of megakaryocytes (+1.4-fold; $P = 0.03$), 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 perivascular fibrosis was rescued, along with the bone phenotype, by systemic administration of an effective $Clcn7^{G213R}$-specific siRNA.
Disclosure
The authors declared no competing interests.
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P190

Development of an *in vitro* model of cancer stem cells from a rare human telangiectatic osteosarcoma
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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 Ricostruttiva 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 sarcosphere 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.
Acknowledgements
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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
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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

ranges from low bone mineral density for age with unexplained fractures to rickets, and premature loss of primary teeth with intact roots. We described a boy with the childhood form of hypophosphatasia presenting with short stature without rickets findings.
Case report
An 11-year-old boy was admitted with short stature. He had no complaints other than short stature. Three fractures developed involving left ankle and left forearm as a result of low energy trauma at age of 9 and 10. His eruption of primary teeth delayed until 2.5 year old. Low alkaline phosphatase (ALP) level (65 IU/ml; the lowest normal level for his age is 130 IU/ml) suggested the diagnosis of hypophosphatasia. High serum pyridoxal 5-phosphate level was found 57 μg/l (reference range 5–50 μg/l). His L1–L4 bone mineral density (BMD) by DEXA was normal for age. *Tissue nonspecific ALP (TNSALP)* gene sequencing revealed the heterozygous pR184W (c.550C > T) mutation. No mutations were detected in both father and mother.
Conclusion
The presence of multipl fractures and delayed primary teeth eruption suggested childhood hypophosphatasia in our case. Although our patient had short stature, there were no findings of rickets and low BMD. The laboratory findings of the patient showed changes at the borderline. But, mutation causing HPP was detected and diagnosis of mild HPP was confirmed. This patient demonstrated that ALP should always be evaluated even if there were no rickets-like findings in every child with short stature.
Disclosure
The authors declared no competing interests.
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P192

Development of an osteogenesis imperfecta specific quality of life measure

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Objectives
Osteogenesis Imperfecta (OI) is a hereditary disorder effecting 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. Concerns 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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P193

Sheffield children's hospital osteogenesis imperfecta service: collaboration and care

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Introduction
Osteogenesis imperfecta (OI) is a clinically heterogeneous heritable connective tissue disorder characterised by increased bone fragility and low bone density, resulting in frequent fractures from little or no trauma and bony deformities such as curvature of the long bones. Type and severity of OI is variable. OI affects the physical, social and emotional well-being of the child, young person and their family. The long term goal for children and young people with OI is independence in all life functions with adaptive devices as needed; in the case of severely affected children, the ability to direct their own care.
Methods
This practice development considers the experience of those living with OI and describes actions taken by the team to improve these. It describes a specialist and integrated multi-disciplinary team with extensive peripatetic involvement with patients and their families, working in partnership with many external agencies, charities and societies. The aim of the service is to provide an equitable, multidisciplinary approach to the diagnosis and management of infants and children aged 0–19 years with mild to severe, complex and atypical OI in the UK. We aim to ensure inclusion is a reality for children and young people living with OI.
Conclusion
Osteogenesis imperfecta is a complicated, long term condition which has far wider implications than merely its symptoms. This practice development reflects a model of working within the UK's National Health Service which achieves optimal outcomes for children, young people and families. By taking a holistic approach and embracing a collaborative model of working, an MDT can deliver the best quality care and improve quality of life.
By reading this poster presentation you will:

- understand the clinical presentation of osteogenesis imperfecta and the associated assessment/treatment aims.
- use a self-reflective approach to developing and delivering your own collaborative model of working.

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

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Background
Familial hypocalciuric hypercalcaemia type 3 (FHH3) is a genetically heterogenous 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.
Discussion
Our case series demonstrates that children affected with FHH3 present with varying degrees of LD. Behavioural difficulties (autistic spectrum disorder and attention deficit hyperactivity disorder) were also observed in several cases. The mechanism by which FHH3 is associated with cognitive and behavioural dysfunction remains unclear. Reports of symptomatic hypercalcaemia in this group of patients could imply that the chronic hypercalcaemia itself may be contributory. It is also postulated that adaptor protein-2 complexes expressed in the brain may affect neurological development by another mechanism. A knock-out mouse model, currently in development, may elucidate the pathogenesis of this phenotype.
Conclusion
We report varying degrees of LD and behavioural issues in children diagnosed with FHH3 and therefore recommend assessing this key phenotype in all patients with FHH3, with provision of appropriate support.
Disclosure
The authors declared no competing interests.
Reference
1. Hannan *et al*., Hum Mol Genet 2015.

Table 1

Case	Age (years)	Sex	*AP2S1* mutation	Learning disabilities	Behavioural issues
1	3	Female	Arg15His	Severe*	Possible evolving ASD
2	11.5	Male	Arg15Leu	Mild*	ADHD, aggression
3	1.5	Female	Arg15Leu	Mild†	None
4	3	Male	Arg15Cys	Severe	None
5	11	Male	Arg15Leu	Mild	ASD

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 intrabony defects in chronic periodontitis in adolescents

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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 and 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 l: OFD alone, group ll: 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-operative. Percentage radiographic intra-bony defect depth reduction was evaluated using computer-aided software at baseline and 6th month. OFD with CGF group showed significant PD and RAL gain than OFD alone group. Group ll sites showed a significantly greater percentage radiographic defect depth reduction ($48.11 \pm 0.019\%$) as comparated to Group 1 ($11.21 \pm 0.082\%$) 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

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Osteogeneses 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 experience 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 mother's 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 transcribe 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)

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Background
Families of children with Osteogenesis Imperfecta (OI) make costly modifications to their home, lifestyle and employment and incur costs of rehabilitative,

preventative and adaptive care for their child. While parents have readily identified that these costs are financially burdensome, the degree of financial burden has not yet been described in families of children with OI.
Objectives
To evaluate the out of pocket, OI related expenses (ExpOI) incurred by families of children with OI. In addition we aimed to evaluate whether there is any association of ExpOI with the perceived impact on the family of caring for a child with OI on the family.
Methods
We undertook a cross-sectional study using self-report questionnaires from families of children with OI followed at SickKids Hospital, Toronto, Canada. Data collected included clinical variables and out of pocket OI related expenses (using modified Expenditures for Health and Social Service utilization questionnaire created by Browne *et al*.). Families also completed the Impact of Family Scale; a validated instrument which measures the impact of chronic health conditions on families.
Results
Of 34 respondents (child's mean age 8.1 ± 4.6 years, 70% female), 38% identified the severity of their child's OI as mild, 38% moderate and 24% severe. The % of net income spent on ExpOI was $10.0 \pm 17.4\%$ with 29.6% of families experiencing financial burden as defined as spending over 10% of their net income on ExpOI. Financially burdened families had greater costs associated with hospital visits ($P=0.019$), bisphosphonate treatments ($P=0.013$) and allied health visits ($P=0.045$). In looking at the impact of OI on the family, higher impact was associated with greater severity of OI, bisphosphonate use, surgery in the previous year, longer travel time to the hospital and increased ExpOI. On multi-regression analysis, greater OI severity, ExpOI and having had a surgical intervention in the preceding year were the greatest predictors of burden experienced by the family unit ($R^2=0.623$.)
Conclusion
Even in Canada, with access to a universal healthcare system, 29.6% of families affected by OI experience financial burden. The results of this study further suggest that the financial cost of caring for a child with OI is a significant contributor to the overall impact the condition has on families.
Disclosure
The authors declared no competing interests.
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P198

Hearing the patient's voice: a focus group listening to the child and parent experiences of living with rare bone diseases
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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 children ($n=19$). The children's group consisted of five patients with rare bone conditions (achondroplasia, acromicric dysplasia, hypophosphataemic rickets, spondylo-epiphyseal dysplasia congenita, multiple epiphyseal dysplasia, hypochondroplasia), and two 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 tertiary 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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P199

Validation of questionnaire for measurement of sunlight exposure in children from Pune, India
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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 ($1\ \text{SED} = 100\ \text{J/m}^2$).
Results
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 25OHD generally estimated among Indian children.
Disclosure
The authors declared no competing interests.
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P200

The platform of expertise for rare diseases Paris-Sud: an innovative model for gathering reference centers and improving care for rare diseases
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Introduction
The platform of expertise for rare diseases Paris-Sud is an organization created at the end of 2014. It brings together 21 reference centers for rare diseases of the university hospitals Paris-Sud, 12 diagnostic and research laboratories, a biological resource center and several patient associations.

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=PLreJu8NovRO3EUfMOQbfaU17yYWyl6qD1), as well as many other communication tools. The bio-informatician, in collaboration with the team working at 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?

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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. Consequent is an increase in the natural lordosis of the lumbar spine which can result in spondylolisthesis/spondylolysis (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 spondylolysis 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 spondylolysis. 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 spondylolysis 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

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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 misdiagnosed as non-accidental injury (NAI)

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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.
Presenting problem
The table summarises patient characteristics, presenting problem, safeguarding assessment and action taken by social services. Café-au-lait lesions were present

in all 3 patients. The diagnosis of NF1 was confirmed by genetic testing in all patients.

Case	Age and gender	Presentation	Radiographic findings	Safe-guarding referral	Safe-guarding investi-gations	Removed from parents	Family history of NF1
1	20M; F	Hard lump above the right lateral malleolus	Fracture of the distal right fibula with pseudoarthrosis	Yes	No	No	Yes
2	7M; F	Anterolateral bow-ing of the right lower leg	Anterolateral bowing of the right distal tibia with fracture and pseudoarthrosis	Yes	Under-taken	Yes	No
3	3M; F	Bilateral bowing of the lower legs from birth Pain when left leg moved	Fracture of the left distal tibia with pseudoarthrosis Antero-lateral bowing of the right tibia & fibula, i.e. bilateral CTP	Yes	Under-taken	Yes	No

Legend: M, months; F, female; Safeguarding referral, 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 awareness of classical radiological features of CTP would 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 pseudarthrosis, 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?

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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 ($\rho=-0.291$, $P=0.003$) and the percentage of tasks classified as 'unable to do' ($\rho=-0.210$, $P=0.036$) and 'not-applicable' ($\rho=-0.617$, $P<0.001$) 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 game 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

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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 D_3 levels <20 ng/ml were observed in 77% of the patients. 38% presented with 25 OH-vitamin D_3 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 osteopathology 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-I deficiency

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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.

Presenting problem
A 7.7 year old girl born to consanguineous parents presented with poor growth; height −3.2 SDS, weight −2.7 SDS, BMI −0.8 SDS. Examination revealed abdominal obesity, thin limbs and a suggestion of mid-face hypoplasia. There was no scoliosis or other dysmorphic features, but a history of recurrent fractures. Investigations showed a 46XX karyotype and negative coeliac screen negative. Pituitary function testing revealed high basal GH of 6.5 μg/l, rising to 25.5 μg/l, but with undetectable IGF-1 (<3.2). An IGF-I generation test performed following 4 days of GH (0.035 mg/kg per day) displayed no increment in IGF-I. DNA analysis for a GHR mutation was negative. Metabolic investigations showed a pattern of amino acids consistent with LPI.
Clinical management
The patient was diagnosed with LPI and commenced on oral citrulline and a low protein diet. Due to the history of fractures and the association between LPI and osteoporosis, the bone health was further investigated. The bone density Z-scores were −5.0 and −3.1 SDS at the Lumbar Spine (Bone Mineral Apparent Density) and total body respectively. A spine X-ray revealed multiple vertebral crush fractures associated with spinal tenderness. Treatment with intravenous pamidronate improved the bone pain significantly. Despite compliance with treatment, our patient's growth remained poor (height −4.0 SDS) and the IGF-I remained low. She was therefore commenced on recombinant IGF-1 therapy at 9.9 years of age.
Discussion
Although low GH levels have been reported in LPI, IGF-I deficiency with high GH has not been described. Furthermore, osteoporosis is a rare presentation of LPI. In the investigation of growth abnormalities or recurrent fractures in childhood, an index of suspicion should be maintained in the presence of prevailing clinical or biochemical findings and LPI should be considered.
Disclosure
The authors declared no competing interests.
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P207

Bone turnover in the obese children is related to gender, body compo-sition and leptin level

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Introduction
Recently published data revealed that bone turnover is related to the body composition in pubertal children and may be impaired in obese adolescents. The aim of the study was to determine the relationship between bone turnover markers, body composition and leptin level in obese children.
Material and methods
In 54 obese adolescents (25 boys and 29 girls) in the mean age of 13.96±2.78 years bone turnover markers – osteocalcin (OC), N-terminal telopeptide of type I collagen (NTx), OC/ NTx ratio and leptin were determined. Anthropometric parameters expressed as BMI Z-score, WHR, W/HtR and body composition was evaluated by bioelectrical impedance analysis (BIA)such as fat mass (FAT), fat-free mass (FMM), predicted muscle mass (PMM) and total body water (TBW). The results were compared to the control group of 75 normal weight children (25 boys and 38 girls).
Results
OC was significantly lower in obese children, particularly in obese girls ($P<0.05$ and $P<0.0001$ respectively). Bone turnover ratio (calculated as OC/NTx) was significantly lower in obese girls only ($P<0.01$). Significant negative correlation was found between the OC level and BMI Z-score in the whole studied population of children. OC and OC/NTx correlated significantly with all anthropometrical parameters only in girls. There was also a significant positive correlation between NTx and leptin 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

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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, whereas 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

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Patients with moderate to severe osteogenesis imperfecta commonly have functional difficulties following fractures or surgery, which generally result in long periods of immobility. 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-facetted 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

LB1

Treatment with a Novel activin receptor IIB ligand trap improves muscle mass and bone geometry in a mouse model of severe Osteogenesis Imperfecta

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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 *Col1a1*^{Jrt}/+ 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 littermates. 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 seem to improve diaphyseal bone geometry in a model of severe OI.
Disclosure
The authors declared no competing interests.

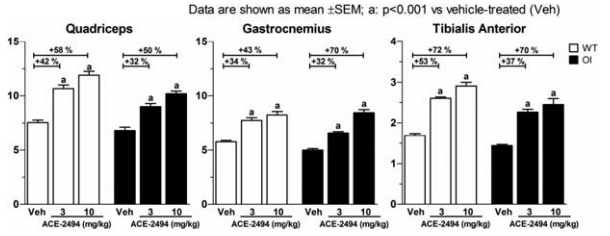

Figure 1 Increase in muscle mass (weight/initial body mass (mg/g)) by ACE-2494 treatment. Data are shown as mean±SEM; a: $P<0.001$ vs vehicle-treated (Veh).

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LB2

The regulation of *Smpd3* expression in skeletal tissues and its role in fracture healing

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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 stronger 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 p*Sox9* or p*Osx* expression vectors. For *Smpd3* promoter studies, a 1.9-kb mouse *Smpd3* proximal promoter was cloned into the pGL4.10 (*luc2*) vector. This construct was then co-transfected with or without p*Sox9* or p*Osx*. **2 -** To investigate the role of SMPD3 in fracture healing, we generated a conditional knockout mouse, *Smpd3*$^{flox/flox}$*;Osx-Cre*, which lacks *Smpd3* in both chondrocytes and osteoblasts. Rodded 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 p*Sox9* and p*Osx* in ATDC5 and MC3T3 cells, respectively. Furthermore, *Smpd3* promoter activity was significantly upregulated in the presence of p*Sox9* and p*Osx*. **2 -** Histological analyses showed a prominent callus at the site of fracture in *Smpd3*$^{flox/flox}$*;Osx-Cre* mice, whereas the fractures induced in the WT mice healed well. Histomorphometric analysis showed a significant increase in osteoid volume in *Smpd3*$^{flox/flox}$*;Osx-Cre* bones compared to WT bones.
Conclusion
Our data provides compelling evidence that SMPD3 activity, regulated by *Sox9* and *Osx*, 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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LB3

Mediating effect of muscle on the relationship of physical activity trajectories and bone outcomes: The Iowa Bone Development Study

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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 (aBMD), aBMD 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 aBMDs (neck, trochanter, intertrochanter, inferomedial and superolateral neck), on the ratio between the inferomedial and superolateral neck aBMD, and on the hip axis length (HAL). The effects of MVPA mediated by leg LST were 43–49% on regional aBMDs in girls ($P<0.01$) and 27–32% in boys ($P<0.05$). On the ratio between the inferomedial and superolateral neck aBMD 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 aBMDs 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

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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 GTCB 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 hypocalcaemia requiring intravenous calcium. Both patients received treatment for vitamin D deficiency.
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 1 Patient characteristics, treatment indication, duration, dosing information and hypercalcaemia management.

	Patient 1 (P1)	Patient 2 (P2)
Age at diagnosis	14 years 9 months	14 years 2 months
Gender	Male	Female
Weight (Kg)	56.5	45.6
Location of GCTB	Sacrum	Sacrum
Denosumab indication	Tumour recurrence following surgery and embolization	Large tumour not amenable to surgery
Denosumab regimen ClinicalTrials.gov Identifier: NCT00680992	120 mg subcutaneously on day 1, 8,15, 28 and then 4 weekly	120 mg subcutaneously on day 1, 8,15, 28 and then 4 weekly
Individual dose (mg/kg)	2.1	2.6
Total number of doses	46	18 (12 pre-, and 6 post-operative)
Cumulative dose over treatment duration (total, and mg/kg)	5,520 mg 98 mg/kg	2,160 mg 47 mg/kg
Total treatment duration	3.6 years	1.3 years
Reason for treatment cessation	Osteonecrosis of the jaw (ONJ)	End of treatment
Rebound hypercalcaemia		
Time from last denosumab dose	7 months	6 months
Calcium at presentation	3.1 mmol/l (nl 2.2–2.7)	3.4 mmol/l (nl 2.2–2.7)
Creatinine at presentation	180 μmol/l (nl 80–120)	137 μmol/l (nl 37–70)
Parathyroid hormone	0.4 pmol/l (nl 1.6–7.5)	< 3 ng/l (nl 11–29)
25 hydroxy-vitamin D	10.5 nmol/l (nl >50)	17 nmol/l (nl >50)
Treatment	Hyperhydration + calcitonin (400IU in 500 mls 0.9% NaCl over 6 hours)	Hyperhydration + pamidronate (0.6 mg/kg, over 8 hours, for 2 days)
Recurrent hypercalcaemia	Yes x2, requiring repeat calcitonin	No

Disclosure
The authors declared no competing interests.
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LB5

Role of type III sodium/phosphate co-transporters in the responsiveness of osteoblasts to extracellular inorganic phosphate

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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 ^{32}P-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* (*Alpl*) 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

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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 radial-ray abnormalities with a tendency to early osteoporosis and osteopenia. 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 *FancC*$^{-/-}$ 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 $FancC^{-/-}$ mice present a 15% decrease in bone mineral content, reduced cortical thickness and diameter combined with a 15% reduction of the bone marrow area. $FancC^{-/-}$ mice also present elevated *tartrate-resistant acid phosphatase* staining as compared to wild-type littermates. In addition, $FancC^{-/-}$ embryos show abnormal skeletal development indicated by decreased bone length and mineralization. Using *in vitro* studies, we found that $FancC^{-/-}$ 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

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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 evaluated retrospectively as an average over the past 5 years.

Results

Relative muscle power (P_{max}/mass) and force (F_{max}/body weight) were significantly decreased in T1D subjects (mean Z-scores -0.4 ± 1.0; $P<0.001$, and -0.3 ± 1.1; $P<0.01$, respectively). The duration of T1D negatively affected P_{max}/mass ($P<0.01$) but not F_{max}/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

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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) days 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 4.12(0.06) 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, $P<0.001$), 18.5 cm^2 lower bone area (BA; 95% CI -26.9 cm^2 to -10.2 cm^2, $P<0.001$) and 6.7g/ cm^2 lower bone mineral density (BMD; 95% CI -10.1g/cm^2 to -3.3g/cm^2, $P=0.041$). 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 cm^2, 95%CI -1.40 cm^2 to -0.15 cm^2, $P=0.015$) 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 cm^2, 95%CI -14.2 cm^2 to 0.3 cm^2, $P=0.083$) and four year hip BA (-0.61 cm^2, 95%CI -1.17 cm^2 to -0.04 cm^2, $P=0.035$).

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

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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.

Method

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 $\alpha 4\beta 7$. 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 $\alpha4\beta7^+CD4^+$ lymphocytes ($P=0.005$). Cases also had a significant reduction in their vBMD (0.29 vs 0.26 g/cm^3; $P=0.002$), 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 $\alpha4\beta7^+CD4^+$ 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 IBD, 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 IBD 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

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Introduction
Whilst it is well-established that sedentary behaviour may increase the risk of paediatric obesity, and potentially result in early onset cardio-metabolic diseases such as type 2 diabetes and cardiovascular disease, there is less consensus about the potential detrimental effects that long-term sedentary behaviour may have on bone health during childhood.
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 ($\leq$100 counts/min) based on at least two objective (Actigraph accelerometer worn for $>$4 days) sedentary behaviour measurements assessed at three year intervals from pre-school age to thirteen years. Peripheral quantitative computed tomography (pQCT) was used to assess the total bone area (ToA), cortical density (CoD), cortical area (CoA), marrow density (MaD), polar strength strain index (SSIp) and total cortical and regional (endo-, mid- and peri-cortical) vBMD at the mid (66%) tibia and radius. Bone outcomes were compared across groups adjusting for maturity offset and gender using analysis of covariance followed by *post hoc* pairwise comparisons with Bonferroni adjustments.
Results
There were no significant differences in height, weight or BMI z-scores between groups. At the tibia, when adjusting for maturity offset and gender, children with more sedentary time across childhood had 7.4% lower ToA, 8.0% lower CoA and 9.0% lower SSIp, and 2.0% greater CoD ($P<0.05$) compared to the active group. Sex-specific analyses showed that these significant differences persisted in boys, but not in girls, with those spending more time in sedentary behaviour having 11.1–14.9% lower CoA and SSIp ($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

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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 ± 3.0y). 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 years). Puberty (tanner staging) was classified as pre-pubertal (tanner stage = 1) and entered puberty (tanner stage $\geq$ 2). pQCT (STRATEC XCT-2000) of the radius of 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 ± 1.6g/dl and ferritin was 3005.3 ± 2183.4 ng/ml (range: 14–17.5g/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.

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LB12

Correlation of the first fracture time and COL1A1/2 mutations in patients with Osteogenesis Imperfecta

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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 causing OI.
Total number of 167 unrelated OI patients from the Osteogenesis Imperfecta database of Tartu University Department of Traumatology and Orthopeadics, 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 $\geq$7 years old. Significance of correlations

between time of the first fracture and *COL1A1/2* mutations was tested with Fisher's test.
Among 167 analysed patients, 29 (17.37%) had intrauterine fractures, 21 (12.57%) had perinatal fractures, 106 (63.47%) had fractures at the age 0–6 years old, 8 patients (4.79%) at $\geq$7 years old, and 3 (1.80%) patients had no fractures. Number of patients harbouring collagen I mutations was 103 (61.68%). The statistically significant correlation between the first fracture time and *COL1A1/2* mutations was highlighted during the study ($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$). Intrauterine fractures did not reveal correlation with presence of the *COL1A1/2* mutations ($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

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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 based 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/d, 2 consecutive days) every 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 I collagen) and alkaline phophatase 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 t.
Disclosure
The authors declared no competing interests.
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LB14

P4HB recurrent missense mutation causing Cole-Carpenter syndrome: exploring the underlying mechanism

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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 metadiaphyseal 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) 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

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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 has an active role to provide input from an OI Community perspective.
2) Forming up 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 be attend a consensus conference prior to the "13th International Conference on Osteogenesis

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
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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.
10. Providing adequate short-term plans and goals.
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
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Bone health among boys with duchenne muscular dystrophy after initiation of glucocorticoids
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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 level 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
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Occurrence of vitamin D and vitamin K deficiency in children with low-energy fractures
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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)

serum concentrations were evaluated in every patient. Ratio of serum under-carboxylated osteocalcin to serum carboxylated osteocalcin ucOC:cOC - 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 ($P<0.000004$). 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: cOC - UCR - have positively and statistically significantly correlated with lower rate of low-energy fracture incidence.
Disclosure
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Selected risk factors of fractures in children -own observation
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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* (rs1544410), *FokI* (rs2228570), *ApaI* (rs7975232) 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%Confidence Interval (95%CI) 1.00–1.02) 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* a alleles (0.08). ORs were 1.01 (95%CI 1.00–1.02) for OC, 0.29 (95%CI 0.07–1.14) for *BsmI*, 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 osteoclacin 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 fracure in children from Central Poland.
Disclosure
The authors declared no competing interests.
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LB20

COL2A1 c.1609G>A (p.Gly537Ser) a pathogenic variant causing multiple skeletal abnormalities and severe short stature
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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. IGF-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 vertebrae. Family history was significant for short stature of the father (150 cms) with bone deformities.
Methods
NGS 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, >350000 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 HiSDefault 2500 SystemTM (Illumina) 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:

Genomic position and genotype	Gene/ Transcript	Nucleotide Change/ Aminoacid change	dbSNP ID/ExAC. Freq.	Exon/Effect	Zygosity[(1)]/ Variant Freq.	In *silico* Pred.[(2)]	Categoriz-ation of the variant[(3)]
Chr12: 48379582 C/T	*COL2A1* NM_001844.4	c.1609G>A p.Gly537Ser	–	Exon 25 missense	Het 42%	8	Pathogenic

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 spondyloepihyseal 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
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LB21

Morbid obesity and respiratory failure in a child with pseudohypoparathyroidism type 1A
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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 levothyroxine, calcium carbonate, alfacalcidiol 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

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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